UNIVERSIDAD DE CÓRDOBA

Facultad de Veterinaria

Departamento de Medicina y Cirugía Animal

Biociencias y Ciencias Agroalimentarias

Tesis Doctoral con Mención Internacional

EVALUACIÓN DE NOCICEPCIÓN INTRAOPERATORIA Y DE TOLERANCIA E HIPERALGESIA INDUCIDA POR REMIFENTANILO

INTRAOPERATIVE NOCICEPTION ASSESSMENT AND DETERMINATION OF TOLERANCE AND HYPERALGESIA INDUCED BY REMIFENTANIL

Directores: María del Mar Granados Machuca y Juan Manuel Domínguez Pérez

Autora: Patricia de la Paz Ruíz López

Córdoba, Noviembre 2019

TITULO: *EVALUACIÓN DE NOCICEPCIÓN INTRAOPERATORIA Y DE TOLERANCIA E HIPERALGESIA INDUCIDA POR REMIFENTANILO*

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Título: Evaluación de nocicepción intraoperatoria y de tolerancia e hiperalgesia inducida por remifentanilo / Intraoperative nociception assessment and determination of tolerance and hyperalgesia induced by remifentanil

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Memoria de Tesis Doctoral titulada **"EVALUACIÓN DE NOCICEPCIÓN INTRAOPERATORIA Y DE TOLERANCIA E HIPERALGESIA INDUCIDA POR REMIFENTANILO / INTRAOPERATIVE NOCICEPTION ASSESSMENT AND DETERMINATION OF TOLERANCE AND HYPERALGESIA INDUCED BY REMIFENTANIL"**, presentada por **Patricia de la Paz Ruíz López**, Licenciada en Veterinaria para optar al grado de DOCTOR.

Los directores:

Dra. María del Mar Granados Machuca

Dpto. Medicina y Cirugía Animal

Facultad de Veterinaria

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Dr. Juan Manuel Domínguez Pérez

Dpto. Medicina y Cirugía Animal

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DÑA. MARÍA DEL MAR GRANADOS MACHUCA Y D. JUAN MANUEL DOMÍNGUEZ PÉREZ, profesores del Departamento de Medicina y Cirugía Animal de la Facultad de Veterinaria de la Universidad de Córdoba.

Informan:

Que la Tesis Doctoral titulada "**EVALUACIÓN DE NOCICEPCIÓN INTRAOPERATORIA Y DE TOLERANCIA E HIPERALGESIA INDUCIDA POR REMIFENTANILO / INTRAOPERATIVE NOCICEPTION ASSESSMENT AND DETERMINATION OF TOLERANCE AND HYPERALGESIA INDUCED BY REMIFENTANIL**", de la que es autora Dña. Patricia de la Paz Ruíz López, ha sido realizada bajo nuestra dirección y asesoramiento en el Departamento de Medicina y Cirugía Animal de la Universidad de Córdoba, y que a nuestro criterio dicho trabajo reúne las condiciones necesarias para ser presentado ante el Tribunal correspondiente a fin de obtener el Grado de Doctor.

Y para que conste, y a los efectos oportunos, firmamos el presente informe en Córdoba a 18 de Noviembre de dos mil diecinueve.

Fdo. María del Mar Granados Machuca Fdo. Juan Manuel Domínguez Pérez

TÍTULO DE LA TESIS: EVALUACIÓN DE NOCICEPCIÓN INTRAOPERATORIA Y DE TOLERANCIA E HIPERALGESIA INDUCIDA POR REMIFENTANILO / INTRAOPERATIVE NOCICEPTION ASSESSMENT AND DETERMINATION OF TOLERANCE AND HYPERALGESIA INDUCED BY REMIFENTANIL

DOCTORANDO/A: PATRICIA DE LA PAZ RUÍZ LÓPEZ

INFORME RAZONADO DEL/DE LOS DIRECTOR/ES DE LA TESIS

(se hará mención a la evolución y desarrollo de la tesis, así como a trabajos y publicaciones derivados de la misma).

La doctoranda Patricia de la Paz Ruíz López está colaborando activamente con nuestro grupo de investigación desde el año 2013. Dicha colaboración se inició con su incorporación al Máster en Medicina, Sanidad y Mejora Animal de la Universidad de Córdoba durante el curso 2013-2014. Durante este periodo inició una labor investigadora que encontró su continuación académica en los estudios de Doctorado. Su Proyecto de Tesis Doctoral está encuadrado en el Programa de Doctorado de la Universidad de Córdoba correspondiente al área de Biociencias y Ciencias Agroalimentarias (P.D. con mención de calidad), estando a su vez adscrito a la línea de investigación de Anestesia y Cirugía Experimental.

La presente Tesis Doctoral, llevada a cabo bajo nuestra supervisión y dirección, se ha desarrollado durante el periodo comprendido entre 2015 y 2019.

Durante dicho periodo la doctoranda ha mostrado una gran dedicación e interés en las tareas de investigación asignadas. De igual modo, ha sido capaz de realizar una labor de investigación con gran validez científica, fruto de la cual dicha Tesis Doctoral ha originado varias publicaciones en revistas de alto interés científico dentro del ámbito de la anestesiología Veterinaria, así como comunicaciones a congresos nacionales e internacionales. La doctoranda ha asistido y presentado comunicaciones en varios congresos del ámbito de interés de la Tesis Doctoral a lo largo de estos años, recibiendo el premio a la mejor comunicación oral el estudio "**Evaluación intraoperatoria del monitor PTA (Parasympathetic Tone Activity) en perras sometidas a cirugía laparoscópica**" presentado en el Congreso de la Sociedad Española de Anestesia y Analgesia Veterinaria (SEAAV) en el año 2016.

Además, con objeto de completar su formación y profundizar en el estudio de la anestesia y monitorización de caballos y pequeños animales, la doctoranda realizó una estancia de cuatro meses en la Universidad de Viena (Austria) y una estancia de tres meses en la Universidad de Londres (Reino Unido).

El trabajo realizado en esta Tesis Doctoral ha generado la siguiente producción científica:

a. Publicaciones en revistas científicas indexadas:

Ruíz-López, P; Domínguez, JM; Granados, MM. **Intraoperative nociception-antinociception monitors: a review from the veterinary perspective.** Veterinary Anaesthesia and Analgesia (2019). DOI: 10.1016/j.vaa.2019.09.006

Ruiz-López, P; Navarrete-Calvo, R; Morgaz, J; Domínguez, JM; Quirós-Carmona, S; Muñoz-Rascón, P; Gómez-Villamandos, RJ; Fernández-Sarmiento, JA; Granados, MM. **Determination of acute tolerance and hyperalgesia to remifentanil constant rate infusion in dogs undergoing sevoflurane anaesthesia.** Veterinary Anaesthesia and Analgesia (2019). DOI: 10.1016/j.vaa.2019.09.005

b. Comunicaciones científicas a congresos internacionales:

Comunicación oral: **Ruiz-López, P**; Quirós-Carmona, S; Morgaz, J; Navarrete-Calvo, R; Domínguez, JM; Gómez-Villamandos, R; Granados, MM (2019) **Evaluation of the Parasympathetic Tone Activity (PTA) monitor in horses** undergoing xylazine-ketamine-isoflurane anaesthesia. Preliminary study. Spring Meeting of the Association of Veterinary Anaesthetists. Association of Veterinary Anaesthetists (AVA). Bristol, Reino Unido.

Pendiente de publicación.

Comunicación oral: Ruiz-López, P; Domínguez-Pérez, JM; Morgaz-Rodríguez, J; Quirós-Carmona, S; Navarrete-Calvo, R; Fernández-Castañer, J; Gómez-Villamandos, RJ; Granados-Machuca, MM (2017) **Intraoperative evaluation of the parasympathetic tone activity (PTA) in dogs undergoing laparoscopic ovariectomy**. Autumn Meeting of the Association of Veterinary Anaesthetists (AVA) Berlin. Berlín, Alemania.

Publicado como "**Abstracts presented at the autumn meeting of the association of the Veterinary Anaesthetists, Berlin, Gerrmany-9-11th November, 2017**". Veterinary Anaesthesia and Analgesia 2018,45:584-588. ISSN/ISBN:1467-2987

c. Comunicaciones científicas a congresos nacionales:

Comunicación oral: **Ruíz López, P**; Quirós Carmona, S; Morgaz J; Navarrete Calvo, R; Caravaca Paredes ME; Granados Machuca, MM (2019) **Evaluación intraoperatoria del monitor "Actividad del Tono Parasimpático" (PTA) en caballos anestesiados con xilacina-ketamina-isoflurano**. XV Congreso Nacional Sociedad Española de Anestesia y Analgesia Veterinaria. Toledo, España.

Comunicación oral: **Ruiz López, P**; Morgaz Rodríguez, J; Navarrete Calvo, R; Quirós Carmona, S; Sánchez de Medina Baena, A; Granados Machuca, MM (2018) **Evaluación intraoperatoria del monitor Parasympathetic Tone Activity (PTA) en el caballo. Estudio preliminar.** XIV Congreso Nacional Sociedad Española de Anestesia y Analgesia Veterinaria. Pamplona, España

Comunicación oral: **Ruiz López, P**; Domínguez Pérez, JM; Quirós Carmona, S; Morgaz Rodríguez, J; Mengual Riera, C; Navarrete Calvo, R; Fernández Castañer, J; Aguilar García, D; Muñoz Rascón, P; Granados Machuca, MM (2016) **Evaluación intraoperatoria del monitor PTA (Parasympathetic Tone Activity) en perras sometidas a cirugía laparoscópica**. XII Congreso Nacional Sociedad Española de Anestesia y Analgesia Veterinaria. Sevilla, España.

Premio a la mejor comunicación oral "Ignacio Cruz" de la Sociedad Española de Anestesia y Analgesia (SEAAV) 2016.

d. Comunicación científica presentada en el Congreso Científico de Investigadores en Formación de la Universidad de Córdoba

Comunicación escrita: **Ruiz López, P**; Granados Machuca, MM (2016) **Evaluación de hiperalgesia y tolerancia tras la administración de una infusión continua de remifentanilo en el perro**. V Congreso Científico de Investigadores en Formación de la Universidad de Córdoba. Córdoba, España

Publicado como "**Creando redes doctorales. Volumen V**" Universidad de Córdoba 2016, 5:321-324. ISBN: 978-84-9927-271-9

Además de trabajar en el desarrollo y progreso de su Tesis Doctoral, la doctoranda ha participado activamente en los diversos proyectos de investigación que llevamos a cabo en nuestra unidad, figurando entre los autores de numerosas comunicaciones y artículos científicos.

Por todo ello, se autoriza la presentación de la Tesis Doctoral.

Córdoba, 18 de Noviembre de 2019

Firma de los directores

Fdo.: María del Mar Granados Machuca Fdo.: Juan Manuel Domínguez Pérez

MENCIÓN DE DOCTORADO INTERNACIONAL

Esta tesis cumple los criterios para la obtención de la mención “Doctorado Internacional concedida por la Universidad de Córdoba regulada por el RD99/2011.

Para ello se presentan los siguientes requisitos:

1. Estancia predoctoral becada por el Campus de Excelencia Internacional Agroalimentario (ceiA3) para la obtención de doctorado internacional en el Departamento de Anestesia y Cuidados Intensivos Perioperatorios de la Universidad de Medicina Veterinaria de Viena (Austria) bajo la supervisión de la Dra. Profa. Paula Larenza Menzies.
2. Esta Tesis está avalada por los siguientes informes de idoneidad realizados por dos Doctoras de otros centros de investigación internacionales: la doctora Carolina Palacios Jiménez y la doctora Eva Rioja Garcia.
3. El texto ha sido redactado en dos idiomas europeos, español e inglés. La defensa de tesis se realizará en los mismos dos idiomas.
4. Entre los miembros del tribunal se encuentra un Doctor procedente de un centro de educación superior europeo.

Córdoba, Noviembre de 2019

Fdo.: Patricia de la Paz Ruíz López

Esta Tesis Doctoral ha sido parcialmente subvencionada por el Programa de Ayudas de Posgrado para la “Formación de Profesorado Universitario” del Ministerio de Educación, Cultura y Deporte (FPU, convocatoria publicada en el BOE Nº313 de 27 de Diciembre de 2014, Orden ECD/1619/2013)

Agradecimientos

Creo que la parte más difícil de escribir es ésta. No hay una guía o un modelo a seguir para esta parte en la que agradecer sobradamente todo lo que me han aportado las personas que me rodean.

“La familia es lo primero”. Por ello, en primer lugar, debo agradecer a mis padres, Lina y Antonio. Ellos siempre me han apoyado, me han dado la libertad de elegir que quería hacer en cada momento de mi vida, casi desde que tengo uso de razón. Se han preocupado por mí y me han ayudado en la medida de lo posible, pero sobre todo, apoyándome y creyendo más en mí, de lo que yo creo. Un ejemplo a seguir de constancia y trabajo infatigable. Junto a ellos han estado siempre mis hermanas, incondicionales en apoyo, animándome en cada paso del camino y aconsejándome. Ana Mary, desde la distancia, los kilómetros nos separan, pero siempre presente en cada día de mi vida. Admiro tu valentía por irte lejos, por formar una vida lejos de tu familia, tu tranquilidad y sosiego. Mónica, en una distancia más corta, madre de dos maravillosas personas, Pablo y Fernando, que con su apoyo, al igual que lo he tenido yo, nunca se sentirán solos. De ti admiro tu pasión y tu forma de ser práctica, resolutiva y mirando el futuro. Quizás demasiado parecidas en carácter para no chocar de vez en cuando, pero siempre ahí. Recuerdo el consejo constante de mis padres y de mis hermanas, “haz lo que te haga feliz, lo que quieras hacer y lo que creas que es mejor para ti”. No sé si lo mejor para mí es lo que hago, pero desde luego, es lo que me hace feliz y lo que quiero hacer.

Dicen que la amistad es la familia que se elige y hay tres personas en mi vida, que desde luego, son la familia que he elegido. Almudena, a ti te conocí la primera, somos amigas, casi desde que tenemos memoria. Aunque cada una tengamos unos recuerdos... Siempre hemos estado presentes la una en la vida de la otra y pase lo que pase, sabes que aunque seas hija única, tienes en mí a una hermana. A Bea la conocí en el primer viaje a COVAP, del que nunca podré olvidar que era lo que más ganas tenía Bea de hacer en la carrera. No puedo mencionar a Bea, sin mencionar a “Beatricia”. Compañeras inseparables de la carrera, demasiado parecidas en unas cosas y tan diferentes en otras, pero siempre un equipo perfectamente coordinado. Estudiar veterinaria, no hubiera sido lo mismo sin ti. Cuánto nos hemos reído en clase, casi hasta llorar. Aún en la distancia, sigues siendo en la persona que más me apoyo cada vez que

necesito a alguien para hablar. Pilar, te conocí como casera y nos convertimos en amigas. Tu sabiduría, tus ganas de vivir, de superar nuevos retos cada día y de saltar al vacío cada vez que quieres dar un giro a tu vida, me parecen admirables. Eres la persona más valiente y decidida que conozco. Gracias a las tres, por estar en mi vida.

Tuve claro desde niña que quería ser veterinaria. El amor que me transmiten los animales es incomparable a cualquier otra cosa y siempre recordaré el día que lo descubrí. Volvía del colegio y al llegar a casa, me encontré con una bolita negra, de pelo rizado que se movía con nerviosismo por el salón. Apenas se distinguía en la oscuridad de debajo de la mesa, salvo por sus ojillos brillantes. Fue amor a primera vista. Campeón, fue mi compañero inseparable, aunque desgraciadamente no por mucho tiempo. Tras Campeón, llegó Copito. Compañero de mi infancia y de mi adolescencia, me enseñó la más valiosa lección “hay que vivir el momento y no dar nada por hecho, porque no sabemos cuándo puede acabar”. Lucero, fue otro de los perros más importantes de mi infancia-adolescencia y junto a ellos algunos más. Todos ellos me enseñaron el amor y la lealtad que te puede ofrecer un animal, solo por una pizca de tu tiempo y cariño. Ellos fueron quienes me llevaron a querer estudiar Veterinaria.

Recuerdo el día, que conocí a María del Mar como estudiante. Aún no había cursado Anestesiología, pero estaba haciendo prácticas en el HCV (Hospital Clínico Veterinario). Ella era la encargada de anestesiar el caso que hubiera en ese momento y se puso a preguntarme por la máquina anestésica, mi respuesta fue “no lo sé, estoy en tercero, todavía no he hecho la asignatura y no sé nada de esto”. No recuerdo exactamente que me contó o que no, pero sí que se puso a explicarme. Recuerdo también, que solo anestesia y cirugía explicaban que hacían, cómo, porqué e implicaban a los alumnos en los procedimientos que realizaban. Después de esas prácticas, supe que si hacía algo, sería con ellos por su calidez humana y ganas de enseñar. María del Mar acabó siendo mi tutora/directora de beca de colaboración, trabajo fin de máster y tesis. Incansable en dedicación a su trabajo y vida personal, siempre dispuesta a explicar, ayudar y buscar soluciones. En este último año, ha sido su positivismo en que “todo saldría bien” lo que más me ha tranquilizado. Es un ejemplo de líder, pues no solo sabe implicarse sino implicar a los demás.

Rafa, eres el “padre” del grupo de anestesia y cirugía. Un referente a seguir, siempre con ideas nuevas, transgresoras y con ganas de innovar. Te quedas con lo mejor de las personas que te rodean y te preocupas por ellas. Sin tu vitalidad, liderazgo y pasión por la vida, este grupo no sería lo mismo.

Rocío es otro pilar de esta familia. Aporta la dulzura, tranquilidad y calma. Recuerdo nuestras conversaciones en el tren, tus consejos durante esos viajes y tu manera de ver solo lo bueno de cada uno. Juan, cirujano infiltrado en los congresos de anestesia, estadístico y bailarín. Sin ti no hubiera sido posible, gran parte de este trabajo. Siempre dispuesto a hacer la estadística cada vez que te lo pedía, a rehacer las gráficas, cada vez que nos lo pide la revista, ya sea fin de semana, festivo o día laborable. Trabajador incansable y siempre con ganas de salir a bailar o explicarle a algún estudiante erasmus alguna nueva palabra. Juanma, riguroso en el quirófano, pero siempre solícito, hemos compartido todas las laparoscopias que fueron necesarias para uno de los estudios de este trabajo, que no fueron pocas. Andrés, tranquilo, meticuloso y calmado. Transmites un gran sosiego y compasión por cada uno de tus pacientes. Sete, organizadora y con gran calma ante situaciones de estrés. Dispuesta a ayudar en lo que sea, ya sea una anestesia u organizar un perol. Carmen, trabajadora silenciosa, no porque no hables sino porque no te gusta hacer ruido. Agradable, incluso cuando estás exasperada. Marga, diferente a todos por tu parte “exótica”, habladora incansable siempre dispuesta a asistir a una quedada o a echar una mano. Dani, serio, comedido, pero siempre amistoso y sin hacer distinciones entre los demás. Julio y Aritz, preocupados por vuestro trabajo y amigos de vuestros amigos. Esther, compañera de trabajo, fuiste compañera de piso, pero sobre todo amiga. Siempre dispuesta a ayudar, incluso cuando estás harta, te preocupas por animales y personas por igual. Has sido un gran apoyo en el último año, sobre todo a nivel personal y una gran tía de Charlotte. Hay muchos más nombres en la lista del departamento y el hospital, que están o han estado ahí cada día. Todos formamos una familia, todos formamos un organismo vivo que es el HCV, en constante evolución en el que todos tenemos un hueco y que no es el mismo si alguien falta.

Me gustaría mencionar a mucha más gente, muchos más compañeros y amigos, que me han aportado inmensamente como persona y profesional. A todos los

que he conocido durante estos años en Ronda, Granada, Córdoba, durante mis estancias en Viena y en Londres o en cualquier otro lugar. Gracias a todos, por vuestro granito de arena, porque cada uno de ellos suma. Aprendo de cada persona que conozco, ocupe el puesto que ocupe, fuera y dentro de Veterinaria. Por ello, gracias también a todos aquellos que no menciono por nombre aquí.

Faltan palabras para describir a todos, pero tenía que reservar este último párrafo para mi gato, Charlotte. Ha sido mi compañero desde meses antes de empezar la Tesis, probablemente ha sido el que más ha sufrido mis ausencias por estancias, cursos, congresos o trabajo. Sin decir una palabra me ha apoyado cada día, gracias a él, no he estado ni un solo día sola. Mi amigo y compañero más leal durante la Tesis.

Gracias a todos y cada uno por todo.

Lista de abreviaturas

ADME: asta dorsal de la médula espinal

AMPA: receptor α-amino-3-hidroxi-5-metilo-4-isoxazolpropiónico

AMPc: adenosina monofosfato cíclico

ANI: analgesia nociception index

ANOVA: analysis of variance

ANS: autonomous nervous system

AOT: acute opioid tolerance

ASIC: canales iónicos sensibles a la acidez

AUC: area under curve

BIS: índice biespectral / bispectral index

BIS1min (Estudio I): value of the BIS 1 minute after the surgical stimulus

BIS2min (Estudio I): value of the BIS 2 minutes after the surgical stimulus

BISbaseline (Estudio I): value of the BIS before the surgical stimulus

BP: blood pressure

CAM: concentración alveolar mínima

CGRP: péptido relacionado con el gen de la calcitonina

CRI: constant rate infusion

DAP: diastolic arterial pressure

Dobutamine event (Estudio II): If a MAP ≤ 62mmHg was observed, dobutamine 0.25 μg kg^{-1} $minute^{-1}$ IV at a CRI was started and increased by 0.25 μg kg^{-1} $minute^{-1}$ IV every 5 minutes until the MAP stably reached a value between 70 and 80 mmHg. Data (PTA, HR and MAP) was recorded before and 5 minutes after dobutamine CRI was either started or increased

EEG: electroencephalography

EMG: electromyography

EtISO: end-expiratory isoflurane

$EtCO_2$: end tidal carbon dioxide

FC: frecuencia cardiaca

$F_{E'}$SEVO: end-expiratory sevoflurane

f_R: respiratory rate

GABA: ácido gamma-aminobutírico

GPCRs: receptores acoplados a proteína G

GSK-3β: glicogenosintetasa quinasa-3β

Haemodynamic response (Estudio I): it was considered if the HR or MAP increased more than 20 % regarding baseline values for the statistical analysis

HF: high frequency

HR: heart rate

HR event (Estudio II): it was considered if the HR increased by 10% or more than 10%. Data (PTA, HR and MAP) were registered before and 1, 3 and 5 minutes after the observation of the event

HR1min (Estudio I): HR 1 minute after the surgical stimulus

HR2min (Estudio I): HR 2 minutes after the surgical stimulus

HRbaseline (Estudio I): HR before the surgical stimulus

HRV: heart rate variability

IASP: International Association for the Study of Pain

IL: interleuquinas

IM: intramuscularly

IPPV: intermittent positive pressure ventilation

IV: intravenously

Ketamine event (Estudio II): Ketamine 0.5 mg kg^{-1} IV was administered if an increase in the palpebral reflex, light nystagmus, spontaneous ventilation or lack of muscle relaxation were observed. Data (HR, MAP and PTA) were registered before and 3 and 5 minutes after ketamine administration

LF: low frequency

LTP: potenciación a largo plazo de la potencia sináptica

MAC: minimum alveolar concentration

MACb1 (Estudio III): baseline MAC before remifentanil administration

MACb2R (Estudio III): baseline MAC determined one week after remifentanil CRI administration

MACb2S (Estudio III): baseline MAC determined one week after 0.9% saline CRI administration

MACpostdrug1R (Estudio III): MAC determined 20 minutes after remifentanil CRI started

MACpostdrug1S (Estudio III): MAC determined 20 minutes after 0.9% saline CRI started

MACpostdrug2R (Estudio III): MAC determined 30 minutes after MACpostdrug1R determination

MACpostdrug2S (Estudio III): MAC determined 30 minutes after MACpostdrug1S determination

MAP: mean arterial pressure

MAP event (Estudio II): it was considered if the MAP increased by 20% or more than 20%. Data (PTA, HR and MAP) were registered before and 1, 3 and 5 minutes after the observation of the event

MAP1min (Estudio I): MAP 1 minute after the surgical stimulus

MAP2min (Estudio I): MAP 2 minutes after the surgical stimulus

MAPbaseline (Estudio I): MAP before the surgical stimulus

MNT (Estudio III): mechanical nociceptive threshold

MNT3 (Estudio III): mechanical nociceptive threshold 3 days after the dogs received remifentanil or 0.9% saline CRI

MNT7 (Estudio III): mechanical nociceptive threshold 7 days after the dogs received remifentanil or 0.9% saline CRI

MNTb (Estudio III): mechanical nociceptive threshold before the first anaesthesia

Morphine event (Estudio III): morphine hydrochloride 0.2 mg kg^{-1} was administered and PTA, HR and MAP were recorded before and 10 minutes after the administration

NGF: factor de crecimiento neuronal

NK-1: receptor de neuoquinina 1

NMDA: receptor N-metil-D-aspartato / N-methyl-D-aspartate

NO: oxido nitroso

NSAID: nonsteroidal anti-inflammatory drug

OIH: opioid-induced hyperalgesia

O_2: oxygen

PAM: presión arterial media

$P_{E´}CO_2$: end-tidal carbon dioxide

PNS: parasympathetic nervous system

PTA: parasympathetic tone activity

PTA event (Estudio I): it was considered if the PTA index decreased more than 20% regarding baseline values for the statistical analysis

PTA event (Estudio II): it was considered if the PTA index decreased by 20 % or more than 20% during the surgery. Data (PTA, HR and MAP) were registered before and 1, 3 and 5 minutes after the observation of the event

PTA1min (Estudio I): value of the PTA index 1 minute after the surgical stimulus

PTA2min (Estudio I): value of the PTA index 2 minutes after the surgical stimulus

PTAbaseline (Estudio I): value of the PTA index before the surgical stimulus

PTAi: instantaneous PTA

PTAm: average/mean PTA

R treatment (Estudio I): dogs that received remifentanil 0.3 µg kg^{-1} min^{-1}

RR: respiratory rate

RE: response entropy

RPD: reflex pupillary dilatation

S treatment (Estudio I): dogs that received 0.9% saline CRI

SE: state entropy

SAP: systolic arterial pressure

SNA: sistema nervioso autónomo

SNS: sympathetic nervous system

SPI: surgical plethysmographic index

SpO_2: oxygen saturation

SQI: signal quality index

SR: supression ratio

T: temperature

TLR4: receptor 4 tipo Toll

TNF-α: factor de necrosis tumoral alfa

TRPA: receptor de potencial transitorio A

TRPM8: receptor de potencial transitorio M8

TRPV1: receptor de potencial transitorio V1

ΔANI: dynamic variation of ANI

ΔPTA: variación dinámica del índice PTA/ dynamic variation of the PTA index

ÍNDICE

Resumen

El dolor aumenta las complicaciones tanto intraoperatorias como postoperatorias, pues es un fenómeno estresante que desencadena la liberación de catecolaminas dando lugar al predominio del Sistema Autónomo Simpático. La monitorización nociceptiva-antinociceptiva intraoperatoria se ha basado fundamentalmente en el control de la frecuencia cardiaca (FC) y la presión arterial, sin embargo, las variables hemodinámicas no solo se afectan por la nocicepción. El monitor Parasympathetic Tone Activity (PTA) ha sido desarrollado para las especies canina, felina y equina basado en el monitor Analgesia Nociception Index (ANI) (índice de nocicepción-analgesia) de aplicación en personas. El ANI está basado en la variabilidad de la FC, que refleja los cambios en el sistema nervioso autónomo (SNA), como respuesta a estímulos nociceptivos intraoperatorios y administración de fármacos.

Los opioides son fármacos analgésicos que actúan sobre todas las fases de la nocicepción, que de manera simple se divide en: Transducción, Transmisión, Modulación y Percepción. No obstante, los opioides tienen efectos secundarios entre los que se encuentran en ratas, ratones y en humanos, el desarrollo de tolerancia e hiperalgesia debida a su uso, manifestadas como el aumento de la nocicepción intraoperatoria y de las necesidades de analgésicos postoperatorios.

Tras realizar una revisión de los monitores con los que contamos en Medicina Veterinaria para la monitorización nociceptiva intraoperatoria, se plantearon dos estudios para evaluar el uso del monitor PTA, único monitor desarrollado hasta el momento para su uso en animales.

En el primer estudio “Evaluation of the parasympathetic tone activity (PTA) index and its dynamic variation (ΔPTA) in dogs undergoing laparoscopic ovariectomy”, se emplearon 32 perras sometidas a ovariectomía laparoscópica para valorar si el índice PTA o su variación dinámica (ΔPTA) precede o coincide con cambios en la frecuencia cardiaca o en la presión arterial media (PAM) tras la aplicación de cuatro estímulos nociceptivos quirúrgicos: insuflación de neumoperitoneo, introducción de trócares, extirpación del ovario izquierdo y extirpación del ovario derecho. Para asegurar un plano anestésico estable se empleó el monitor de índice biespectral (BIS). Se registraron los parámetros de PTA, BIS, FC y PAM antes y 1 y 2 minutos tras la realización de cada uno de los

estímulos quirúrgicos. Los datos se analizaron estadísticamente para detectar "eventos PTA" y "respuestas hemodinámicas" ocurridas durante el estudio, considerandose como evento PTA una disminución del índice PTA mayor o igual a un 20% y como respuesta hemodinámica un incremento de la FC o la PAM mayor o igual a un 20%. El índice PTA disminuyó significativamente (p = 0,007) 1 y 2 minutos después de la insuflación. Durante los eventos PTA, la PAM se incrementó significativamente (p = 0,001) tras 1 y 2 minutos, pero no la FC o el BIS. La ΔPTA fue significativamente diferente en los perros que presentaron respuesta hemodinámica durante la insuflación del pneumoperitoneo. Las curvas ROC mostraron un valor de corte de PTA basal (previo al estímulo quirúrgico) ≤ 51 para detectar una respuesta hemodinámica, con una sensibilidad y especificidad del 69 y 52% respectivamente.

En el segundo estudio "Assessment of the autonomic nervous system activity by monitoring parasympathetic tone activity (PTA) in horses after a nociceptive stimulus and subsequent administration of morphine, ketamine and dobutamine", se emplearon 20 caballos sometidos a cirugía electiva bajo anestesia general para valorar los cambios en el SNA, observados como cambios en los valores del índice PTA, tras un estímulo nocieptivo o la administración de morfina, ketamina o dobutamina. Para ello, se valoraron los parámetros de índice PTA, FC y PAM antes y 1, 3 y 5 minutos tras la incisión quirúrgica. Dichos parámetros también se registraron antes y después de la administración de fármacos: antes y a los 10 minutos de la administración de morfina, antes y a los 5 minutos del inicio de una infusión continua de dobutamina, y antes y a los 3 y 5 minutos de la administración de un bolo de ketamina. La morfina se administró siempre una vez registrados los parámetros estudiados tras la incisión quirúrgica. La dobutamina se administró cuando la PAM fue inferior a 62 mmHg y la ketamina si el animal presentó aumento del reflejo palpebral, nistagmo o ventilación espontánea. Se consideró evento PTA si el índice PTA disminuyó un 20% o más, evento FC si la FC se incrementó un 10% o más y evento PAM si la PAM se incrementó un 20% o más, momento en el que se registraron los parámetros de índice PTA, FC y PAM antes (retorspectivo) y 1, 3 y 5 minutos después de que se observara el evento. El índice PTA disminuyó significativamente a los 3 minutos de la administración de

ketamina (p = 0,042) y 1 minuto después de la identificación de un evento PTA (p = 0,016). La PAM disminuyó significativamente a los 10 minutos de la administración de morfina (p = 0,009) y 5 minutos tras la administración de ketamina (p = 0,010).

En el tercer estudio “Determination of acute tolerance and hyperalgesia to remifentanil constant rate infusion in dogs undergoing sevoflurane anaesthesia”, se emplearon 9 perros de raza beagle para determinar si la infusión continua de remifentanilo produce tolerancia aguda o hiperalgesia en el perro. Se observó si la infusión continua de remifentanilo disminuyó la reducción de la concentración alveolar mínima (CAM) de sevoflurano en el perro (tolerancia aguda), así como si disminuyó el umbral nociceptivo al aplicar un estímulo mecánico a los 3 o 7 días o si aumentó la CAM basal una semana después de la administración de remifentanilo (hiperalgesia). Para ello se realizó la inducción y mantenimiento de la anestesia con sevoflurano. Se determinó la CAM basal 1 (MACb1) y se inició la administración de remifentanilo 0,3 μg kg^{-1} $minuto^{-1}$ o suero salino. Veinte minutos tras el inicio de la administración de remifentanilo o suero salino se determinó la CAM post fármaco 1 (MACpostdrug1) y 30 minutos después de la determinación de MACpostdrug1, se determinó la CAM post fármaco 2 (MACpostdrug2). Una semana más tarde, los perros se anestesiaron de nuevo con sevoflurano para la determinación de la CAM basal 2 (MACb2). Antes de la primera anestesia, a los 3 y a los 7 días se determinó la respuesta al estímulo mecánico. La infusión continua de remifentanilo redujo la MACb1 en un 43,7%. No hubo diferencias significativas entre MACpostdrug1 y MACpostdrug2, MACb1 y MACb2, ni entre los estímulos mecánicos en el grupo del remifentanilo ni del salino.

La monitorización nociceptiva intraoperatoria es una tarea compleja, que se complica por el posible desarrollo de tolerancia e hiperalgesia a los opioides. En estos tres estudios, se encontró que el índice PTA no fue efectivo para determinación de nocicepción intraoperatoria en relación a cambios en la FC y la PAM en perros anestesiados. Además, la ΔPTA no predijo las respuestas hemodinámicas tras estímulos nociceptivos en el perro. En el caballo, no se observaron cambios en la actividad del SNA tras el estímulo quirúrgico o la administración de morfina o dobutamina empleando el índice PTA. Solo la

ketamina produjo una disminución del mismo, que se interpreta como un aumento de la actividad del sistema nervioso simpático. Bajo las circunstancias del estudio III, no se hallaron indicios de tolerancia e hiperalgesia tras el uso de remifentanilo, ya que no se alteró la reducción de la CAM de sevoflurano ni se disminuyó el umbral nociceptivo para la respuesta al estímulo mecánico.

Abstract

Pain increases intraoperative and postoperative risks. Since, it is a stressful process that triggers catecholamines release that increases predominance of the sympathetic nervous system. Intraoperative nociception monitor is based mainly in heart rate (HR) and blood pressure changes. However, there are more players involved in haemodynamic changes. The Parasympathetic Tone Activity (PTA) index has been developed for dogs, cats and horses. It is the homologous of the Analgesia Nociception Index (ANI) monitor for humans. It is based on the HR variability that reflects the changes on the autonomous nervous system (ANS) as consequence of intraoperative nociceptive stimuli and drugs administration.

Opioids are analgesic drugs that acts at the different phases of the nociception: transduction, transmission, modulation and perception. Nevertheless, opioids show side effects as development of tolerance and hyperalgesia. These phenomena can be observed as an intraoperative nociception increase and higher needs of postoperative analgesic drugs. These phenomenons have been demonstrated in rats, mice and humans.

After reviewing the intraoperative nociception monitors, two studies were planned using the PTA monitor, the only one developed for veterinary medicine.

The objective of the first study “Evaluation of the parasympathetic tone activity (PTA) index and its dynamic variation (ΔPTA) in dogs undergoing laparoscopic ovariectomy”, was to assess if the PTA index or its dynamic variation (ΔPTA) coincide or precede with changes on heart rate or mean arterial pressure (MAP) after nociceptive surgical stimuli: insufflation, introduction of the trocars, removal of the left and right ovaries. A total of 32 bitches undergoing laparoscopic ovariectomy were included in this study. The bispectral index (BIS) was used to ensure that a stable depth of anaesthesia was maintained. Data for PTA, BIS, HR and MAP were registered before and after 1 and 2 minutes from the nociceptive surgical stimulus. For the statistical analysis, A PTA event was considered if the PTA index decreased by 20% or more than 20% and a haemodynamic response was considered if HR and/or MAP increased 20% or more than 20% regarding baseline values. The PTA index decreased significantly (p = 0.007) 1 and 2 minutes after insufflation. During PTA events, the MAP increased significantly 1 minute (p = 0.001) and 2 minutes (p = 0.001) later but

HR (p = 0.192) and BIS (p = 0.245) did not change. The ΔPTA was significantly different between dogs that presented a haemodynamic response and dogs that did not present it at pneumoperitoneum insufflation (p = 0.005). ROC curves showed a threshold value of PTAbaseline ≤ 51 to detect a haemodynamic response (sensitivity 69%, specificity 52%).

The second study “Assessment of the autonomic nervous system activity by monitoring parasympathetic tone activity (PTA) in horses after a nociceptive stimulus and subsequent administration of morphine, ketamine and dobutamine”, determined if ANS activity changes in response to a surgical nociceptive stimulus or during the administration of morphine, ketamine and dobutamine observed as changes on PTA index. A total of 20 horses undergoing general anaesthesia for elective surgeries were included in this study. HR, MAP and PTA index were monitored before and 1, 3 and 5 minutes after the surgical incision, and before and 10 minutes after morphine administration. If an increase of the palpebral reflex was noted or nystagmus or spontaneous ventilation was observed, a ketamine bolus was given and the three variables were registered before, 3 and 5 minutes after the administration. If the MAP fell below 62 mmHg, a dobutamine infusion was administered and the three variables were registered before and 5 minutes after the infusion was started or increased. If the PTA index decreased ≥ 20% (PTA event), HR increased ≥ 10% (HR event) or MAP increased ≥ 20% (MAP event), the three variables were registered before and 1, 3 and 5 minutes after each event. The PTA index decreased significantly 3 minutes after the ketamine bolus (p = 0.042) and 1 minute after a PTA event was identified (p = 0.016). The MAP decreased significantly 10 minutes after morphine administration (p = 0.009) and 5 minutes after ketamine administration (p = 0.010).

The third study of this Thesis “Determination of acute tolerance and hyperalgesia to remifentanil constant rate infusion in dogs undergoing sevoflurane anaesthesia”, determined if acute opioid tolerance (AOT) or opioid-induced hyperalgesia (OIH) could develop and limit the remifentanil-induced reduction in the sevoflurane minimum alveolar concentration (MAC). The response to mechanical nociceptive threshold (MNT) was evaluated and related to OIH. A total of nine beagle dogs were included in this study. The dogs were

anaesthetized with sevoflurane. Baseline sevoflurane MAC was measured (MACb1). Remifentanil (0.3 μg kg^{-1} $minute^{-1}$) or 0.9% saline constant rate infusion (CRI) was administered intravenously (IV). Sevoflurane MAC was determined 20 minutes after CRI was initiated (MACpostdrug1), 30 minutes after MACpostdrug1 determination (MACpostdrug2) and after 1 week (MACb2). The MNT was determined at baseline (before anaesthesia), 3 and 7 days after anaesthesia. Remifentanil CRI reduced sevoflurane MACpostdrug1 by 43.7% with respect to MACb1. MACpostdrug2 was no different with respect to MACpostdrug1 in the saline (p = 0.62) or remifentanil (p = 0.78) treatments. No significant differences were observed in the saline (p = 0.99) or remifentanil (p = 0.99) treatments between MACb1 and MACb2, or for MNT values between baseline, 3 and 7 days.

The intraoperative nociception monitor is a complex duty that is complicated with the possible development of tolerance and hyperalgesia to opioids. Among these studies, it was found that the PTA index did not effectively assess intraoperative nociception regarding haemodynamic changes, neither ΔPTA predicted a haemodynamic response in anaesthetised dogs. Furthermore, no changes in ANS activity, using the PTA index, were registered in anaesthetised horses after a surgical nociceptive stimulus or dobutamine and morphine administration. The PTA index demonstrated an increase in sympathetic nervous system activity after ketamine administration.

In dogs, under the third study conditions, remifentanil efficacy in reducing sevoflurane MAC did not diminish in the short term, suggesting remifentanil did not induce AOT. Hyperalgesia was not detected 3 or 7 days after the administration of remifentanil. Contrary to data from humans and rodents, development of AOT or OIH in dogs is not supported by the findings of this study.

Capítulo I

INTRODUCCIÓN GENERAL

1. Definiciones

La anestesia se define de manera simple como una combinación de analgesia, inconsciencia e inmobilidad (Gruenewald & Illies 2013), siendo necesario el componente analgésico para evitar el dolor y sus consecuencias tras el estímulo quirúrgico (Gruenewald & Dempfle 2017).

Según la definición de la Asociación Internacional para el Estudio del Dolor (International Association for the Study of Pain, IASP) el dolor es “una experiencia sensorial o emocional desagradable asociada a un daño real o potencial en un tejido, o descrito en términos de dicho daño” (Boyd et al. 1994). Más tarde, basada en esta definición, se puntualizó que el dolor es una experiencia subjetiva debida al daño en una parte del cuerpo (Williams & Craig 2016). Además, la IASP especifica que la incapacidad de expresar verbalmente ese dolor, no exime de la necesidad de aliviarlo.

Estas definiciones de dolor requieren que haya una implicación de estructuras cerebrales para su percepción, mientras que la nocicepción se define como la codificación y procesamiento del estímulo nociceptivo en el sistema nervioso (Bell 2018).

Se diferencian varios tipos de dolor en función de su duración, fisiología y cambios en el umbral doloroso, como se describe a continuación.

Dependiendo de su duración, se diferencia entre dolor agudo y dolor crónico. El primero, el **dolor agudo**, responde a un estímulo nociceptivo de duración limitada en el tiempo y en consonancia al daño tisular o afectación orgánica que lo ha producido. Se considera que tiene una función protectora, ya que previene daños en el organismo. En segundo lugar, el **dolor crónico**, se define como aquél que no previene daños en el organismo y va más allá en el tiempo del daño tisular o afectación orgánica (Basbaum et al. 2009). Las cirugías electivas son eventos que producen dolor agudo y que pueden dar lugar a dolor crónico como consecuencia del dolor persistente postquirúrgico, que se define como un dolor persistente que se extiende más allá de dos meses desde la cirugía y que no se puede explicar por otra causa (Chapman & Vierck 2016). Los mecanismos por los que el dolor agudo se perpetúa están en relación con el desarrollo de tolerancia e hiperalgesia, y se desarrollarán posteriormente.

Dependiendo de su fisiología, se diferencia entre **dolor nociceptivo**, si se origina por la estimulación de nociceptores, que son las terminaciones de las fibras aferentes primarias que actúan como receptores cutáneos o subcutáneos especializados en la detección de estímulos potencialmente dañinos; y **dolor neuropático**, si lo causa una lesión o enfermedad del sistema nervioso somatosensorial, este tipo de dolor carece de una función protectora (Smith 2018).

El umbral doloroso se define como la mínima intensidad de un estímulo que se percibe como dolor (van Ganzewinkel et al. 2017). Si un estímulo que normalmente produce dolor, no lo hace, se denomina **analgesia**. Si la ausencia de dolor no es completa, sino que se produce un aumento del umbral doloroso, se conoce como **hipoalgesia** (Boyd et al. 1994). Aunque, de manera práctica, se emplea analgesia indistintamente para analgesia e hipoalgesia.

Si ese mismo estímulo produce un aumento de la sensibilidad al dolor o hay una disminución del umbral doloroso, se define como **hiperalgesia** (Chu et al. 2008) La hiperalgesia puede ser primaria, secundaria o referida (Sandkhuler 2009):

- Primaria: si se produce adyacente al punto de la lesión, causada principalmente por alteraciones en las terminaciones de las fibras sensitivas.

- Secundaria: si se produce dentro del área de la lesión tisular o remota, debido a alteraciones en el procesamiento de la información sensorial en el sistema nervioso central (SNC).

- Referida: si existe dentro del área de la lesión tisular y remota a ésta en la piel.

Una variante de la hiperalgesia es la **alodinia,** situación en la que se produce dolor a consecuencia de estímulos no dolorosos para un individuo normal (Sandkuhler 2009)

A veces confundida con la hiperalgesia, encontramos el término **tolerancia**. A diferencia de la hiperalgesia, el umbral doloroso para un estímulo nociceptivo es el mismo que en un individuo normal. Se denomina tolerancia cuando el efecto terapéutico del fármaco disminuye o desaparece tras una o más administraciones, necesitando aumentar la dosis para conseguir el mismo efecto

(Bekhit 2010). Ésta puede ser aguda si se desarrolla rápidamente en el transcurso de horas, como ocurre con la administración intraoperatoria de opioides de corta duración en la rata (Gómez de Segura et al. 2009; Benito et al. 2010), o retardada cuando se produce tras varios días de tratamiento (Ballantyne & Mao 2003). Si se desarrolla a las semanas o meses de iniciar un tratamiento se denomina tolerancia crónica, suele darse en pacientes con dolor crónico por cáncer, normalmente tratados con opioides (Viet et al. 2017).

En el caso de fármacos con mecanismos de acción comunes, como los opioides, puede producirse **tolerancia cruzada**. De manera sencilla se puede decir, que la tolerancia cruzada se produce cuando la administración de un primer fármaco condiciona el desarrollo de tolerancia al segundo fármaco. Si se produce en un corto plazo de tiempo como, por ejemplo, durante el postoperatorio, se observa un incremento en las necesidades del segundo analgésico empleado (Guignard et al. 2010). Un mayor consumo de analgésicos en el postoperatorio, ha hecho que hiperalgesia y tolerancia puedan confundirse (Kim et al. 2014). En el caso de estrategias de rotación de analgésicos como los opioides en pacientes con cáncer puede producirse tolerancia cruzada a largo plazo (Inturrisi 2002; Mercadante et al. 2009).

Desde el punto de vista farmacológico, en función de cómo se produce, se diferencia entre tolerancia farmacocinética y farmacodinámica. La **tolerancia farmacocinética** implica menores concentraciones del fármaco en plasma o a disposición del receptor, en la mayoría de los casos por un incremento del metabolismo como ocurre con opioides (Chang et al. 2007). La **tolerancia farmacodinámica** se produce cuando disminuye la densidad de receptores o se producen cambios conformacionales en los mismos que provocan su desensibilización, este mecanismo se relaciona con los receptores N-metil-D-aspartato (NMDA) y también se relaciona con opioides y sus receptores (Chang et al. 2007).

Tolerancia e hiperalgesia se han usado indistintamente (Richebe et al. 2012). Sin embargo, la tolerancia implica una mayor necesidad de un fármaco para producir la misma analgesia, ante un mismo estímulo doloroso. Mientras que, la hiperalgesia se trata de una mayor respuesta nociceptiva ante un mismo estímulo doloroso (Bekhit 2010; Roeckel et al. 2016).

2. Nocicepción

La nocicepción comienza cuando un estímulo térmico, mecánico o químico (estímulo nociceptivo) activa la neurona aferente primaria en la periferia que lo transduce a señales eléctricas que se transmiten al sistema nervioso central, asta dorsal de la médula espinal (ADME) o al ganglio trigémino, si la información proviene de la cara. Es en este nivel donde se produce la integración de la información nociceptiva a nivel central y donde se modula para proyectarse a través de la neurona de segundo orden a la médula ventrolateral caudal, médula ventromedial craneal, área parabraquial, sustancia gris periacueductal, núcleo del tracto solitario y tálamo. Desde aquí, la neurona de tercer orden transmite la información hacia la corteza cerebral para su procesamiento final que conlleva la percepción, momento en el cual el individuo se hace consciente (Bell 2018). El dolor es una de las posibles manifestaciones de la nocicepción, no siempre reconocible, especialmente en aquellas circunstancias en las que el animal se encuentra bajo sedación o anestesia general.

De las distintas fases de la nocicepción, en este capítulo profundizaremos en la transducción, transmisión y modulación nociceptiva.

2.1. Transducción y transmisión nociceptiva

Los nociceptores son las terminaciones libres de las fibras aferentes primarias que pueden ser fibras Aδ (mielinizadas y de conducción rápida, 5-40 m s^{-1}) que transmiten información mecánica y térmica; o fibras C (desmielinizadas y de conducción lenta, 0,5- 2 m s^{-1}) que transmiten información mecánica, térmica y química (Sneddon 2018). Los cuerpos celulares de estas fibras se encuentran en el ADME. Según los canales que se expresen se produce sensibilidad al calor (receptor de potencial transitorio V1 o TRPV1), al frío (receptor de potencial transitorio M8 o TRPM8), al medio ácido (canales iónicos sensibles a la acidez o ASIC) o a irritantes químicos (receptor de potencial transitorio A o TRPA). Neuronas sensitivas que no transmiten los estímulos nociceptivos, sino el tacto, emplean las fibras Aβ (mielinizadas y de conducción rápida, 35-90 m s^{-1}). Por último, las fibras Aα (mielinizadas y de conducción

rápida, 80-120 m s^{-1}) transmiten la propiocepción, por ejemplo, información referente al movimiento de músculos y cápsulas articulares (Sneddon 2018).

El potencial de acción de la fibra aferente primaria transduce la señal nociceptiva propagando el potencial de acción generado a través de canales iónicos activados de sodio, potasio y calcio hasta hacer sinapsis con las neuronas de segundo grado en las láminas del ADME (Bell 2018). Las fibras Aδ llegan a las láminas I y V, mientras que las fibras C llegan a las láminas más superficiales, I y II, y las fibras Aβ llegan a las láminas II, IV y V (Basbaum et al. 2009).

Los potenciales postsinápticos generados son de corta duración y se encuentran mediados por transmisores excitatorios como el glutamato (implicado en el desarrollo y mantenimiento de la tolerancia e hiperalgesia a opioides), que actúa sobre receptores N-metil-D-aspartato (NMDA), α-amino-3-hidroxi-5-metilo-4-isoxazolpropiónico (AMPA) y kainato entre otros, y por péptidos como la sustancia P, que actúa sobre los receptores de neuroquinina 1 (NK-1) entre otros. Es aquí donde las interneuronas localizadas en el ADME realizan la mayor parte de los procesos de modulación de la información nociceptiva.

2.2. Modulación nociceptiva

Durante los eventos deportivos o en el campo de batalla, se produce una disociación en la que el individuo no muestra signos de dolor. Esta disociación hizo pensar que existía algún tipo de modulación nociceptiva y que puede conferir una ventaja para la supervivencia (Patel 2010). La modulación nociceptiva puede suprimir o exacerbar la respuesta dolorosa. Se realiza por tres mecanismos: inhibición segmental, sistema opioide endógeno y sistema inhibitorio descendente.

Inhibición segmental: las interneuronas localizadas en el ADME, modulan la transmisión de la información nociceptiva y tactil. Dependiendo del neurotransmisor implicado pueden ser excitatorias, mediadas por glutamato, o inhibitorias, medias por ácido γ-aminobutírico (GABA) y glicina (Zeilhofer et al.

2012). Las fibras descendentes supraespinales (excitatorias e inhibitorias) actúan también sobre las interneuronas localizadas en el ADME.

La teoría del control de puerta, propuesta por Melzack y Wall en 1965, modificada pero aún válida, sostiene que las interneuronas inhibitorias disminuyen o bloquean la transmisión de la información entre las fibras nerviosas aferentes y las células del ADME (Moayedi & Davis 2013). Las fibras Aδ y C, que transmiten la información nociceptiva, realizan sinapsis con estas interneuronas inhibitorias, de manera que cuando un estímulo nociceptivo llega, inhibe la acción de las interneuronas inhibitorias y la transmisión nociceptiva continua su camino hacia centros superiores. Mientras que las fibras Aβ activan las neuronas inhibitorias y, por tanto, inhiben la transmisión sináptica, es decir, se interrumpe el ascenso hacia centros superiores donde se produce la percepción de la información nociceptiva (Zeilhofer et al. 2012).

Sistema nervioso inhibitorio descendente: desde la sustancia gris periacueductal y la médula rostral ventromedial parten las fibras nerviosas descendentes hacia la médula espinal. El estrés activa el sistema nervioso inhibitorio descendente, que modula la actividad neuronal y controla el ascenso de información nociceptiva a áreas superiores. Los neurotransmisores implicados son la serotonina y la noradrenalina principalmente, los cuales pueden ser modificados farmacológicamente (Patel 2010). Se ha demostrado en ratas que el ondasentron facilita la acción de este sistema nervioso inhibitorio descendente en animales sanos (Bannister et al. 2015). El neurotransmisor GABA es inhibido por la activación de estas vías, lo que paradójicamente puede traducirse en una desensibilización local y aumento de la percepción del dolor.

Sistema opioide endógeno: las encefalinas, dinorfinas y endorfinas son neurotransmisores opioides producidos en el SNC. Estos neurotransmisores se unen a los receptores opioides (Patel 2010) que se encuentran en la periferia, la médula espinal y el cerebro actuando sobre las distintas fases de la nocicepción y, por lo tanto, modulándola.

Capítulo II
MONITORIZACIÓN NOCICEPTIVA INTRAOPERATORIA

1. Introducción

Intraoperative nociception-antinociception monitors: a review from the veterinary perspective

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Perspective

Intraoperative nociception-antinociception monitors: a review from the veterinary perspective

Patricia Ruíz-López, Juan Manuel Domínguez, María del Mar Granados

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Abstract

Intraoperative nociception-antinociception monitors: a review from the veterinary perspective

Objective To review monitors currently available for the assessment of nociception-antinociception in veterinary medicine.

Databases used PubMed, Web of Science and Google Scholar. The results were initially filtered manually based on the title and the abstract.

Conclusions The provision of adequate antinociception is difficult to achieve in veterinary anaesthesia. Currently, heart rate and arterial blood pressure are used to monitor the response to a noxious stimulus during anaesthesia, with minimum alveolar concentration sparing effect and stress-related hormones used for this purpose in research methods. However, since none of these variables truly assess intraoperative nociception, several alternative monitoring devices have been developed for use in humans. These nociceptive-antinociceptive monitoring systems derive information from variables, such as electroencephalography (EEG), parasympathetic nervous system (PNS) response, sympathetic nervous system (SNS) response and electromyography (EMG). Several of these monitoring systems have been investigated in veterinary medicine, although few have been used to assess intraoperative nociception in animals. There is controversy regarding their effectiveness and clinical use in animals. A nociceptive-antinociceptive monitoring system based on the PNS response has been developed for use in cats, dogs and horses. It uses the parasympathetic tone activity (PTA) index which claims detect inadequate intraoperative nociception-antinociception balance in veterinary anaesthesia. Nonetheless, to date there are limited published studies, and cardiovascular variables remain the gold standard. Consequently, further studies in this area are warranted.

Keywords analgesia, antinociception, intraoperative, monitor, nociception, veterinary

Intraoperative nociception-antinociception monitors: a review from the veterinary perspective

Background

The Triad of Anaesthesia includes unconsciousness, antinociception and immobility (Gruenewald & Ilies 2013). The assessment of nociception is one of the most challenging components of anaesthesia, especially during the intraoperative period (Gruenewald & Ilies 2013). While antinociceptive drugs are used to alleviate pain, their overuse may prolong postanaesthetic recovery, and induce hyperalgesia, tolerance or immunodepression (Gruenewald & Ilies 2013).

The responses to nociceptive stimuli described 50 years ago, as an increase in heart rate (HR) or arterial blood pressure, are still used to assess intraoperative nociception (Breazile & Kitchell 1969). Although, intraoperative cardiovascular variables can be altered by drugs such as β-blockers, vasoactive medications, lack of unconsciousness or concomitant illness.

Stress-related hormones, such as catecholamines (epinephrine, norepinephrine) and cortisol are utilised in research studies as markers of nociception. Samples collection can itself induce more stress (Ishibashi et al. 2013). Extraction and evaluation of the samples are also time consuming, thus they are of little clinical utility.

Minimum alveolar concentration (MAC) sparing effect has been used to evaluate the antinociceptive effects of drugs, such as opioids (Monteiro et al. 2010), lidocaine (Rezende et al. 2011) or ketamine (Wilson et al. 2008), mainly for research purposes. Likewise, the MAC to block autonomic reflexes to nociceptive stimuli (MAC BAR) has been used (Roizen et al. 1981), but physiological and pathological factors may influence the MAC value (Aranake et al. 2013).

The investigation of accurate and non-invasive methods with which to assess nociception-antinociception is ongoing. In human medicine, electroencephalography (EEG) (March & Muir 2005; Morgaz et al. 2011; Jensen et al. 2014), parasympathetic nervous system (PNS) response (Jeanne et al. 2009; Mansour et al. 2017), sympathetic nervous system (SNS)

response (Gruenewald et al. 2009; Funcke et al. 2017) and electromyography (EMG) (Bergadano et al. 2006; Spadavecchia et al. 2016) have all been studied.

The objective of this manuscript is to review the monitors, which are currently available for the assessment of nociception-antinociception in human and veterinary medicine.

Methods

A literature search was performed in PubMed, Web of Science and Google Scholar using the following keywords: intraoperative monitor, bispectral index, spectral entropy, qNOX index, qCON index, nociceptive withdrawal reflex, RIII-reflex, nociceptive flexion reflex, pupillometry, reflex pupillary dilatation, surgical plethysmographic index, surgical stress index, cardiovascular depth of analgesia (CARDEAN) index, skin conductance, analgesia nociception index or parasympathetic tone activity combined with one each of the following words: veterinary, animal, dog, cat, horse, equine, canine, feline, rat, mice, rabbit, ruminant, sheep, cattle, goat and pig. All types of articles were included. The exclusion criteria were: articles for which the full text was unavailable, articles not in English or articles not focused on nociception-antinociception assessment. The results were filtered manually, initially based on the title and the abstract. All monitors associated with nociception-antinociception assessment were included.

Nociception-antinociception monitors

Monitoring systems based on electroencephalography

These systems were developed mainly to enable the anaesthetist to assess hypnosis and avoid human patient awareness, but they have also been used to assess nociception.

- *Bispectral index*

The bispectral index (BIS; Covidien, CO, USA) based on EEG was the first monitor approved by the American Food and Drug Administration (FDA) to measure the hypnotic effect of anaesthesia in humans. The BIS value is a dimensionless number between 0 and 100, with 0 meaning total absence of encephalogram activity and 100 representing complete awareness (March & Muir 2005). However, BIS is not correlated with the application of nociceptive stimuli nor with the effects of analgesics, therefore it is a poor marker of antinociception in humans (Funcke et al. 2017). Furthermore, numerous clinical conditions, such as hypoglycaemia, cerebral ischaemia, neurological disorders, or the administration of inhalant anaesthetics such as halothane, sevoflurane or isoflurane, injectable anaesthetics for example ketamine or muscle relaxants, might influence BIS values because of their effects on the EEG (Dahaba 2005).

The BIS monitor, which was originally developed for humans has been employed during anaesthesia of pigs (Haga et al. 1999; Haga et al. 2001), horses (Haga & Dolvik 2002; Williams et al. 2016), dogs (Morgaz et al. 2009), sheep (Verhoeven et al. 2015), goats (Antognini et al. 2000), cats (March & Muir 2003a; March & Muir 2003b) and calves (Deschk et al. 2016). However, adequate nociception-antinociception assessment has not been proved.

- *Spectral entropy*

Spectral entropy (GE Healthcare, Finland) is also based on the EEG and EMG signals measured on the forehead and its algorithm was published more than a decade ago (Viertiö-Oja et al. 2004). Spectral entropy includes two values, namely, state entropy (SE) and response entropy (RE), that reflect the hypnosis and nociceptive levels, respectively, during general anaesthesia. The SE reflects the animal's cortical activity and values range from 0 (deep hypnosis) to 99 (awake), whereas the RE measures the EEG and the frontal EMG, with values ranging from 0 to 100. The difference between RE and SE indicates EMG activation, which has been related to the level of nociception. However, an increase in the RE and SE difference may represent the motor response to a noxious stimulus, so it is not a good indicator of nociception (Takamatsu et al. 2006). In dogs, RE and SE did not detect deep planes of anaesthesia, furthermore, RE and RE-SE differences were not found to be

adequate nociceptive indicators (Morgaz et al. 2011). In contrast, a recent publication demonstrated a change in RE and SE in response to tail clamping during sevoflurane MAC determination in dogs (Mahidol & Thengchaisri 2015). In view of these results, spectral entropy might have some utility as nociception-antinociception monitor.

- *qNOX index*

The qNOX (registered name) index is obtained using four EEG spectral ratios, while the qCON index (qCON 2000, Quantium Medical, Spain) also adds the burst suppression ratio. Both indexes are fed into an Adaptive Neuro Fuzzy Inference System (ANFIS), a mathematical model resulting from an artificial neural network and a fuzzy logic system (Jensen et al. 2014). The qNOX index is believed to indicate nociception, while the qCON index represents hypnosis. A 0–99 scale is provided, with the highest values of qNOX indicating high levels of nociception, and values nearer to 0 indicating low nociception.

In humans, the qNOX index predicts patient movement as a response to noxious stimulation, even when the measured end-tidal anaesthetic concentrations were similar (Jensen et al. 2014). Furthermore, qNOX values increased rapidly after anaesthetic administration was stopped, which suggests a higher probability of response to noxious stimulation before the complete recovery of consciousness (Melia et al. 2017). This indicates that the qNOX can rapidly detect changes between unresponsive and responsive states. No published studies have been found concerning the use of the qNOX index in veterinary medicine, so this index is based on human data.

Monitoring systems based on electromyography

- *Nociceptive withdrawal reflex*

The nociceptive withdrawal reflex, nociceptive flexion reflex or normalised RIII-reflex threshold, as it is known in human medicine, is a polysynaptic withdrawal response to nociceptive stimuli. It is based on the measurement of spinal reflexes because they are relatively stable, stimulus-induced and physiological responses (Skljarevski & Ramadan 2002). The

electrical stimulation is performed randomly every 5–15 seconds to avoid stimulus habituation, delivering trains of 5–10 rectangular-wave pulses of 1.0 ms duration at a frequency of 200–300 Hz. Stimulus habituation has been proven and is dependent on the stimulus intensity (von Dincklage et al. 2013). In humans, the RIII-reflex has shown a similar prediction probability for reactions to noxious stimuli under inhalant (von Dincklage et al. 2010b) and intravenous anaesthetics (von Dincklage et al. 2010a). The RIII-reflex threshold is modified by various factors, such as sex, diurnal rhythms, activity of baroreceptors, stimulation site selection, and drugs, which have been detailed previously (Skljarevski & Ramadan 2002) and may be a limitation in veterinary medicine.

The nociceptive withdrawal reflex has been demonstrated experimentally in cats (Schomburg et al. 2000) and rats, where it was employed as a tool to detect antinociception properties of ketorolac tromethamine (Bustamante & Paeile 1993).

It has been used in conscious animals as a non-invasive model of nociception threshold monitoring in several studies. Bergadano et al. (2006) described the use of this method in conscious dogs, where electrodes were placed on the forelimbs, *musculus deltoideus* and *musculus cleidobrachialis,* and over the hind limbs, *musculus biceps femoris* and *musculus tibialis anterior*. It was found that the nociceptive withdrawal reflex correlated with behavioural reactions, enabling quantification of nociceptive system excitability in individual dogs (Bergadano et al. 2006). Moreover, the nociceptive withdrawal reflex has been used experimentally in awake horses to assess the antinociception provided by different drugs, by placing the stimulating electrodes over the skin of the plantar and palmar nerves (Spadavecchia et al. 2016). Therefore, the nociceptive withdrawal reflex is used to measure the nociceptive threshold, but it has not been used to assess intraoperative nociception.

Monitoring systems based on sympathetic nervous system

- *Pupillometry*

Pupillometry is based on the reflex pupillary dilatation (RPD) pathway and it is used in humans as a measurement of nociception, since a nociceptive stimulus increases sympathetic tone, thus leading to an increase in pupil diameter.

The pupillometer is positioned in front of the pupils, while the eyes are held open with goggles (Grozdanic et al. 2007) or an eyelid speculum (Whiting et al. 2013). Time and light stimuli are used to record the changes in the pupillary diameter (mm). Several tools are used to monitor RPD in humans, such as the NeurOptics PLR-100 (NeurOptics Inc., CA, USA), VideoalgesiGraph (Synapsis, France) and Algiscan (IDMed, France).

RPD may be useful in evaluating nociception during opioid and inhalation anaesthesia in humans (Larson et al. 1997). It has been described as a more sensitive method of measuring the nociceptive stimuli response than haemodynamic variables or BIS in children anaesthetized with sevoflurane (Constant et al. 2006). The superiority of RPD over clinical signs (HR and mean arterial pressure) to detect standardised noxious stimuli response under sedation with propofol has also been demonstrated (Funcke et al. 2017). Moreover, a good correlation between RPD and the dose of infused remifentanil has been shown (Funcke et al. 2017). Nonetheless, RPD has some limitations related to its methodology, mainly eye bulb movement during light anaesthesia or sedation, as well as interference from ambient light or ophthalmological abnormalities, which might complicate the measurements. Moreover, sympathomimetic and parasympathomimetic drugs or antagonists of the dopaminergic receptors might delay the RPD (Gruenewald & Ilies 2013).

No nociception-antinociception studies have been performed in veterinary species. In dogs, pupillometry has been used to quantify the canine pupillary light reflex under different chemical restraint protocols and between healthy and diseased canine eyes using a custom made apparatus (Whiting et al. 2013). The pupillary light reflex has also been recently quantified in

awake dogs and dogs under anaesthesia using a handheld pupillometer (NPI-100; NeurOptics, Inc., CA, USA) (Kim et al. 2015). However, differing pupillary responses between animal species to drugs such as opioids would render pupillometry useless to assess intraoperative nociception in veterinary medicine.

- *Surgical plethysmographic index*

The surgical plethysmographic index (SPI; GE Healthcare, Finland) was first introduced as a surgical stress index. The SPI is derived from the pulse oximeter using an optional software licence (GE Ohmeda SpO_2 technology; GE Healthcare, Finland) and compatible sensors are required. The SPI is obtained by applying a published algorithm (Huiku et al. 2007) to the photoplethysmographic amplitude and pulse interval. The SPI is a dimensionless number between 0 and 100, reflecting low and high sympathetic activity, respectively, with a SPI range between 20 and 50 considered to reflect adequate antinociception during general anaesthesia in adult human. Since the SPI reflects sympathetic activity, it was suggested that the SPI value might be used to monitor the response to nociceptive stimuli and antinociceptive medications during general anaesthesia. SPI is reduced by remifentanil administration (Gruenewald et al. 2009), and it had been used to guide opioid administration in humans, thereby reducing opioid use and accelerating return to consciousness extubation of patients (Bergmann et al. 2013). However, in a more recent study, no clinical advantages were found using SPI as an opioid administration guide compared with clinical signs (HR and non-invasive blood pressure) (Colombo et al. 2015). It has been suggested that SPI values greater than 30 obtained at the end of surgery might predict postoperative pain (Ledowski et al. 2016).

It is important to note that the SPI is influenced not just by nociceptive stimuli, but also by factors stimulate the sympathetic component of the autonomic nervous system; such as hypothermia, hypovolaemia, mental stress (Ilies et al. 2010). To the best of the authors' knowledge, no studies have been published describing the use of the SPI in veterinary medicine.

- *Suppression of the cardiac baroreflex: CARDEAN index*

The cardiovascular depth of analgesia (CARDEAN) index (Alpha-2 Ltd., France) is based on the suppression of the cardiac baroreceptor reflex. The physiological basis of this reflex consists of a decrease in HR owing to an increase in systemic arterial blood pressure. Nevertheless, during nociceptive stimulation, autonomic responses cause a minor increase in blood pressure with a short duration (10–20 second) increase in HR. These changes are too subtle for the anaesthetist to perceive, thus making the CARDEAN index a useful device to monitor these haemodynamic changes. Blood pressure is measured using the oscillometric method every 5 minutes and HR is obtained from the ECG; all data are stored on a computer for analysis. For a further description of the index performance, see Rossi et al. (2012). The index range is between 0 and 100, and values over 60 indicate tachycardia as well as hypertension. Studies performed in humans using the CARDEAN index showing that the index is more sensitive to trends rather than to isolated nociceptive events (Rossi et al. 2012). When the CARDEAN index was used for opioid guidance administration, the incidence of unpredictable movements during anaesthesia was reduced by 51% in patients administered a similar alfentanil dose (Martinez et al. 2010). Recently, the combination of the CARDEAN index and the analgesia nociception index (ANI) was found to allow clinicians to better assess pain in conscious burns patients (Papaioannou et al. 2016). No published data have been found regarding the use of the CARDEAN index in veterinary medicine.

- *Skin conductance*

Skin conductance has been used as a stress indicator in humans. The Med-Storm Stress Detector (Med-Storm Innovation AS, Norway) is based on the electrodermal activity principle. The SNS controls the activity of the sweat glands, when stress increases, SNS stimulation activates the sweat glands, therefore the electrical impedance decreases in the sweat glands, thus leading to an increase in skin conductance (Storm et al. 2002). The Med-Storm Stress Detector consists of three electrodes with a measuring electrode, a countercurrent electrode and a reference voltage electrode, which applies a constant voltage. Electrodes are applied to the palmar areas in adults and children older than 1 year, or on the soles of children younger than

1 year. The skin conductance provides calculated peaks, peaks per second, and the area under the curve for 15 seconds prior to measurement. There are different algorithms for adults, children, postoperative pain, intensive care units, general anaesthesia and research purposes. The intensity of the nociception is given as a colour on a five-colour-scale (white, light yellow, yellow, orange and red), which ranges from 'no nociception' to 'severe nociception'. Although changes in skin conductance have been shown after a fentanyl bolus with respect to baseline, it has a poor predictive ability (Ledowski et al. 2010). Moreover, it has also been found that skin conductance is insensitive in patients administered neuromuscular blockers (Solana et al. 2015). Furthermore, skin conductance did not prove to be a more sensitive or faster indicator of nociception than clinical scales, granting no advantages over cardiovascular variables, clinical scales or BIS in clinical practice (Solana et al. 2015).

In dogs, skin conductance (Echorode III ECG electrode; Fukuda Denshi Co, Ltd, Japan) has been compared to cortisol, adrenaline and noradrenaline plasma concentrations after the administration of medetomidine, acepromazine or fentanyl. The investigators used electrodes attached to the metacarpal pads and found skin conductance reflects different degrees of stress, similar to the changes in stress-related hormones in dogs (Ishibashi et al. 2013). In another study, the skin conductance failed detecting pain in dogs with chronic cranial cruciate ligament disease (Giordano et al. 2018). A limitation could be the application of the electrodes in veterinary medicine.

Monitoring systems based on the parasympathetic nervous system

- *Analgesia nociception index - parasympathetic tone activity monitor*

The analgesia nociception index (ANI) (PhysioDolorys; MetroDolorys, France) is a non-invasive device, which uses heart rate variability (HRV) derived from a standard ECG signal. The monitor measures the amplitude between the R waves of the ECG; the amplitude between R waves is modified by the HR frequency and depends on the activity of the autonomic nervous system. High frequency changes in HRV (0.15–0.5 Hz) are controlled by

parasympathetic activity, while low frequency changes are mediated by sympathetic and parasympathetic activity. The index is obtained by application of a Fast Wavelet Transformation, a quantitative analysis of the HRV that allows analysis of non-stationary signals (Pichot et al. 1999; Jeanne et al. 2009).

During 'adequate' nociception-antinociception balance, only small variations in HRV occur, mainly because of the respiratory sinus arrhythmia. Due to the influence of the autonomous nervous system on the respiratory rate (f_R), if parasympathetic tone decreases, HR increases, and the effect of respiration on the RR period interval can be used to assess sympathetic tone, therefore the nociception-antinociception balance (Jeanne et al. 2009). Thus, a stable respiratory pattern is necessary to ensure the correct performance of the ANI, since apnoea does not allow ANI assessment.

The index is shown as a numerical scale, where 0 represents a very low parasympathetic activity or a high sympathetic activity (high stress level) and 100 represents a high parasympathetic activity (low stress level). The monitor describes two ANI values updated every second, the instantaneous ANI (ANIi) and the average ANI (ANIm). The ANIm is the variable used to correlate with haemodynamic events, so it was utilised in studies conducted to assess the nociceptive-antinociceptive state. The ANI seems to reflect different levels of stimulation during isoflurane general anaesthesia (Ledowski et al. 2014), although it appears to perform better during propofol anaesthesia (Boselli et al. 2013; Boselli & Jeanne 2014). The behaviour of the ANI is similar to cardiovascular changes during nociceptive stimuli (Kommula et al. 2017), while the ANI value in the immediate postoperative period is also significantly correlated with postoperative pain intensity (Boselli et al. 2013). Nevertheless, the use of ANI in awake patients is limited, not only because of movement, but also because of the influence of stress and the patient's emotional state (Jess et al. 2016). Other authors found that ANI was unable to predict changes in the autonomous nervous system as consequence of nociceptive stimuli (Gruenewald et al. 2013) and had little predictive value for cardiovascular changes (Ledowski et al. 2014). The effects of β-blockers, adrenergic agonists and adrenergic antagonists drugs on the functionality and

efficacy of the ANI are still unknown and could modify the understanding of the ANI (Kommula et al. 2017).

The parasympathetic tone activity (PTA) monitor (MDoloris Medical Systems, France) is the veterinary medicine equivalent to the ANI. The PTA contains software from three different species: dogs, cats and horses. The PTA monitor records a surface ECG (lead II) using a three-lead system with flattened crocodile clips attached to the skin. The monitor provides the same numerical scale (0: high stress – 100: low stress) and displays two PTA values updated every second, the instantaneous PTA (PTAi) calculated on the 54 previous PTA values and the average PTA (PTAm) calculated on the 176 previous values. The limitations of the PTA monitor are the same as those of the ANI in human medicine.

The evidence demonstrating the ability of the PTA monitor to assess nociception is still limited, since just three papers have been published. A correct assessment of the nociceptive stimuli and prediction of the haemodynamic response have been shown in dogs with a sensitivity of 77% and a specificity of 72% (Mansour et al. 2017). In a separate study, PTA was found to differentiate between antinociceptive treatments in pigs (Leitão et al. 2018). In a recent study in dogs, PTA detected low intensity stimuli better than HR or blood pressure, but haemodynamic response was quicker than PTA changes to nociceptive stimuli of higher intensities (Aguado et al. 2019). Nonetheless, further studies are necessary to determine the usefulness of the PTA index to assess intraoperative nociception/antinociception balance in veterinary medicine.

Combination index

- *The nociception level index*

Due to limitations in the reliability of individual monitors, it is suggested that a combination of them may provide more consistent information. The nociception level (NoL) index is based on a non-linear combination of heart rate, HRV, photoplethysmograph wave amplitude, skin conductance fluctuations and their time derivatives. This index is able to discriminate

between noxious and non-noxious stimuli with high sensitivity and specificity, showing better results than its individual components (Edry et al. 2016). To date, it has been implemented only in human medicine.

Limitations of the monitors

No single monitor is currently available in human or veterinary medicine for the detection of adequate intraoperative nociception/antinociception; when a wide range of surgical procedures and anaesthesia techniques are used. Some of the monitors described above are specifically designed to assess nociception; however, the stress response may also influence the variables measured by monitors based on sympathetic and parasympathetic nervous system. The stress response related to emotional perception due to manipulation and hospitalisation has been described in humans, even when a non-painful situation exists (Mirilas et al. 2010). After evaluation of the effects of surgery on endocrine and metabolic response during anaesthesia, it was found that surgery had little additional effect on the stress response to halothane anaesthesia in Equidae (Taylor 1998). Therefore, the influence of the stress response due to manipulation and the anaesthesia itself should not be dismissed. In order to use these monitors to assess the administration of antinociceptive drugs, it should be remembered that nociception alleviation itself may not necessarily lead to an important modification of the stress response (Kehlet 1988). For example, adequate postoperative pain relief failed to reduce the surgical stress response in conscious human patients (Moller et al. 1988).

Furthermore, many monitors developed for use in humans cannot be applied directly to veterinary species like it happens in children owing to differences in resting HRs and variability in blood vessel distensibility (Park et al. 2015).

In addition, monitors such as the CARDEAN index or the SPI, where nociception indexes are based on the sympathetic nervous system activity, a decrease in intravascular volume reduces the cardiovascular response to

nociceptive stimuli (Rossi et al. 2012). Dehydration or administration of a fluid bolus that may also interfere with the accuracy of these monitors. Drugs used during anaesthesia may affect the interpretation of data recorded for example, opioids cause pupillary constriction in humans and pupillary dilatation in cats, which is unrelated to nociception.

In veterinary medicine, only the PTA monitor has been developed for animals with limited supported publications. Regarding the monitors designed for humans, only the Bispectral and Spectral Entropy have been used to assess intraoperative nociception with limited results. Pupillometry and skin conductance have been used in dogs, but nociception was not assessed. There are no studies describing the use of SPI, CARDEAN index and qNOX in veterinary medicine to date. The RIII-reflex determines nociceptive thresholds rather than intraoperative nociception assessment.

Currently, there is no consensus in human medicine for the most suitable nociception-antinociception monitor. Furthermore, no single monitor has been proven to be superior to the use of cardiovascular variables (HR and arterial blood pressure) for the assessment of intraoperative nociception despite the numerous studies performed. Therefore, cardiovascular variables are still the gold standard method to assess the nociception-antinociception balance.

While there is much literature regarding the use of monitors assessing nociception-antinociception balance in humans, there is a lack of studies in veterinary medicine. Nonetheless, it is impossible to include all the papers investigating intraoperative nociception-antinociception balance in humans, thus, this perspective review might present some unconscious bias.

Conclusion

It is the responsibility of the veterinary anaesthetist to avoid perioperative nociception in animals. Nonetheless, despite current advances in human medicine, the search for the perfect method to assess nociception-antinociception in veterinary is ongoing. From the veterinary perspective, the options are limited, with only one available for veterinary species, namely, the

PTA monitor with limited supporting studies. Currently, cardiovascular variables remain the gold standard to assess the intraoperative nociception-antinociception balance. Consequently, there is a need for further studies in veterinary medicine, where veterinarians work with a limited variety of tools in a more exigent anaesthesia field.

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2. Hipótesis y objetivos

Monitoring intraoperative nociception is a very complex task. Recently, a new monitor for veterinary purpose, the PTA monitor has been developed in order to measure changes on the ANS as consequence of nociceptive stimuli or drug administered. It was hypothesised that changes on the PTA index or its dynamic variation will precede or coincide with changes on heart rate and blood pressure after a nociceptive stimulus in dogs or after a nociceptive stimulus or drug administration in horses.

The next objectives were defined and developed in two studies:

1. To determine the performance of the PTA index in relation to the haemodynamic variables, HR and direct MAP to assess its utility to monitor intraoperative nociception in dogs undergoing laparoscopic ovariectomy.
2. To determine if the PTA index or dynamic variation of PTA (ΔPTA) predict the intraoperative haemodynamic response during nociceptive stimuli.
3. To determine in horses if ANS activity changes in response to a surgical nociceptive stimulus or during the administration of morphine, ketamine and dobutamine in horses under general anaesthesia.
4. To assess in horses how changes in ANS activity might interfere with the PTA index to monitor intraoperative nociception.

3. Estudio I

Evaluation of the parasympathetic tone activity (PTA) index and its dynamic variation (ΔPTA) in dogs undergoing laparoscopic ovariectomy

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Abstract

Evaluation of the parasympathetic tone activity (PTA) index and its dynamic variation (ΔPTA) in dogs undergoing laparoscopic ovariectomy

Objective. To determine if parasympathetic tone activity (PTA) index or its dynamic variation (ΔPTA) could predict intraoperative haemodynamic responses during surgical nociceptive stimuli.

Study design Prospective, observational, clinical study.

Animals A total of 32 client-owned, adult, bitches undergoing laparoscopic ovariectomy.

Methods PTA and bispectral (BIS) indexes, HR and direct MAP were assessed before (baseline), 1 (1 min) and 2 minutes (2 min) after four surgical time points: insufflation, introduction of trocars and removal of the left and right ovary. A generalised linear mixed model was performed for PTA, HR, BIS and MAP. A PTA event was considered if the PTA index decreased more than 20% and a haemodynamic response was considered if HR and/or MAP increased more than 20% regarding baseline values. The performance of PTAbaseline, PTA1min and ΔPTA to predict hemodynamic response to the surgical time points were assessed by calculation of the area under the curve of a receiver operating characteristic (ROC) curve. Significant differences were considered if $p < 0.05$.

Results The PTA index decreased significantly ($p = 0.007$) 1 and 2 min after insufflation. During PTA events, the MAP increased significantly 1 min ($p = 0.001$) and 2 min ($p = 0.001$) later but HR ($p = 0.192$) and BIS ($p = 0.245$) did not change. The ΔPTA was significantly different between dogs that presented a haemodynamic response and dogs that did not present it at pneumoperitoneum insufflation ($p = 0.005$). ROC curves showed a threshold value of PTAbaseline ≤51 to detect a haemodynamic response (sensitivity 69%, specificity 52%).

Conclusion and clinical relevance In this study, the PTA index and ΔPTA did not effectively assess intraoperative nociception and predict a haemodynamic response in anaesthetised dogs. Further research should be performed to assess the usefulness of PTA monitoring in dogs of different breeds using different anaesthesia protocols.

Keywords Analgesia, nociception, parasympathetic tone activity, PTA, dog

Introduction

Anaesthesia is the result of a combination of different hypnotic, antinociceptive and myorelaxing drugs to avoid consciousness, nociception and movement (Ledowski 2017). Nociception assessment is one of the most challenging aspects of anaesthesia and its intraoperative monitoring is commonly based on changes in heart rate (HR), blood pressure (BP) and/or respiratory rate (RR) (Jeanne et al. 2012; Gruenewald & Ilies 2013). Cardiorespiratory variables can be modified by diverse circumstances such as cardiovascular or central nervous system illness, drugs administered or surgical procedure (Mansour et al. 2017). Thus, nociception assessment based on clinical variables could be inaccurate in human (Gruenewald et al. 2013) and in veterinary (Mansour et al. 2017) medicine. Furthermore, the nociceptive stimulus can abolish the cardiac baroreceptor reflex (Gruenewald & Ilies 2013), thus the HR and the BP increase simultaneously instead of compensating for each other (Rossi et al. 2012). This reflex suppression might cause unappreciated changes which might escape the anaesthetist's attention, justifying the need for an accurate method to assess the analgesia-nociception balance (Gruenewald & Dempfle 2017).

Advanced nociception monitoring has been developed for human medicine based on electroencephalography, electromyography and autonomous nervous system (ANS) activity (Gruenewald et al. 2013; De Jonckheere et al. 2015). The analgesia nociception index (ANI) evaluates the ANS activity based on the registration of the human heart rate variability (HRV), which has shown promising results improving nociceptive assessment compared to HR or BP, allowing for more accurate antinociceptive drug administration (Jeanne et al. 2009; Boselli et al. 2015). The dynamic variation of ANI (ΔANI) was defined by (Boselli et al. 2016), showing that ΔANI was better than ANI to predict haemodynamic changes.

The parasympathetic tone activity (PTA) (PhysioDoloris; Mdoloris Medical Systems, France) index is homologous to ANI in veterinary medicine and has been developed based on the HRV of dogs, cats and horses. However, literature about the use of PTA index in veterinary species is limited and to the authors' knowledge, just two papers regarding the nociception-antinociception balance

have been published to date. The PTA index and its dynamic variation (ΔPTA) predicted haemodynamic reactivity in anaesthetised dogs undergoing different surgeries (Mansour et al. 2017). It was also found that PTA is useful to optimise antinociceptive drugs delivery, since it was able to recognise antinociception levels between treatments in swine (Leitão et al. 2018).

In view of the importance of assessing intraoperative nociception and the limited studies published in dogs using this monitor, the primary aim of this study was to determine the performance of the PTA index in relation to the haemodynamic variables, HR and direct mean arterial pressure (MAP), to assess its utility to monitor intraoperative nociception in dogs undergoing laparoscopic ovariectomy. The secondary aim was to determine if the PTA index or ΔPTA could predict the intraoperative haemodynamic response during nociceptive stimuli. It was hypothesised that changes in the PTA index or ΔPTA would coincide with or precede the haemodynamic response.

Materials and Methods

This observational, prospective, clinical study was performed at the Veterinary Teaching Hospital, University of Cordoba and was approved by the ethical committee for animal welfare of the Teaching Hospital of the University of Cordoba (CEBAHCV32/2016). All procedures were conducted in compliance with the ethical principles of good practice in animal experimentation and with previous informed consent of the owners.

Animals

Thirty-two client-owned adult female dogs undergoing laparoscopic ovariectomy were enrolled in this study. Health status was assessed by means of physical examination, electrocardiography, haemogram and serum biochemical analyses. Only ASA I or ASA II patients without previous treatments were included and brachycephalic breeds, due to their high vasovagal tonus index (Doxey & Boswood 2004), were excluded.

Anaesthetic protocol and monitoring

Food, but not water, was withheld for 12 hours prior to surgery. Dogs were premedicated with 0.004 mg kg^{-1} dexmedetomidine (Dexdomitor 0.5mg ml^{-1}, Ecuphar, Spain) and 4 mg kg^{-1} pethidine (Dolantina 50 mg ml^{-1}, Kern Pharma, S.L., Spain) intramuscularly (IM). Prior to the induction phase, an oxygen flow rate of 4 L min^{-1} was delivered via a face mask and an adult-size circle breathing circuit system. Twenty-gauge catheter (VasoVet, B Braun Vet Care GmbH, Germany) was placed in the cephalic vein and the venous access was used for administering LR (5 ml kg^{-1} h^{-1}) by use of a peristaltic pump (NIKI V4 Volumetric Infusion Pump, EVEREST Veterinary Technology S.L., Spain). Anaesthesia was induced with propofol (Propofol Lipuro 10 mg ml^{-1}, B Braun VetCare S.A., Spain) intravenously (IV) until orotracheal intubation was accomplished. Following placement of a cuffed orotracheal tube, the flow rate was reduced to 50 ml kg^{-1} min^{-1} of 50% oxygen and 50% air. Anaesthesia was maintained with isoflurane (IsoVet, B Braun VetCare, S.A., Spain) using end-tidal isoflurane (EtISO) 1.3–1.8%. Dogs were positioned in dorsal recumbency and were mechanically ventilated to maintain an end-tidal carbon dioxide pressure ($EtCO_2$) between 35 and 45 mmHg (4.7–6 kPA). Controlled ventilation was established considering that respiration is related to cardiac vagal changes (Smith et al. 2013) and respiratory sinus arrhythmia affects the R-R interval, therefore the HRV and stable breathing is needed for correct performance of the monitor (Jeanne et al. 2009). Fentanyl 0.002 mg kg^{-1} IV (Fentanest 0.05 mg ml^{-1}, Kern Pharma, S.L., Spain) was administered when the animal breathed spontaneously and/or HR and/or MAP increased more than 20%. A twenty or eighteen-gauge catheter (VasoVet, B Braun Vet Care GmbH, Germany) was placed in the dorsal pedal artery and connected to a pressure system filled with heparinised saline (1UI ml^{-1}) solution to display systolic arterial pressure (SAP), diastolic arterial pressure (DAP) and MAP on the monitor. The zero-reference level of the pressure transducer was set at the level of the heart. Oesophageal temperature was obtained by a probe positioned at the thoracic portion of the oesophagus and maintained within a narrow range (37ºC to 38ºC) by an electric blanket. Adhesive

surface electrodes were attached according to a lead II ECG monitor (Datex Ohmeda Multiparameter Monitor, GE Healthcare, Finland).

After induction, the hair of the forehead was clipped and the skin degreased to position the disposable BIS-XP Sensor according to (Campagnol et al. 2007) and it was connected to the bispectral monitor (BIS Pediatric XP, Aspect Medical System Inc., MA, US).

Surgical procedure

All dogs were positioned in dorsal recumbence during the laparoscopic ovariectomy. Pneumoperitoneum was obtained by mechanical insufflation of CO_2 (14 mmHg) through a Verres needle. After that, two 5 mm cannulas were positioned as operating channels and abdominal pressure was decreased to 10 mmHg.

Study design

The PTA measurement was obtained by the PTA Monitor (PhysioDoloris, MetroDoloris Medical System, France), a non-invasive device that registers values derived from the electrocardiogram (ECG). The PTA index is calculated from the analysis of the HRV, which is based on small beat-to-beat oscillations of the R-R interval due to the influence of the ANS (Mansour et al. 2017). The calculated values of PTA range from 100 to 0, based on the degree of parasympathetic activation, with 100 indicating a high parasympathetic modulation and 0 means extremely low parasympathetic modulation that could correlate with nociception.The PTA monitor displays two PTA values updated every second, the instantaneous PTA calculated on the 54 previous PTA values and the average PTA calculated on the 176 previous values. The average PTA is considered the anaesthesiologist number. Therefore, the manufacturer indicates that average PTA should be considered intraoperative, since it is more reliable than instantaneous PTA to evaluate the PTA index. .

Flattened crocodile clips were attached to the skin using a 3-lead system according to a lead II ECG after premedication. Yellow and red electrodes were

place on the left and right underarm respectively, while the black electrode was placed over the craneal of the right hindlimb similar to Mansour et al. (2017).

The bispectral index (BIS), signal quality index (SQI), electromyography (EMG), suppression ratio (SR), mean parasympathetic tone activity (PTA), heart rate (HR), systolic arterial pressure (SAP), mean arterial pressure (MAP), diastolic arterial pressure (DAP), temperature (T^a), end-tidal isoflurane (EtISO), end-tidal carbon dioxide ($EtCO_2$) and respiratory rate (RR) were monitored and registered every 5 minutes during the procedure. If SQI was under 90, the BIS values were rejected.

The values of PTA, HR, MAP and BIS were recorded before (baseline) (PTAbaseline, HRbaseline, MAPbaseline, BISbaseline), and 1 minute (PTA1min, HR1min, MAP1min, BIS1min) and 2 minutes (PTA2min, HR2min, MAP2min, BIS2min) after four surgical time points considered as nociceptive stimuli: pneumoperitoneum insufflation (insufflation), introduction of trocars (trocar), and removal of the left (left ovary) and right (right ovary) ovaries.

Statistical analysis

A sample size of 32 animals was determined (G*Power, v.3.1.9.2. Germany) considering a significance of 0.05 and a power of 0.8 to identify an increment of 20% from a PTA value of 50 and a difference deviation of 19 with an effect size of 0.5. The statistical analysis was performed using IBM Statistics SPSS v25. Normality of distribution was assessed using a Shapiro-Wilk test. A generalised linear mixed model was performed for PTA, HR, MAP and BIS according the surgical time points (insufflation, trocar, left ovary, right ovary) and different times (baseline, 1 and 2 min) with a Bonferroni as post hoc test.

For the statistical analysis, a PTA event was defined as a decrease of PTA higher than 20% after any of the surgical time points regarding the baseline values. When these PTA events were identified, it was considered a haemodynamic response to nociception if HR and/or MAP increased more than 20% regarding their baseline values. An ANOVA for repeated measures with a Bonferroni test was performed for PTA, HR, MAP and BIS during the PTA events.

The ΔPTA was calculated at each of surgical time points [(PTA1min–PTAbaseline)/(PTA1min + PTAbaseline)/2]*100 as previously described by Mansour et al. (2017). The performance of PTAbaseline, PTA1min and ΔPTA to predict haemodynamic responses at the surgical time points was assessed by calculation of the area under curve (AUC) of a receiver operating characteristic (ROC) curve using pooled data from the different time points. Data were expressed as mean ± standard deviation. A p value < 0.05 was considered statistically significant.

Results

Thirty-two different breeds of bitches undergoing laparoscopic ovariectomy aged 2.9 ± 2.3 years and weighing 17 ± 6.6 kg were included in this study. Fentanyl boluses were required once in eight animals.

The variation of HR, MAP, PTA and BIS during the four predetermined surgical time points of interest and during the PTA event are shown in Table 1. A significant decrease was observed for PTA (p = 0.007) only 1 minute (16: CI 95% 3–29; p = 0.010) and 2 minutes (13: CI 95% 0.5–27; p = 0.045) after pneumoperitoneum insufflation.

The HR increased significantly 1 minute after introduction of trocars compared to baseline (8 bpm: CI 95% 2–14 bpm; p = 0.007) and decreased 2 minutes after introduction of trocars compared to 1 minute (7 bpm: CI 95% 1–14 bpm p = 0.018). After the removal of the left ovary, a significant elevation of HR after 1 minute was detected compare to baseline (8 bpm: CI 95% 2–13 bpm; p = 0.004) and 2 minutes later (6 bpm: CI 95% 1–8 bpm; p = 0.015). A significant elevation of HR 1 minute after removal of right ovary was detected with respect to the baseline value (6 bpm: CI 95% 1–10 bpm; p = 0.009).

Significant differences were observed for MAP (p = 0.013), which increased significantly 1 minute (10 mmHg: CI 95% 4–17 mmHg; p = 0.001) and 2 minutes (8 mmHg: CI 95% 3–14 mmHg; p = 0.003) after pneumoperitoneum insufflation. A similar trend was observed after the introduction of trocars (1 minute: 9 mmHg, CI 95% 5–14 mmHg, p = 0.001; 2 minutes: 11 mmHg, CI 95%

6–16 mmHg, p = 0.001) and after removal of the left ovary (1 minute: 8 mmHg, CI 95% 3–13 mmHg, p = 0.001; 2 minutes: 9 mmHg, CI 95% 4–15 mmHg, p = 0.001). There were no changes in BIS (p = 0.088).

Thirty-seven PTA events were detected throughout the study, with significant reduction of PTA values 1 minute (30: CI 95% 22–37; p = 0.001) and 2 minute (27: CI 95% 19–35; p = 0.001) with respect to the baseline value. During PTA events, the MAP increased significantly 1 minute (11 mmHg: CI 95% 7–17 mmHg; p = 0.001) and 2 minutes (10 mmHg: CI 95% 6–14 mmHg; p = 0.001) later compared to baseline. However, HR (p = 0.192) and BIS (p = 0.245) were not significantly different.

Baseline PTA (PTAbaseline) (Figure 1) and the value of PTA 1 minute after nociceptive stimuli (PTA1min) (Figure 2) were not different between dogs that presented a haemodynamic response and animals that did not, whereas the ΔPTA was significantly different between dogs that presented haemodynamic response and dogs that did not presented haemodynamic response at pneumoperitoneum insufflation (40: CI 95% 12–68; p = 0.005) but ΔPTA did not change during the other surgical time points or during the PTA event (p = 0.104) (Figure 3).

ROC curves for PTAbaseline, PTA1min and ΔPTA are shown in Figure 4, with only AUC values of PTAbaseline (AUC=0.609: CI 95% 0.499–0.718; p = 0.048) and ΔPTA (AUC=0.323: CI 95% 0.209–0.437; p = 0.002) being statistically significant, despite PTAbaseline having an AUC value higher than 0.5. At the threshold value of PTAbaseline ≤ 51, the sensitivity and specificity of PTA to detect a haemodynamic response within 1 minute were 69% and 52% respectively.

Discussion

The aim of this study was to determine the performance of the PTA index in relation to the haemodynamic variables HR and MAP to assess its utility in monitoring intraoperative nociception. It was hypothesised that changes in the PTA index or ΔPTA may coincide with or even predict haemodynamic response.

The PTA index decreased significantly only during insufflation and MAP increased significantly at the same time. Haemodynamic responses were detected throughout the surgery without changes in the PTA. The PTAbaseline and PTA1min values were similar in dogs that had a haemodynamic response and in dogs that did not. During the PTA events detected, the MAP increased significantly, but the HR did not change.

The ΔPTA could not predict intraoperative haemodynamic changes during nociceptive stimuli. A threshold value of PTAbaseline ≤ 51 was able to predict a haemodynamic response with moderate sensitivity and low specificity (69% and 52% respectively).

Sudden increments in HR and/or MAP intraoperatively are clinically interpreted as a nociceptive response (Jeanne et al. 2009; Gruenewald et al. 2013; Boselli et al. 2016; Mansour et al. 2017; Theerth et al. 2018). It is useful for the anaesthetist to have a validated monitor to assess nociception, since it is known that these parameters can be influenced by the drugs used, the animal condition and the anaesthesia plane (Mansour et al. 2017) as in humans (Gruenewald & Ilies 2013). Jeanne et al. (2012) found the ANI to be more sensitive to intraoperative stimuli than HR or BP during laparoscopic surgery in humans.

To the author's knowledge, only one paper has described the use of the PTA index to determine nociception in dogs (Mansour et al. 2017). However, the results of our study do not agree with their results. Under the study conditions, considering the intraoperative increments of 20% of the HR and/or the MAP as a nociceptive response (Jeanne et al. 2014; Boselli et al. 2016; Mansour et al. 2017), the PTA index did not decrease significantly during a cardiovascular response. Mansour et al. (2017) found a significant decrease of PTA within 1 minute after predetermined times, which was associated with a significant haemodynamic response within 5 minutes. However, the PTA index showed a poor ability to predict a haemodynamic response (Mansour et al. 2017). In our study, the PTA index decreased significantly only 1 and 2 minutes after insufflation, accompanied by a significant increase in the MAP.

If PTA index values change instantaneously, then it would be more appropriate to compare them with data provided by direct BP measurement rather than data obtained by oscillometric arterial BP monitoring. This may account for the differences found with respect to the study of Mansour et al (2017), in which they monitored HR and SAP 5 minutes after nociceptive stimulus, so it might increase the ability of the PTA index to anticipate a haemodynamic response. In the authors' experience, 5 minutes is a relatively long time and cardiovascular changes could be influenced by other stimuli later in the surgery. Similar to our findings, (Ledowski et al. 2014) compared ANI before and 30 seconds after stimulation, reporting a poor predictive probability to indicate an increase of 10% in HR and SAP within 3 minutes.

Specificity and sensitivity must be considered to evaluate the PTA assessment. Boselli et al. (2015) reported that ANI ≤55 predicted an increase in HR and/or SAP by >20% within the next 5 minutes with a sensitivity and specificity of 88% and 83% respectively. Recently, it was found that the correlation between BP and HR with ANI are good in neurosurgical patients undergoing elective supratentorial craniotomy, however, the r values were very low (Kommula et al. 2019). The ΔANI seems to be a better parameter to predict haemodynamic reactivity in humans, since a ΔANI threshold of -19% predicts hemodynamic reactivity with 85% sensitivity and 85% specificity (Boselli et al. 2016).

In dogs, the ROC curve of the PTA index after 1 minute of predefined times for the prediction of haemodynamic events indicated a threshold value of <46, with a sensitivity and specificity of 60% and 63%, respectively. However, the ΔPTA showed better results, since a ΔPTA <-18% was showed to correctly predict the haemodynamic response within 5 minutes, with a sensitivity and specificity of 76% and 72%, respectively (Mansour et al. 2017). In our study, the ΔPTA showed an AUC of 0.323, therefore, only the PTAbaseline value was useful to predict the haemodynamic response within 1 minute. However, a threshold value of PTAbaseline ≤51 showed a moderate sensitivity (69%) with a low specificity (52%). Variability between ΔPTA in both studies could be explained by the different breeds used and surgeries performed, mainly due to the difference in haemodynamic data registration times.

The PTA index is influenced by instant variations, thus the dynamic variations ΔANI or ΔPTA may provide a better reliability (Boselli et al. 2016; Mansour et al. 2017). However, the ΔPTA seems more interesting for retrospective studies, since it needs to be calculated based on the PTA index provided by the monitor. This might be considered a limitation for clinical purposes given that the main utility of the PTA monitor should be to help the anaesthetist to maintain an adequate intraoperative nociception-antinociception balance. It would be useful if the ΔPTA is given by the PTA monitor based on the recorded data.

To discard the variability on the depth of anaesthesia led to changes in HR, MAP or PTA, a stable depth of anaesthesia was maintained and monitored using the BIS monitor (March & Muir 2005; Morgaz et al. 2009; Gruenewald et al. 2013). Since the BIS values did not change significantly during surgery or any PTA events, any change in HR, MAP or PTA was considered to be produced by nociceptive stimuli.

However, isoflurane was used for maintenance, which could influence the study results considering that the ANI monitor seems to perform better in detecting moderate nociceptive stimuli in humans under propofol/fentanil anaesthesia than under halogenated agents (Boselli et al. 2013). It is possible that inhaled anaesthetic is associated with higher sympathetic activity (Boselli & Jeanne 2014). Alpha2-agonists may influence the sympathovagal balance (Khan et al. 1999), however their use is necessary in a clinical context to decrease stress previous to surgery. Nevertheless, a standardised anaesthetic protocol was used in all dogs and a low dose of dexmedetomidine was used 90 ± 19 minutes before the surgery.

There was no breed restriction, except for brachycephalic dogs, in the present study. Nonetheless, autonomic nervous system variability may be diverse between breeds (Doxey & Boswood 2004), so could be considered as a limitation, but the purpose of the study was to evaluate the PTA monitor performance under clinical conditions and not only for a specific breed.

The nature of the nociceptive stimulus should be considered since differences between stimuli might influence results (Valverde et al. 2003). In the

present study, nociceptive stimuli were always similar since only laparoscopic ovariectomies were included, which could explain partially the differences with previous results reported in dogs (Mansour et al. 2017) or in humans (Boselli et al. 2016) which included different types of surgeries. Standardised electrical nociceptive stimuli for the assessment of the ANI during anaesthesia have been used in human research (Luginbuhl et al. 2010; Gruenewald et al. 2013), however most ANI performance results are from clinical studies that used different stimuli (Jeanne et al. 2012; Ledowski et al. 2014; Boselli et al. 2015), so could influence the results. In the present study performed in a clinical context, the response to four fixed stimuli during the same surgical procedure was performed by the same surgeon and analysed to standardise noxious stimuli.

Conclusion

In the present study, the PTA index and ΔPTA were not effective to assess intraoperative nociception and predict a haemodynamic response in anaesthetised dogs. Further research should be performed in veterinary medicine to assess the usefulness of PTA monitor in dogs of different breeds using different anaesthesia protocols.

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3.1. Tablas y figuras del estudio I

Table 1 Mean ± standard deviation for parasympathetic tone activity (PTA) index, heart rate (HR) (beats minute^{-1}), mean arterial pressure (MAP) (mmHg) and bispectral index (BIS), before (baseline) and 1 and 2 minutes after four surgical time points: pneumoperitoneum insufflation (Insufflation), introduction of trocars (Trocar) and removal of the left (Left ovary) and right (Right ovary) ovaries, and when PTA decreased more than 20% after any of the surgical time points compared to baseline (PTA event). *Statistically different from baseline (p < 0.05), †Statistically different from 2 minutes (p < 0.05).

PTA		**Baseline**	**1 minute**	**2 minutes**
	Insufflation	67 ± 21	51 ± 21*	53 ± 21*
	Trocar	54 ± 22	48 ± 19	46 ± 22
	Left ovary	49 ± 22	54 ± 21	49 ± 21
	Right ovary	56 ± 21	58 ± 21	54 ± 21
	PTA event	64 ± 22	35 ± 14*	37 ± 17*
HR (beats minute^{-1})				
	Insufflation	86 ± 17	82 ± 17	83 ± 14
	Trocar	83 ± 14	91 ± 12*†	83 ± 15
	Left ovary	83 ± 16	91 ± 13*†	87 ± 14
	Right ovary	81 ± 15	87 ± 12*	85 ± 13
	PTA event	85 ± 14	88 ± 14	84 ± 13
MAP (mmHg)				
	Insufflation	67 ± 12	78 ± 12*	76 ± 11*
	Trocar	77 ± 11	86 ± 14*	88 ± 13*
	Left ovary	86 ± 14	95 ± 14*	96 ± 15*
	Right ovary	89 ± 13	91 ± 13	91 ± 13
	PTA event	78 ± 15	89 ± 14*	88 ± 13*
BIS				
	Insufflation	56 ± 11	54 ± 11	55 ± 10
	Trocar	56 ± 12	56 ±12	53 ± 12
	Left ovary	53 ± 11	55 ± 13	54 ± 12
	Right ovary	54 ± 12	53 ± 12	51 ± 12
	PTA event	56 ± 8	55 ± 8	54 ± 10

Figure 1 Mean PTA baseline values in dogs that showed a haemodynamic response (Haemodynamic response) and in dogs that did not (No haemodynamic response) 1 minute after the four surgical time points: pneumoperitoneum insufflation (Insufflation), introduction of trocars (Trocar), removal of the left ovary (Left ovary), removal of the right ovary (Right ovary) or when PTA value decreased more than 20% after any of the surgical time points compared to baseline (PTA event).

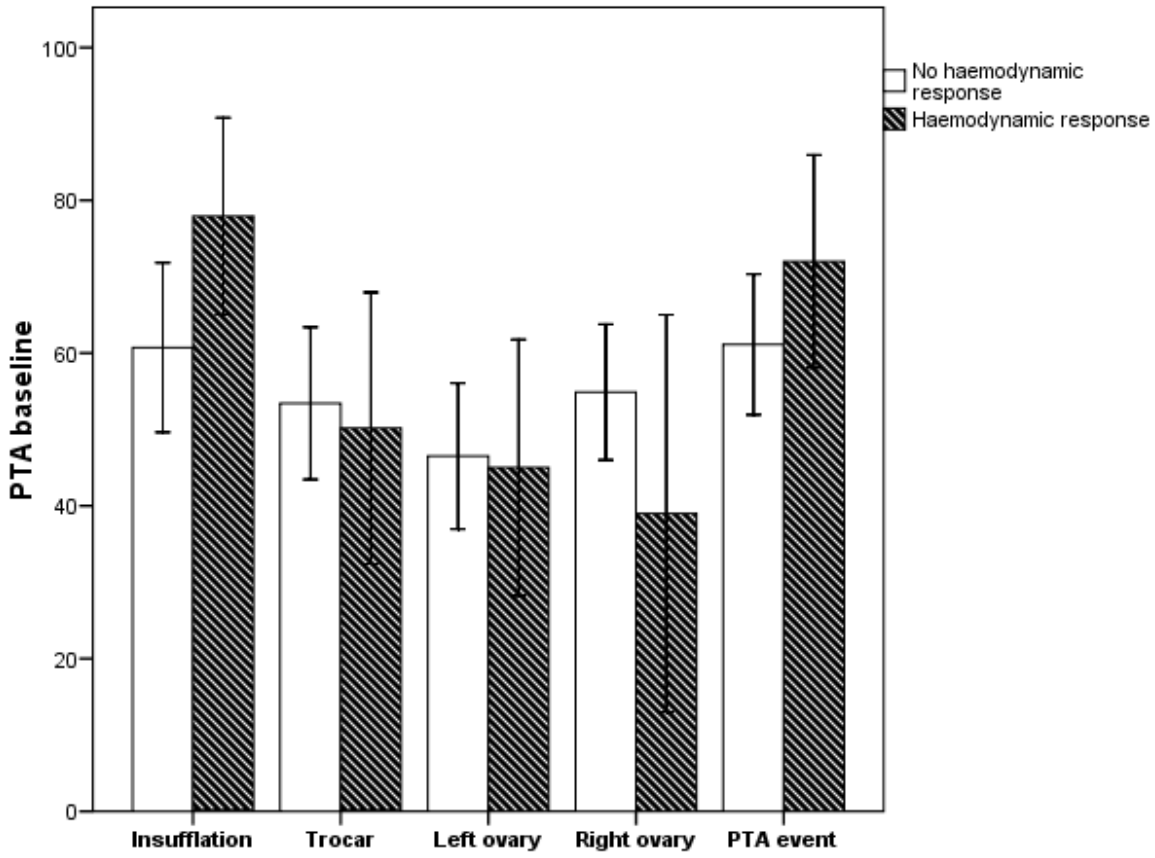


Figure 2 PTA values 1 minute after the surgical stimulus (PTA1min) in dogs that showed a haemodynamic response (Haemodynamic response) or in dogs that did not (No haemodynamic response) at four surgical time points: pneumoperitoneum insufflation (Insufflation), introduction of trocars (Trocar), removal of the left ovary (Left ovary), removal of the right ovary (Right ovary) and when PTA value decreased more than 20% after any of the surgical time points compared to baseline (PTA event).

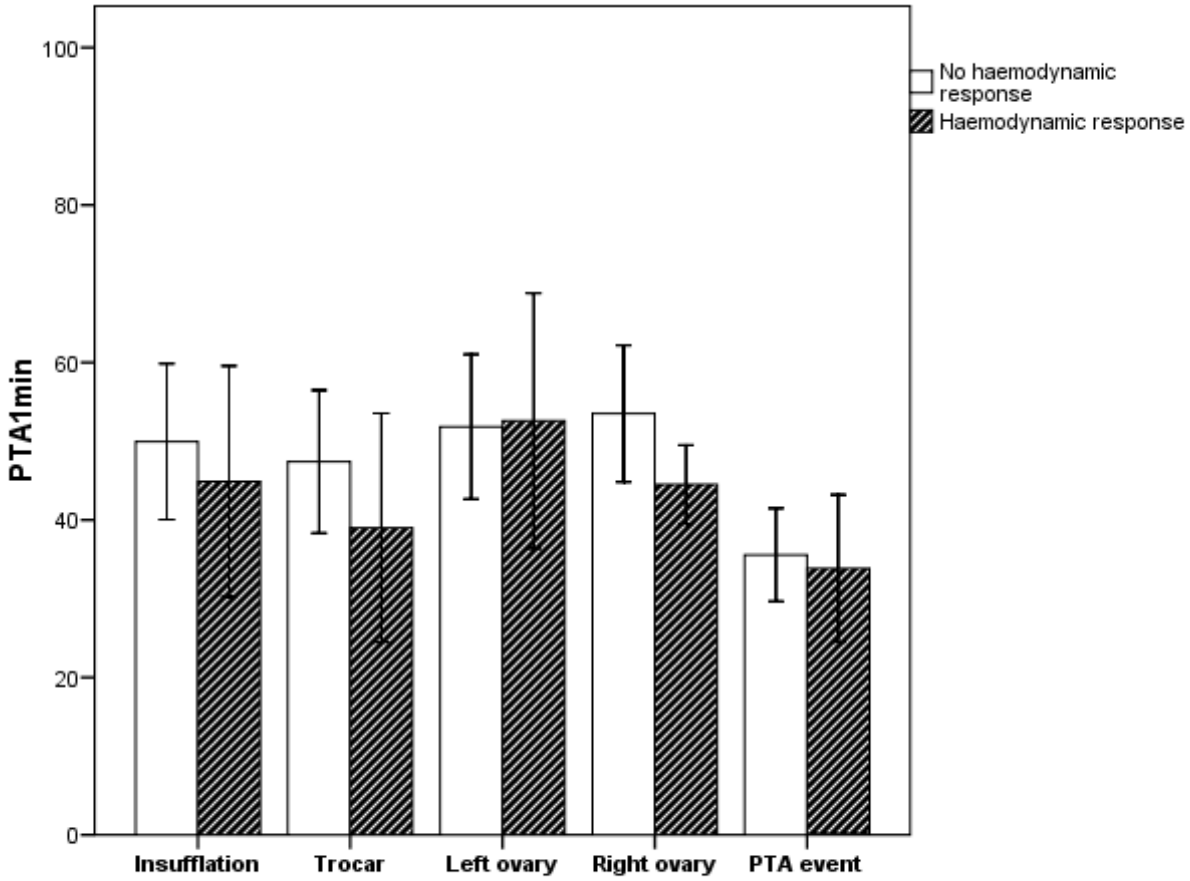


Figure 3 Dynamic variations of PTA (ΔPTA) in dogs that showed a haemodynamic response (Haemodynamic response) and in dogs that did not (No haemodynamic response) at four surgical time points: pneumoperitoneum insufflation (Insufflation), introduction of trocars (Trocar), removal of the left ovary (Left ovary), removal of the right ovary (Right ovary) and when PTA value decreased more than 20% after any of the surgical time points compared to baseline (PTA event).*Statistically different from the no haemodynamic response group ($p < 0.05$).

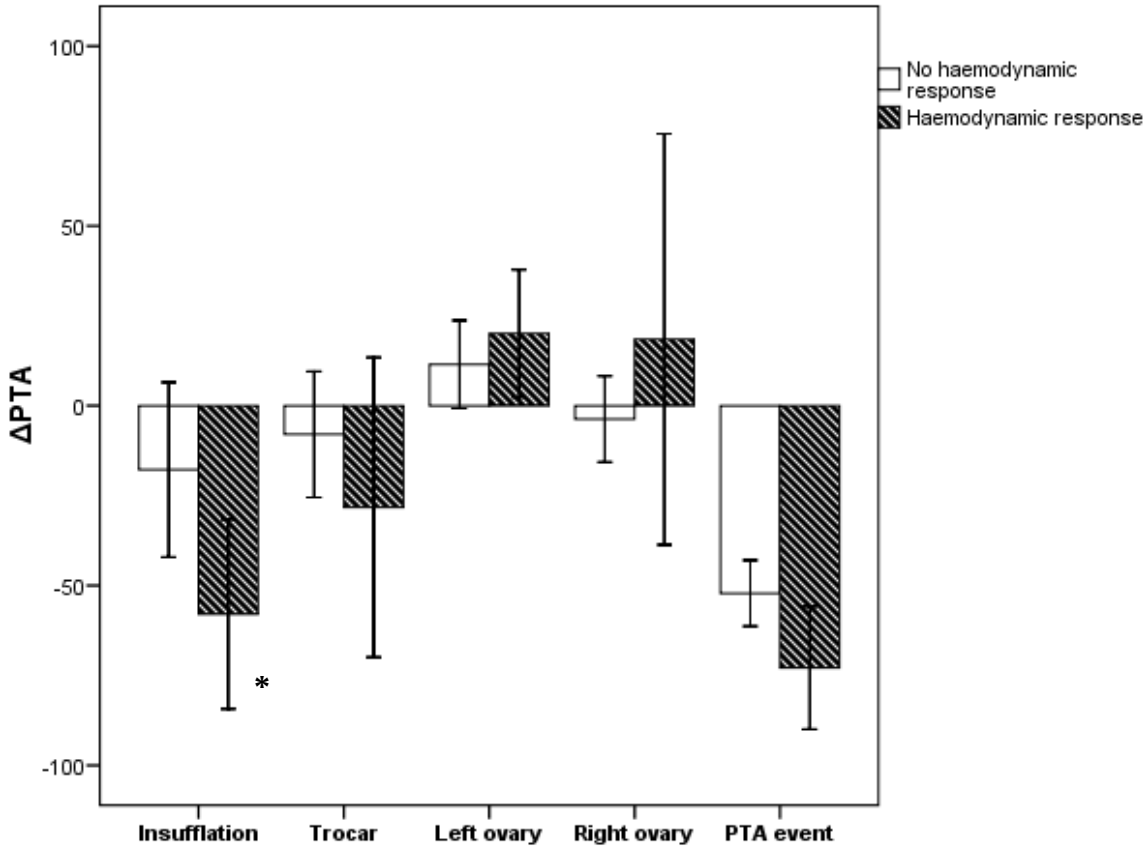
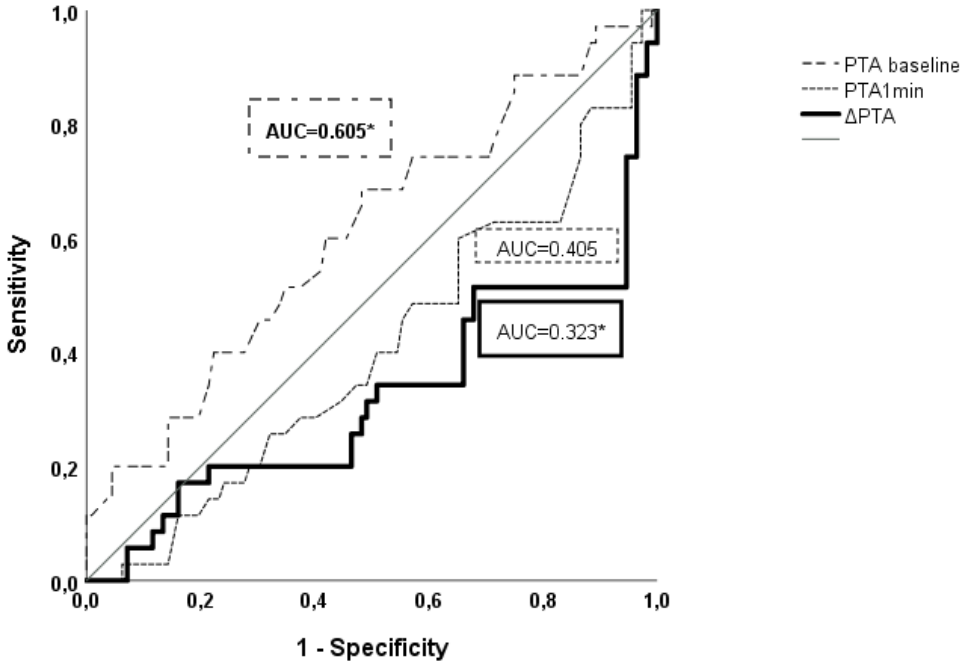


Figure 4 Comparison ROC curves (n=147). Performance of PTA previous to the surgical time points (PTA baseline), PTA 1 minute after the surgical time points (PTA1min) and dynamic variation of PTA (ΔPTA) to predict haemodynamic response. Area Under the Curve (AUC). *Statistically different ($p < 0.05$).



4. Estudio II

Assessment of autonomic nervous system activity by monitoring parasympathetic tone activity (PTA) in horses after a nociceptive stimulus and subsequent administration of morphine, ketamine and dobutamine

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Abstract

Assessment of autonomic nervous system activity by monitoring parasympathetic tone activity (PTA) in horses after a nociceptive stimulus and subsequent administration of morphine, ketamine and dobutamine

Objective To determine whether a surgical nociceptive stimulus and the administration of morphine, ketamine and dobutamine would modify the autonomous nervous system activity monitored using the parasympathetic tone activity (PTA) index in horses under general anaesthesia.

Study design Prospective, observational, clinical study.

Animals Twenty adult horses anaesthetised for elective surgeries.

Methods Heart rate (HR), mean arterial pressure (MAP) and PTA index were monitored before and 1, 3 and 5 minutes after the surgical incision, and before and 10 minutes after morphine administration. If an increase of the palpebral reflex was noted or light nystagmus or spontaneous ventilation was observed, a ketamine bolus was given and the three variables were registered before, 3 and 5 minutes after the administration. If the MAP fell below 62 mmHg, a dobutamine infusion was administered and the three variables were registered before and 5 minutes after the infusion was started or increased. If the PTA index decreased ≥ 20% (PTA event), HR increased ≥ 10% (HR event) or MAP increased ≥ 20% (MAP event), the three variables were registered before and 1, 3 and 5 minutes after each event.

Results The PTA index decreased significantly 3 minutes after the ketamine bolus ($p=0.042$) and 1 minute after a PTA event was identified ($p=0.016$). The MAP decreased significantly 10 minutes after morphine administration ($p=0.009$) and 5 minutes after ketamine administration ($p=0.010$).

Conclusion and clinical relevance No changes in ANS activity, using the PTA index, were registered in anaesthetised horses after a surgical nociceptive stimulus. Neither dobutamine nor morphine modified ANS activity. The PTA index demonstrated an increase in sympathetic nervous system activity after ketamine administration. Further studies should be performed to determine whether changes in ANS activity under different conditions could affect the PTA index.

Keywords Parasympathetic tone activity, horse, nociceptive, morphine, dobutamine, ketamine

Introduction

The autonomic nervous system (ANS) plays an important role in modifying heart rate (HR), blood pressure (BP) (Gruenewald et al. 2013) and heart rate variability (HRV) (Jeanne et al. 2009). During operations, HR and BP, among other variables, are monitored in order to ensure adequate depth of anaesthesia and adequate nociception-antinociception balance (Gruenewald & Ilies 2013). The analgesia nociception index (ANI), based on HRV and a reflection of the autonomic nervous system's behaviour, was developed for human medical applications (Boselli et al. 2016). For that reason, an increase in parasympathetic nervous system activity decreases HR, consequently increasing the R-R interval, changing HRV (Paris et al. 2001). In veterinary medicine, the parasympathetic tone activity (PTA) index, based on the HRV of dogs, cats and horses (Mansour et al. 2017), has been developed as a tool to monitor ANS activity. The PTA index is largely correlated with ANS activity, with values ranging from 0 to 100. Values close to 0 reflect high sympathetic tone activity and low parasympathetic tone activity, while values close to 100 reflect high parasympathetic tone activity and low sympathetic tone activity (Mansour et al. 2017; Leitão et al. 2018). The PTA index and the dynamic variation thereof correctly reflect nociception-antinociception balance and predicted cardiovascular reactivity in anaesthetised dogs (Mansour et al. 2017). Another study showed that the PTA index was able to determine analgesic levels in treatments in pigs (Leitão et al. 2018). To our knowledge, no studies have yet been performed on the use of the PTA index in horses.

Nociceptive stimuli can alter haemodynamic variables (HR, BP) (Gruenewald & Ilies 2013) and HRV (Jeanne et al. 2009). Furthermore, there are also different drugs that might affect ANS activity, such as dissociative anaesthetics such as ketamine, due to their sympathomimetic properties (Chernow et al. 1982; Bollag et al. 2015), analgesics such as opioids, due to their stimulation of the central nervous system (Clutton 2010), and sympathomimetic drugs such as dobutamine (Schauvliege & Gasthuys 2013; Dancker et al. 2018). The effect of esmolol on the ANS was evaluated in piglets undergoing septic shock. Esmolol affected HR and BP, but not HRV or ANI (Boselli et al. 2018). Furthermore, it is necessary to elucidate how drugs commonly used for equine

anaesthesia might interfere with PTA index assessment due to their effect on the ANS.

The aim of this study was first to determine if ANS activity changes in response to a surgical nociceptive stimulus or during the administration of morphine, ketamine and dobutamine in horses under general anaesthesia. The study's second aim was to assess how changes in ANS activity might interfere with the PTA index to monitor intraoperative nociception. We hypothesised that a nociceptive stimulus and the administration of morphine, ketamine and dobutamine would modify autonomous nervous system activity and it would be observed as a change in the PTA index.

Materials and Methods

Twenty adult horses (17 males and 3 mares) between 1 and 11 years old and 529.5 ± 68.9 kg were enrolled in this prospective, observational, clinical study performed at the Veterinary Teaching Hospital, University of Córdoba. The horses fasted for 8 hours prior to elective surgery that included fifteen arthroscopies, one tenoscopy, three castrations and one umbilical hernia repair. Horses were determined to be healthy by physical examination, a complete blood count and a serum biochemistry panel. Horses in which any alterations were found were excluded from the study. The study was approved by the Ethical Committee of Animal Welfare of the Veterinary Teaching Hospital, University of Córdoba (CEBAHCV60/2018). All procedures were conducted in compliance with the ethical principles of good practice in animal experimentation and with previous informed consent from the owners.

Anaesthesia

All animals received phenylbutazone 4.4 mg kg^{-1} intravenously (IV) (Butasyl, Zoetis, Spain), sodium benzyl penicilin 22,000 UI kg^{-1} IV (Penilevel, Laboratories ERN, SA, Spain) and gentamicine 6.6mg kg^{-1} IV (Genta-Equine, Dechra, Spain) one hour prior to the surgery. Premedication consisted of xylazine 1.5 mg kg^{-1} IV (Xilagesic, Laboratories Calier, SA, Spain). Ten minutes later, anaesthesia was induced with ketamine 2.7 mg kg^{-1} (Imalgene, Merial, France)

and diazepam 0.1 mg kg^{-1} (Roche Farma, SA, Spain) IV to allow for endotracheal intubation. Horses were positioned in dorsal recumbence. Anaesthesia was maintained with isoflurane at 1.3-1.6% (IsoVet, Piramal Healthcare UK Limited, UK) in 100% oxygen. Normocapnia was maintained using intermittent positive pressure ventilation (IPPV). The facial artery was catheterised using a 20-gauge cannula (VasoVet, B Braun Melsungen AG, Germany) for invasive measurement of blood pressure. The depth of anaesthesia was assessed by monitoring palpebral reflex, nystagmus and muscle relaxation.

Respiratory and cardiovascular variables were registered over the intraoperative period every 5 minutes (Datex Ohmeda Multiparameter Monitor, GE Healthcare, Finland). Furthermore, heart rate (HR), invasive mean arterial pressure (MAP) and mean parasympathetic tone activity (PTA) were registered for their statistical analysis before and after the evaluated events. The PTA monitor (MetroDoloris, France) is a non-invasive device based on electrocardiography, since it applies a mathematical formula on the distance between two consecutives R (R-R interval) waves that reflects the HRV. The PTA monitor displays two PTA values updated every second, the instantaneous PTA calculated on the 54 previous PTA values and the average PTA calculated on the 176 previous values. The manufacturer indicates that average PTA should be considered intraoperative, since it is more reliable than instantaneous PTA to assess the PTA index. Since breathing is also affected by the autonomous nervous system (Jeanne et al. 2012), controlled ventilation is necessary to evaluate the PTA index. The three electrodes of the PTA monitor used to register the electrocardiogram were applied in a similar manner to the lead II of the electrocardiography apparatus. The red and yellow electrodes were positioned in a similar manner to the same colours of the lead II, while the black electrode from the PTA monitor was placed at the right underarm, opposite the green electrode of the lead II.

Evaluated events

The nociceptive stimulus was established as the first incision for soft tissue surgeries or the first incision plus the introduction of the scope for the arthroscopies (incision event). Therefore, the three variables (HR, MAP and PTA) were registered before and at 1 minute, 3 minutes and 5 minutes after the incision

event. After data were registered, morphine hydrochloride 0.2 mg kg^{-1} (Morfina, B Braun Medical SA, Spain) was administered (morphine event) and the three variables were recorded 10 minutes (Knych et al. 2014) after the administration.

Ketamine (Imalgene, Merial, France) 0.5 mg kg^{-1} IV was administered if an increase in the palpebral reflex, light nystagmus, spontaneous ventilation or lack of muscle relaxation were observed (ketamine event). The HR, MAP and PTA were registered before and 3 and 5 minutes after ketamine administration (Kaka et al. 1979).

If a MAP ≤ 62mmHg was observed, dobutamine (Dobutamina, Inibsa Hospital, SLU, Spain) 0.25 µg kg^{-1} $minute^{-1}$ IV at a continuous rate of infusion (CRI) was started (dobutamine event) and increased to 0.25 µg kg^{-1} $minute^{-1}$ IV every 5 minutes until the MAP stably reached a value between 70 and 80 mmHg. If the MAP increased to over 80 mmHg, the dobutamine CRI was either reduced by the same proportion or stopped. Data for the dobutamine event was recorded before and 5 minutes after dobutamine CRI was either started or increased.

If either a decrease in PTA ≥ 20% (PTA event), an increase in HR ≥ 10% (HR event) or an increase in MAP ≥ 20% (MAP event) were observed, data from before and 1, 3 and 5 minutes after the event were registered.

Thiopental (Tiobarbital, B Braun VetCare, SA, Spain) 1 mg kg^{-1} IV was administered when the animal presented intense nystagmus or if it was determined that there was a risk of movement. Data acquired in the 15 minutes (Abass et al. 1994) after thiopental administration were not considered for the study.

If more than one event happened at the same time, they were excluded from the study.

Statistical analysis

A sample size of 20 animals was chosen (G*Power, v.3.1.9.2. Germany), based on a significance level of 0.05 and a power level of 0.95, to identify a decrease in 15% from a PTA value of 50, a deviation of 12, an effect size of 0.9 and considering a 10% loss. The statistical analysis was performed using IBM

Statistics SPSS v25. The distribution was confirmed to be normal using a Shapiro-Wilk test. Each variable (PTA, HR and MAP) was analysed using a Friedman's test with a Bonferroni post hoc test before and after each event. If significant differences were observed, a Wilcoxon's test was conducted. Data are expressed as mean ± standard deviation or median (25-75th percentile). A Spearman's correlation was performed for PTA, HR and MAP using the incision event data. A p value < 0.05 was considered statistically significant.

Results

Twenty incision and morphine events, fifteen ketamine events and twenty-eight dobutamine events were evaluated. No differences were found for any of the variables (PTA, HR or MAP) before (baseline) and 1, 3 or 5 minutes after the incision (Table 1). A significant decrease in MAP was found 10 minutes after administration of morphine (- 6 mmHg: CI95% -2/-10 mmHg; p=0.009) (Table 1). When ketamine was administered, the PTA decreased significantly after 3 minutes (-16: CI95% -2.5/-27.5; p=0.042) and the MAP decreased significantly after 5 minutes (-5.5 mmHg: CI95% -12/ -2 mmHg; p=0.010) (Table 1). The MAP increased significantly 5 minutes after CRI of dobutamine was started or increased as expected (10 mmHg: CI95% 7-14 mmHg; p=0.001) (Table 1).

Seven PTA events were registered, and a significant decrease was identified 1 minute after the PTA event compared to its previous value (-24: CI95% -29/-20; p=0.016) (Table 1). The HR and MAP did not change during the PTA events. Only three MAP events and two HR events were identified, so data were not statistically analysed.

The PTA and HR showed a weak Spearman's correlation (r=0.252: p=0.024), while no correlation was found between PTA and MAP (r=0.079: p=0.489) at the incision event.

Thiopental was needed on 2 occasions, and no event was registered after thiopental administration.

Discussion

The ANI is based on the analysis of the HRV that depends on the relation between low frequency (LF), mainly related to sympathetic tone activity, and high frequency (HF), mainly related to parasympathetic tone activity (LF/HF). Therefore, changes in ANS activity can be reflected as changes in ANI (Boselli et al. 2018). Since ANS activity can be affected under general anaesthesia by nociception or drug administration, we hypothesised that the PTA index would change after a surgical nociceptive stimulus and after ketamine, morphine and dobutamine administration. However, the PTA index decreased after ketamine administration only, remaining stable during the rest of events. An increased sympathetic activity was identified on more occasions due rather to a decrease in the PTA index than due to an increase of HR or MAP.

Morphine was administered intravenously after the incision event. It was decided in order to increase the nociceptive response since a preliminary study was done administering morphine prior to the incision, and HR, MAP or PTA indices did not change after the surgical stimulus (data no published). The MAP decreased significantly 10 minutes after morphine administration, although it was not considered a clinically relevant decrease. The MAP decrease could be due to the time spent in dorsal recumbency (Stegmann & Littlejohn 1987), the use of isoflurane (Steffey et al. 1987) and/or the use of IPPV (Edner et al. 2005). The use of IPPV was required since the respiratory pattern influences the HRV (Jeanne et al. 2009); a controlled ventilation therefore improves the PTA index assessment. The MAP decrease could also be a result of decreased nociception. In this case, it would be expected that a decrease in sympathetic activity would register as an increase in the PTA index.

The administration of opioids to horses stimulates the central nervous system (Clutton 2010). Therefore, it would be interesting to know how opioids affect ANS activity, and therefore how PTA index is influenced in order to consider the values provided by this monitor. It has been reported that fentanyl increases vagal tone and decreases sympathetic nervous system activity in healthy humans (Vettorello et al. 2008). Remifentanil has also been reported to increase parasympathetic tone activity in humans, observed as an increase in ANI

(Gruenewald et al. 2013). The use of morphine has been poorly documented but has been suggested to alter HRV (Bressan et al. 2014). On the contrary, trimebutine, a peripheral opioid receptor agonist, did not change HRV compared to a placebo in humans (Ellidokuz et al. 2008). These results may be explained by the different distribution of opioid receptors, which are species-dependent (Thomasy et al. 2007).

Dobutamine has sympathomimetic properties (Dancker et al. 2018) and significantly increases MAP 5 minutes after the CRI of dobutamine is started or increased. Hypoxia, dobutamine and phenylephrine were found to affect sympathetic tone activity (Xhaet et al. 2008). However, dobutamine did not change HRV in horses under a sevoflurane anaesthetic (Ohmura et al. 2006). The effects of dobutamine on the ANS during myocardial ischemia were studied in humans (Sharma et al. 2015). It was found that dobutamine was predominantly related to parasympathetic activity in the absence of myocardial ischemia, but to sympathetic activity under myocardial ischemia conditions (Sharma et al. 2015). The frequent use of dobutamine during anaesthesia in horses to treat hypotension (Schauvliege & Gasthuys 2013) means that it is important to know whether the PTA values are influenced by this drug. Boselli et al. (2018) observed that the administration of the B-blocker esmolol significantly changed the HR and MAP, without significantly affecting the ANI or HRV in piglets undergoing sepsis. According to our results, the lack of any change in PTA index during dobutamine CRI administration suggests that the PTA monitor could be used intraoperatively to assess ANS balance without the effect of dobutamine's interference. Thus, changes in PTA monitor may help identify nociception stimuli in horses, as was proven in dogs (Mansour et al. 2017).

Ketamine has sympathomimetic properties (White & Ryan 1996) which shift the balance of ANS towards sympathetic tone activity (Komatsu et al. 1995). In our study, the PTA index decreased significantly 3 minutes after the administration of ketamine. This result is inconsistent with those of a previous human medical study in which ANI did not change after a ketamine bolus administration (Bollag et al. 2015). Bollag et al. (2015) administered the ketamine right after intubation, while the horses received ketamine when they showed an increased palpebral reflex, light nystagmus, spontaneous ventilation or lack of

muscle relaxation due to a lighter depth of anaesthesia. It cannot be ruled out that the PTA monitor would not register changes in HRV early enough, thereby showing a normal PTA value before ketamine administration. However, it must be pointed out that the increment in sympathetic tone activity registered could be due to the sympathomimetic properties of ketamine. In any case, no change in the PTA index was registered 5 minutes after administration, suggesting that at the dose used the sympathetic effect of ketamine would last for a short period of time and changes in the PTA index during this time should not be used to assess nociception. The decrease in MAP 5 minutes after ketamine administration could be due to the analgesic effect of ketamine (Muir 2010) or to the deeper level of anaesthesia obtained (Hans et al. 2005). The change was not clinically significant since the horses remained normotensive.

To homogenise the type of nociceptive stimulus, only the first incision plus the introduction of the scope for the arthroscopies or the first incision for the soft tissue surgeries were considered. No significant change in PTA index, HR or MAP was found during nociceptive stimulation in contrast to Mansour et al. (2017), who showed a significant decrease in PTA index values after the surgical incision in dogs. These authors found a correlation between the decrease in the PTA index and the increase in HR and SAP, suggesting an increase in sympathetic nervous system activation. It has been said that HRV in humans may change after nociceptive stimuli and predict cardiovascular reactions to the nociceptive stimuli (Jeanne et al. 2009). Later studies showed that ANI changed after nociceptive stimuli (Jeanne et al. 2012), and that HR and blood pressure increments could be predicted (Boselli et al. 2015; Boselli et al. 2016). However, another study found that ANI had little predictive value for cardiovascular changes (Ledowski et al. 2014).

A lack of activation of the sympathetic nervous system during surgical incision could indicate adequate intraoperative anaesthesia depth, avoiding any response from the ANS that could change the haemodynamic variables or the HRV in this study. The behaviour of the ANS is difficult to assess during the intraoperative period, considering that the drugs used, the surgical manoeuvres and other factors could influence ANS balance (Gruenewald et al. 2013). The ANI has shown to perform adequately with an inhaled anaesthetic in human studies

(Kommula et al. 2017; Theerth et al. 2018). Also, the PTA index previously demonstrated a correct assessment of nociceptive stimuli in dogs maintained with an isoflurane anaesthetic (Mansour et al. 2017). Due to the clinical aspect of our study, we decided to maintain anaesthesia with isoflurane. The inhaled isoflurane concentration was stably maintained between 1 and 1.23 times the isoflurane MAC in horses (1.3 and 1.6%). It would be better to evaluate the response of the ANS during a lighter depth of anaesthesia to avoid any interference, but this was not possible since these were clinical cases. Furthermore, phenylbutazone, a COX-1 selective nonsteroidal anti-inflammatory drug (NSAID) (Knych 2017), was given to all the animals prior to general anaesthesia, which provided analgesia and could have contributed to making the sympathetic response to the stimulus more difficult.

Alfa-2-agonists can modify sympathovagal balance (Valverde 2010). That is why the anaesthesia was only maintained with isoflurane. Xylazine was used for its shorter half-life (31.4±8.9 minutes) in horses (Santonastaso et al. 2014) compared to other alfa-2-agonists. A minimum of 60 minutes passed before the incision event was registered to ensure xylazine's effects were insignificant.

During this study, the PTA index decreased on more occasions (7) than HR increased (2 occasions) or MAP increased (3 occasions). This was similar to a study in humans where 95 ANI events (ANI decreased ≥ 20%), but only one HR event (HR increased ≥ 20%) and 14 SBP events (Systolic Blood Pressure increased ≥ 20%), were identified in a group of 30 patients (Ledowski et al. 2014). This may mean that the PTA index was able to identify changes produced by sympathetic tone activation on more occasions. Since the PTA was significantly decreased only 1 minute after the PTA event, it could be showing a punctual activation of the sympathetic activity that had not repercussion on cardiovascular variables. It has been shown that ANI changes do not necessarily coincide with cardiovascular changes (Weber et al. 2018). Some studies have shown that ANI measurements are well correlated with nociceptive stimuli (Logier et al. 2010; Jeanne et al. 2014). In our study, the correlation between HR and PTA was weak, and no correlation between MAP and PTA was found at the incision event. However, there was no significant change in any variable during the incision event. This lack of correlation should therefore be carefully considered.

The ANS balance is more than a mathematical equilibrium between the predominance of sympathetic and parasympathetic tone activity (Reyes del Paso et al. 2013). The HRV includes low frequency (LF) that reflects parasympathetic and sympathetic nervous system activity and high frequency (HF) that depends predominantly on parasympathetic nervous system activity (Manzo et al. 2009) . The LF/HF ratio is a reflection of sympathetic nervous system activity (Manzo et al. 2009). After reviewing several studies on HRV and ANS balance, Reyes del Paso et al. (2013) found that the LF is determined mainly by parasympathetic tone activity, making it really important for cardiac regulation. HRV is a well-established non-invasive measure of cardiac ANS control that can be modified by maturation, environmental conditions and drugs (Faye et al. 2010). The use of the PTA monitor to assess HRV should be considered carefully and, since not only nociceptive stimuli can affect the PTA index, could be a limitation. However, only ketamine modified the behaviour of PTA index during this study. No changes after dobutamine or morphine were found, meaning that the PTA index could be useful for assessing changes in ANS, reflecting sympathetic tone activity, following nociceptive stimuli. Further studies should be performed to clarify the effect of different drugs, doses and circumstances.

Conclusion

No change in ANS activity was observed in anaesthetised horses after a surgical nociceptive stimulus using the PTA index. Ketamine increased the sympathetic activity but neither dobutamine nor morphine modified the ANS. An increased sympathetic activity was identified on more occasions due to a decrease in the PTA index rather than an increase in HR or MAP. Results could suggest the utility of the PTA index for monitoring intraoperative nociception in a clinical context.

Further studies should be performed to determine how different drugs and administration doses might affect ANS balance in anaesthetised horses and how these could interfere with PTA index assessment in a clinical context.

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4.1. Tabla del estudio II

Table 1 Incision event: Data from before the incision (baseline) and 1, 3 and 5 minutes after the incision; Morphine event: Data from before the administration of morphine (baseline) and 10 minutes after the administration of morphine; Dobutamine event: Data from before a continue rate of infusion (CRI) of dobutamine was started or increased (baseline) and 5 minutes after the CRI of dobutamine was started or increased; Ketamine event: Data from before the administration of ketamine (baseline) and 3 and 5 minutes after the administration of ketamine; PTA event: Data from before a decrease in parasympathetic tone activity index ≥ 20% of the previous value was identified (baseline) and 1, 3 and 5 minutes after that. Data representing HR at the incision event is reported as mean ± standard deviation. The rest of the data are reported for heart rate (HR), mean arterial pressure (MAP) and mean parasympathetic tone activity (PTA) as medians (25-75th percentile). *Statistically different from baseline ($p < 0.05$)

Variable	Baseline	1 minute	3 minutes	5 minutes	10 minutes
Incision event					
HR (beat minute^{-1})	37±4	37±5	38±6	38±6	--
PTA	82 (71-90)	79 (63-89)	81 (68-93)	86 (76-94)	--
MAP (mmHg)	76 (69-86)	71 (68-79)	70(67-78)	71(64-79)	--
Morphine event					
HR (beat minute^{-1})	37 (36-39)	--	--	--	37 (35-39)
PTA	83 (71-92)	--	--	--	85 (70-94)
MAP (mmHg)	73 (66-80)	--	--	--	67 (60-77)*
Dobutamine event					
HR (beat minute^{-1})	38 (35-39)	--	--	38 (34-41)	--
PTA	73 (59-91)	--	--	67 (61-78)	--
MAP (mmHg)	55 (48-60)	--	--	65 (61-73)*	--
Ketamine event					
HR (beat minute^{-1})	41 (34-45)	--	38 (34-45)	39 (34-44)	--
PTA	71 (59-86)	--	56 (44-65)*	65 (60-76)	--
MAP (mmHg)	78 (67-90)	--	73 (67-85)	69 (67-85)*	--
PTA event					
HR (beat minute^{-1})	37 (34-43)	39 (34-45)	41 (36-45)	42 (36-44)	--
PTA	86 (80-92)	61 (59-70)*	68 (54-79)	69 (53-81)	--
MAP (mmHg)	86 (76-89)	83 (70-86)	81 (69-86)	83 (67-85)	--

5. Conclusiones

As we can see monitoring intraoperative nociception is a very complex task. The hypothesis was rejected since most of the changes on the PTA index or ΔPTA did not precede or coincide with changes on heart rate and blood pressure after a nociceptive stimulus in dogs or after a nociceptive stimulus or drug administration in horses.

The next conclusions were obtained:

1. The PTA index was not effective to assess intraoperative nociception in relation to changes on haemodynamic variables, HR and direct MAP in anaesthetised dogs.
2. The ΔPTA did not predict the intraoperative haemodynamic response during nociceptive stimuli in anaesthetised dogs.
3. No changes in autonomous nervous system activity were observed after a surgical nociceptive stimulus using the PTA index. Ketamine increased the sympathetic activity observed as a decrease in PTA index, but neither dobutamine nor morphine modified the ANS in horses under general anaesthesia.
4. An increased sympathetic activity was identified on more occasions due to a decrease in the PTA index rather than an increase on HR or MAP.

Further research should be performed in veterinary medicine to assess the usefulness of PTA monitor in dogs of different breeds using different anaesthesia protocols. In addition, further studies should be performed to determine how different drugs and administration doses might affect ANS balance in anaesthetised horses and how these could interfere with PTA index assessment in a clinical context.

Capítulo III

TOLERANCIA E HIPERALGESIA INDUCIDA POR OPIOIDES

1. Introducción

1.1. Fisiopatología de la sensibilización central y periférica

El estímulo nociceptivo permanente asociado con heridas o enfermedades puede producir inflamación y daño nervioso que propicien cambios dinámicos en el sistema nervioso periférico (sensibilización periférica) y central (sensibilización central), lo que da lugar a fenómenos que se traducen en el desarrollo de dolor patológico. El proceso de sensibilización consiste en un aumento de la respuesta de las neuronas nociceptivas al estímulo nociceptivo o a estímulos por debajo del umbral normal de respuesta (Basbaum et al. 2009; Chu et al. 2008, Roeckel et al 2016).

1.1.1. Sensibilización periférica: daños en la conducción o en las propias fibras provocan cambios en las propiedades de los nervios periféricos. Durante la inflamación que acompaña a la lesión tisular, los nociceptores y células no neurales (plaquetas, queratinocitos, fibroblastos y células del sistema inmune) liberan factores endógenos que pueden activar los nociceptores (Bell 2018). Algunos de estos factores endógenos son: neurotransmisores, péptidos (sustancia P, bradiquinina, péptido relacionado con el gen de la calcitonina o CGRP), eicosanoides y sustancias relacionadas (prostaglandinas, tromboxanos, leucotrienos, endocanabinoides), neurotropinas, citoquinas, proteasas y protones. El factor de crecimiento neuronal (NGF) activa las fibras C propiciando hipersensibilidad mecánica y al calor. Además, la respuesta inflamatoria es potenciada por las interleuquinas (IL) 1β y 6, y el factor de necrosis tumoral (TNF-α) estimulando la producción de sustancias pronociceptivas. Este tipo de moléculas activan canales tipo TRPV1, TRPA1 y ASIC en los nociceptores, de manera que actúan como proinflamatorios o pronociceptivas (Basbaum et al. 2009). La liberación de todas estas sustancias que estimulan los nociceptores disminuye el umbral doloroso dando lugar a la sensibilización periférica (Bell 2018)

1.1.2. Sensibilización central: en su desarrollo y mantenimiento juega un papel muy importante el ADME, donde se producen los siguientes mecanismos:

- Potenciación a largo plazo de la potencia sináptica (LTP): un daño tisular severo hace que las fibras C generen una fuerte y constante señal nociceptiva, lo que conlleva cambios pro y postsinápticos en la sinapsis entre las fibras C y las neuronas del ADME. Esta señal nociceptiva duradera produce una mayor liberación de sustancias excitatorias como el glutamato y la sustancia P en el SNC. La suma de estos potenciales excitatorios origina la sensibilización central. Algunos de los mecanismos implicados en la LTP son la síntesis y liberación de neurotransmisores, así como, cambios de densidad, distribución y activación de los receptores (Sandkuhler & Gruber-Schoffnegger 2012). Muchos de estos cambios están relacionados con el desarrollo de tolerancia e hiperalgesia como veremos en el siguiente apartado.

- Plasticidad intrínseca neuronal: consiste en cambios de las propiedades eléctricas de la membrana de las neuronas y, por tanto, en su excitabilidad. Lo que se traduce en una alteración del patrón de descarga de los potenciales de acción. En ello están involucrados canales de sodio y potasio dependientes de voltaje (Sandkuhler 2009). Los potenciales plateau son mecanismos intrínsecos que amplifican la señal nociceptiva y su sobreexpresión se ha visto asociada a situaciones de sensibilización al dolor (Reali et al. 2011).

- Cambios en el control inhibitorio: las neuronas nociceptivas de la médula espinal están bajo controles inhibitorios permanentes. Estos controles inhibitorios tienen 4 funciones principales: atenuar la respuesta nociceptiva para que no se produzca hiperalgesia, silenciar las neuronas nociceptivas para mantener una respuesta ajustada al estímulo nociceptivo y evitar el dolor espontáneo, separar la información nociceptiva y no nociceptiva para evitar alodinia, limitar la extensión de la información nociceptiva a las áreas adecuadas para evitar el dolor radiado, en espejo o referido (Sandkuhler 2009). Entre los mecanismos que se encuentran implicados están los sistemas relacionados con los receptores GABA y los receptores de glicina (Sandkuhler 2009).

- Modulación descendente: se estimula la facilitación descendente y se reduce la inhibición descendente lo que contribuye a la hiperalgesia, alodinia y dolor crónico (Sandkuhler 2009).

Además de estos mecanismos, las células gliales participan en el inicio y mantenimiento de la sensibilización central. La microglía libera citoquinas (TNF-α, IL-β e IL-6) en presencia de lesión nerviosa aumentando la sensibilización central neuronal y la hiperalgesia. Más tarde, parece que se activan los astrocitos manteniendo esta sensibilización, actuando las células gliales del mismo modo en el tronco del encéfalo lo que estimula la facilitación descendente. (Basbaum et al. 2009; Sandkuhler 2009; Grace et al. 2018).

1.2. Tolerancia Aguda a Opioides (TAO) e Hiperalgesia Inducida por Opiodes (HIO)

Durante los procedimientos quirúrgicos se producen estímulos nociceptivos que pueden desencadenar una respuesta nociceptiva en el paciente. Los analgésicos más usados para el tratamiento del dolor de moderado a intenso son los opioides (Angst and Clark 2006; DuPen et al2007), aunque su uso no queda libre de efectos secundarios, entre los que se ha descrito el desarrollo de tolerancia e hiperalgesia (Corder et al. 2017; Lueptow et al. 2018; Stein et al. 2018). Los opioides de corta duración, como el fentanilo y el remifentanilo, son ampliamente usados durante el periodo intraoperatorio, contribuyendo al desarrollo de tolerancia e hiperalgesia en distintas especies como la especie humana (Guignard et al. 2000; Kim et al. 2014), la rata (Gómez de Segura et al. 2009; Benito et al. 2010; Abreu et al. 2015b) y los ratones (Ishida et al. 2012; Aguado et al. 2018).

Los mecanismos que desencadenan la hiperalgesia inducida por opiodes son similares a los de la tolerancia, produciéndose cambios a nivel central y periférico (Dupen et al. 2007; Chu et al. 2008; Lee et al. 2011). Se han observado cambios celulares asociados a tolerancia e hiperalgesia en las neuronas aferentes, la médula espinal, el cerebro y las vías moduladoras descendentes (DuPen et al. 2007; Coder et al. 2017; Lueptow et al. 2018). En estos cambios celulares entra en juego cambios en los propios receptores, que se han descrito en el ADME, la

médula ventromedial rostral y la sustancia gris periacueductal (Lueptow et al. 2018).

Los receptores opioides son de la familia de los receptores acoplados a proteína G (GPCRs). El opioide se une al receptor y la proteína G se activa, lo que provoca un descenso de la excitabilidad de la membrana. Ello conlleva una disminución de adenosina monofosfato cíclico (AMPc) que suprime los canales de sodio y calcio, mientras que los de potasio se activan, produciendo analgesia (DuPen et al 2007; Lueptow et al. 2018; Stein 2018). El uso reiterado de opioides produce una desensibilización del receptor debido a que proteínas reguladoras intracelulares (GPCRquinasas, β- arrestinas, adenilato ciclasa) activadas por los opioides producen el "desacople" de la proteína G al receptor (DuPen et al 2007; Lueptow et al. 2018). Otro mecanismo sería la inclusión del propio receptor en la membrana celular, ya que los receptores opioides son regulados por endocitosis cuando se desensibilizan. El problema se desarrolla cuando esta endocitosis supera a la reposición de los receptores en la membrana (DuPen 2007; Lueptow et al. 2018). Opioides con alta potencia intrínseca, como el fentanilo, parecen tener un mecanismo dependiente de la β- arrestina (Bobeck et al. 2016).

La distribución de los receptores de los opioides varía según las especies (Thomasy et al. 2007), lo que explica las diferencias encontradas entre especies, sumado a la variable afinidad de los opioides a los distintos receptores (kappa, mu y delta). La interacción entre un opioide y un subtipo de receptor puede dar lugar a fenómenos de tolerancia, que al emplear otro opioide, se verá como tolerancia cruzada, por una disminución de la efectividad del segundo opioide empleado (Lynch 2005). Además, existe una alta variabilidad en cuanto a la farmacocinética del opioide entre individuos (Lynch 2005) sumado a un polimorfismo genético, lo que explicaría los diferentes resultados encontrados en los estudios sobre el uso de opiodes dentro de una especie. El uso de remifentanilo en la especie humana presenta efectos muy variables (Kim et al. 2014).

Debido al uso de opioides se produce un aumento de citoquinas que actúan sobre las células de la microglía aumentando el óxido nitroso (NO), factor de necrosis tumoral alfa (TNF-α) e interleukinas (Merighi et al. 2013). Tanto las

células de la microglía como los astrocitos se consideran una fuente importante de citoquinas, otros tipos de células como las células endoteliales que liberan mediadores inflamatorios también contribuyen al desarrollo de tolerancia en el SNC (Grace et al. 2018). El receptor 4 tipo Toll (TLR4) regula estos mecanismos de tolerancia y se ha relacionado con el desarrollo de hiperalgesia en el ratón (Aguado et al. 2018).

En el equilibro entre el control inhibitorio descendente hacia sistemas pronociceptivos, están implicados los receptores de serotonina 5-HT3 y 5-HT2 (Bannister et al. 2009).

A nivel periférico, se produce una activación de receptores periféricos como el TRPV1, lo que contribuye a los fenómenos de hiperalgesia y tolerancia inducida por opioides (Vardanyan et al. 2009). Se ha comprobado que la tolerancia e hiperalgesia puede iniciarse en los receptores mu que se encuentran en la periferia (Corder et al. 2017). Como consecuencia del desarrollo de alguno de estos mecanismos, se ha descrito en seres humanos un mayor consumo de opioides postoperatorios tras el tratamiento intraoperatorio con remifentanilo (Guignard et al. 2000). Tras una revisión sobre el desarrollo de tolerancia e hiperalgesia al remifentanilo en el ser humano, no se pudieron evidenciar indicios suficientes para aconsejar su desuso (Kim et al. 2014).

En ratas se ha demostrado una disminución en la reducción de la concentración alveolar mínima (CAM) de gases inhalatorios por el uso de infusiones continuas de remifentanilo (Gómez de Segura et al. 2009; Abreu et al. 2015b), lo que se traduce como tolerancia aguda a este opioide. Además, tras el tratamiento con infusión continua de remifentanilo en ratas se ha descrito una disminución del umbral nociceptivo, lo que equivale al desarrollo de hiperalgesia (Abreu et al. 2015b). Se ha demostrado el desarrollo de hiperalgesia asociado a la presencia del receptor Tlr4 en el ratón (Aguado et al. 2018). Aunque en un estudio previo en ratones tratados con infusión continua de remifentanilo no se hallaron indicios de tolerancia ni de hiperalgesia (Ishii et al. 2014)

El desarrollo de tolerancia e hiperalgesia a opiodes en el perro ha sido poco descrito debido a la ausencia de estudios al respecto. Hall et al. (1987) observó el desarrollo de cierta tolerancia tras el uso de sufentanilo durante la

anestesia con enflurano como un aumento en la CAM de enflurano. Mientras que, estudios más recientes centrados en la determinación de la reducción de la CAM por el uso de remifentanilo o fentanilo, no observaron una disminución en el tiempo en la reducción de la CAM de isoflurano y sevoflurano respectivamente (Monteiro et al. 2010; Reilly et al. 2013). El fenómeno de hiperalgesia debido al uso intraoperatorio de opioides no ha sido demostrado en el perro.

2. Hipótesis y objetivos

Remifentanil plays an important role as intraoperative analgesic drug in dogs. In addition, tolerance and hyperalgesia have been developed by the use of remifentanil in rats and humans. No evidence has been shown about development of tolerance or hyperalgesia to remifentanil in dogs. Therefore, it is necessary to study if acute tolerance or hyperalgesia by the use of remifentanil can be demonstrated in a canine model.

The next objectives were defined:

5. To determine if the effect of remifentanil constant rate infusion (CRI) on the minimum alveolar concentration (MAC) of sevoflurane decreases over a short period of time (Acute opioid tolerance, AOT).
6. To determine if remifentanil CRI reduces the nociceptive threshold or increases the MAC of sevoflurane, days after remifentanil administration (Opioid induces hyperalgesia, OIH).

3. Estudio III

Determination of acute tolerance and hyperalgesia to remifentanil constant rate infusion in dogs undergoing sevoflurane anaesthesia

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Research Paper

Determination of acute tolerance and hyperalgesia to remifentanil constant rate infusion in dogs undergoing sevoflurane anaesthesia

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Abstract

Determination of acute tolerance and hyperalgesia to remifentanil constant rate infusion in dogs undergoing sevoflurane anaesthesi

Objective To determine if acute opioid tolerance (AOT) or opioid-induced hyperalgesia (OIH) could develop and limit the remifentanil-induced reduction in the sevoflurane minimum alveolar concentration (MAC). The response to mechanical nociceptive threshold (MNT) was evaluated and related to OIH.

Study design A crossover, randomized, experimental animal study.

Animals A total of nine beagle dogs.

Methods The dogs were anaesthetized with sevoflurane in 50% oxygen. Baseline sevoflurane MAC was measured (MACb1). Remifentanil (0.3 μg kg^{-1} $minute^{-1}$) or 0.9% saline constant rate infusion (CRI) was administered intravenously (IV). Sevoflurane MAC was determined 20 minutes after CRI was initiated (MACpostdrug1), 30 minutes after MACpostdrug1 determination (MACpostdrug2) and after 1 week (MACb2). The MNT was determined at baseline (before anaesthesia), 3 and 7 days after anaesthesia. An increase of MACpostdrug2 greater or equal to 0.25% compared to MACpostdrug1 was considered evidence of AOT. A decrease in MNT at 3 and 7 days or an increase in MACb2 or both with respect to MACb1 were considered evidence of OIH.

Results Remifentanil CRI reduced sevoflurane MACpostdrug1 by 43.7% with respect to MACb1. MACpostdrug2 was no different with respect to MACpostdrug1 in the saline ($p = 0.62$) or remifentanil ($p = 0.78$) treatments. No significant differences were observed in the saline ($p = 0.99$) or remifentanil ($p = 0.99$) treatments between MACb1 and MACb2, or for MNT values between baseline, 3 and 7 days.

Conclusion and clinical relevance In dogs, in the study conditions, remifentanil efficacy in reducing sevoflurane MAC did not diminish in the short term, suggesting remifentanil did not induce AOT. Hyperalgesia was not detected 3 or 7 days after the administration of remifentanil. Contrary to data from humans and rodents, development of AOT or OIH in dogs is not supported by the findings of this study.

Keywords dog, hyperalgesia, opioid, remifentanil, tolerance.

Introduction

Perioperative use of remifentanil as an analgesic is controversial because its administration is associated with both acute opioid tolerance (AOT) and opioid-induced hyperalgesia (OIH) in small mammals such as rats (Gómez de Segura et al. 2009; Aguado et al. 2011) and mice (Aguado et al. 2018). The mechanisms underlying AOT and OIH have previously been described in humans (Lee et al. 2011; Yu et al. 2016). However, despite the results published, there is insufficient evidence to reduce the use of remifentanil (Kim et al. 2014) and new studies are still necessary to explain this phenomenon.

Studies using canine models of AOT and OIH are limited. Acute tolerance is described as an increase in the drug dose required to produce a given pharmacological effect over the course of several hours to days (Rang et al. 2001). Acute tolerance to sufentanil was observed in enflurane anaesthetized dogs (Hall et al. 1987); nevertheless, no evidence of AOT or OIH was reported after the administration of remifentanil or fentanyl constant rate infusion (CRI) during the use of isoflurane (Monteiro et al. 2010) or sevoflurane (Reilly et al. 2013) anaesthesia respectively.

OIH is described as an increased sensitivity to nociceptive stimuli (Angst & Clark 2006). Enhanced postoperative sensitivity to painful pressure stimulation after an intraoperative high dose of remifentanil in human patients has been described (Schmidt et al. 2007). To the authors' knowledge, only one study has evaluated this effect in dogs. Hyperalgesia was found after the administration of escalating doses of remifentanil (Freye & Levy 2010), but it was only evaluated 15 minutes after each dose using somatosensory-evoked potentials. Measurement of the mechanical nociceptive threshold (MNT) is considered an objective method for grading nociceptive thresholds in dogs (Conzemius et al. 1997) and it has been used previously to investigate the development of hyperalgesia (Lascelles et al. 1998).

Many factors may influence AOT and OIH determination, such as the opioid chosen or the volatile anaesthetic agent used (Aranake et al. 2013). The duration of remifentanil CRI administration (Ishida et al. 2012), the dose administered (Cabañero et al. 2009) and even the withdrawal of remifentanil

(Comelon et al. 2016) may influence the results. The tests applied to evaluate AOT and OIH can also play a role (Comelon et al. 2016). Previously, a decrease in the MAC reduction (Hall et al. 1987; Monteiro et al. 2010; Reilly et al. 2013), but not MNT has been used to evaluate AOT and OIH in dogs.

Tolerance to remifentanil was observed at 90 minutes after its continuous administration to conscious rabbits (Hayashida et al. 2003) and rats (Gómez de Segura et al. 2009). But no studies have determined if acute remifentanil-induced tolerance occurs over the relatively short term intraoperative period in dogs, or if OIH is present days after remifentanil administration.

To evaluate the possible development of AOT and OIH due to the administration of remifentanil CRI, we hypothesized that the effect of remifentanil CRI on the MAC of sevoflurane would decrease over a short period of time (approximately 90 minutes) (AOT). We also hypothesized that remifentanil CRI may reduce the nociceptive threshold, or increase the MAC of sevoflurane, days after remifentanil administration (OIH).

Methods

Ethical approval for this study (Ethical Committee No. 21/06/2016/110) was provided by the Junta de Andalucía, Spain. The study was performed according to the ARRIVE (Animals in Research: Reporting In Vivo Experiments) statement.

Animals

A total of nine healthy adult beagle dogs (four males and five females) weighing 13 ± 1.5 kg (mean ± standard deviation) were used in this prospective, blind, cross-over, randomized, experimental study. The dogs were sourced from different litters provided by the Experimental Animal Service, University of Córdoba. They had not participated in previous experimental studies. Dogs were assessed to be healthy based on physical examination findings, and blood and serum biochemistry analyses including haemogram, total protein, albumin, creatinine, urea, alanine aminotransferase, aspartate aminostransferase, gamma-glutamyl transpeptidase, total bilirubin, total cholesterol, triglycerides,

amylase and phosphorus. Orthopaedic examination including radiographs were performed to rule out any orthopaedic abnormality. Exclusion criteria were an existing orthopaedic condition or clinical signs of pain (based on the Short Form of the Glasgow Composite Pain Scale), since acute or chronic pain may have affected the results.

Experimental design and drug treatments

Equipment maintenance calibration was performed prior to the study. Food, but not water, was withheld for 12 hours prior to each experiment. The dogs were anaesthetized on two occasions in a prospective crossover design and were randomly assigned, by one researcher tossing a coin, to assign each treatment, remifentanil (R treatment) or saline 0.9% (S treatment) and the other treatment after a month washout period. All the procedures were performed at the Experimental Unit, Veterinary Teaching Hospital, University of Córdoba, and were started at 8 am.

Anaesthesia was induced with sevoflurane (SevoFlo, Laboratories Dr. Esteve, Spain) in 100% oxygen (O_2), delivered via a face mask and an adult-size circle breathing system (Adult patient circuit, Datex Ohmeda Division Instrumentarium AB, Sweden). The vaporiser was adjusted to deliver 5% sevoflurane at an oxygen flow rate of 4 L minute^{-1} until orotracheal intubation could be accomplished. Orotracheal intubation was performed once the animal lost palpebral and swallowing reflexes. Following placement of a cuffed orotracheal tube, O_2 flow rates were reduced to 0.5 L minute^{-1} mixed with air flow rates of 0.5 L minute^{-1}. Dogs were positioned in lateral recumbency and were mechanically ventilated to maintain an end-tidal carbon dioxide ($PE´CO_2$) between 35 and 45 mmHg (4.7–6 kPa). During instrumentation, sevoflurane delivered by the vaporiser was adjusted to maintain an end-tidal sevoflurane concentration (FE´Sevo) of 2.4% (Kazama & Ikeda 1988). A 20 -gauge cannula (VasoVet, B Braun. B Braun Vet Care GmbH, Germany) was placed into a cephalic vein and the dorsal pedal artery, 30 minutes after the application of lidocaine 2.5%-prilocaine 2.5% cream (EMLA, Laboratorio Astra SA, Spain) to reduce nociceptive stimuli. A peristaltic pump (NIKI V4 Volumetric Infusion

Pump. EVEREST Veterinary Technology SL, Spain) was used to administer 0.9% saline (FisioVet, B Braun. B Braun Vet Care GmbH, Germany) or the remifentanil (Ultiva, GlaxoSmithKline SA, Spain) infusion at a rate of 3mL kg^{-1} $hour^{-1}$. The arterial cannula was connected to a pressure system filled (Combitrans arterial monitoring kit, B Braun. B Braun Vet Care GmbH, Germany) with a heparinised 0.9% saline solution (1 UI mL^{-1}) to display systolic, diastolic and mean arterial pressures (SAP, DAP, MAP). The zero-reference level of the pressure transducer was set at the manubrium of the sternum. Adhesive surface electrodes were attached to the skin according to a lead II ECG to monitor heart rate (HR) using a multiparameter monitor (Datex Ohmeda Multiparameter Monitor, GE Healthcare, Finland). This monitor also measured haemoglobin oxygen saturation using pulse oximetry (SpO_2) and $PE´CO_2$. Temperature was taken by means of a probe with the tip positioned at the thoracic portion of the oesophagus. Body (oesophageal) temperature (T) was maintained between 37 and 38 º Celsius (ºC) using a forced warm air blanket (Equator, Smiths Medical ASD, Inc. MA, USA). Values of HR (beats $minute^{-1}$), SAP (mmHg), DAP (mmHg), MAP (mmHg), *f*R (breaths $minute^{-1}$), $PE´CO_2$ (mmHg) and T (ºC) were noted every 10 minutes during anaesthesia. Blood samples were collected from the arterial cannula into heparinised syringes at the time of each MAC determination, and were immediately analysed using an automated blood gas system to display pH, PaO_2 (kPa and mmHg) and $PaCO_2$ (kPa and mmHg) values (Gasometer Ciba-Corning, Model 850 Chiron Diagnostic, Spain). Blood gases were corrected based on the oesophageal temperature and O_2 inspired fraction. Cardiovascular and acid-base variables were assessed to confirm that MAC determinations were performed under the same conditions.

MAC determination

Following the induction of anaesthesia and instrumentation of the animals, a baseline MAC (MACb1) was determined. The MACb1 was used as their own control for both experiments. MACb1 was maintained for a 15-minute equilibration period and remifentanil 0.3 µg kg^{-1} $minute^{-1}$ without a loading dose (R treatment) or 0.9% saline (S treatment) at the same volume (3mL kg^{-1} hour-

^{1}) were administered IV as a CRI. When the CRI had been administered for 20 minutes, the MAC was determined again (MACpostdrug1R and MACpostdrug1S). In the R treatment, the end-tidal sevoflurane (FE´SEVO) was reduced by 50% to reduce the time of MAC determination. We used the 59% reduction of isoflurane MAC described by Monteiro et al. (2010) as our reference point during the administration of 0.3 μg kg^{-1} minute^{-1} remifentanil CRI. The MAC was determined again 30 minutes after MACpostdrug1 determination was finished (MACpostdrug2R and MACpostdrug2S). Finally, the infusion was stopped, and the dogs were allowed to recover from anaesthesia. Following a 1-week washout period, a new baseline MAC for each treatment (MACb2R and MACb2S) was determined under the same conditions of MACb1. The times for MAC determinations were recorded. A schematic view of the experimental design for MAC determination is shown in Figure 1.

In order to determine the MAC, the FE´SEVO was maintained constant in the 15 minutes before a supramaximal noxious stimulus (50 V, 50 Hz for 10 ms) was delivered using previously calibrated equipment. The noxious stimulus was applied (Grass S-48 Stimulator. Grass Medical Instruments, MA, USA) via two 25-gauge needle electrodes, placed subcutaneously 5 cm apart on the lateral aspect of the ulna. Initially, two single stimuli were delivered, followed by two continuous stimuli of 5 second duration, with a second interval between stimuli (Valverde et al. 2003). The MAC was defined as the minimum FE´SEVO concentration that abolished all motor movements in response to noxious stimulation. Withdrawal or twitching of the non-stimulated limbs or movement of the head or both were considered a positive response. However, chewing, licking, blinking, swallowing or twitching of the stimulated limb was not considered a positive response. If a positive response was elicited, the FE´SEVO was increased by 0.2%. Conversely, if the response was negative, the FE´SEVO was decreased by 0.2% and the noxious stimulus was reapplied following a 10-minute equilibration period. The MAC was taken as the concentration midway between the highest concentration that permitted movement in response to the stimulus, and the lowest concentration preventing such movement. The observer of the electrical stimuli response was blind to the treatment given.

MNT determination

The MNT was performed using a dynamometer (PCE-FM50, PCE Deutschland GmbH, Germany) as previously described (Gutiérrez-Bautista et al. 2018) to evaluate hyperalgesia. It consisted of a portable cell with a pen-like metal probe with a plane surface and a screen that showed the applied force in one point. The force applied to the end of the probe was transmitted to the load cell and a numerical value was obtained in Newtons (N). The mechanical stimulus was applied between the olecranon and the lateral epicondyle of the humerus at a constant rate of 1.6 N seconds^{-1} until the animal showed a response. Any sudden movement from the animal, such as avoidance of the device, shaking the head, withdrawing the limb, vocalisation or attempting to bite was considered a positive response and the end point of reading. Dogs were standing while the contralateral limb was held up off the ground to avoid the animal tilting and thereby changing the intensity of the stimulus. To obtain an average value three positive measures were taken.

Thresholds were determined at baseline before anaesthesia (MNTb), and at 3 and 7 days after anaesthesia (MNT3 and MNT7) in each treatment (R and S). A reduction of MNT3 or MNT7 values with respect to the MNTb value was interpreted as a nociceptive threshold reduction due to remifentanil-induced hyperalgesia. The observer to the MNT response was blind to the treatment given.

Statistical analysis

A sample size of eight animals per treatment was determined (G*Power, v.3.1.9.2. Germany) assuming two treatments with four comparisons each, to achieve a power of 0.95, with significance at 0.05 for a mean MAC difference of 0.25% with a standard deviation of 0.35% and the effect size 0.7. The sample size was increased to 9 in anticipation of possible dropouts.

Statistical analysis was performed using standard statistical software (IBM SPSS Statistics vs 25.0 for Windows, SPSS Inc. IL, USA). Data were

assessed for normality using the Shapiro Wilks test before applying statistical parametric tests.

To examine the effect of remifentanil on MAC and the response to the MNT into and between treatments over time, a two-way repeated measures analysis of variance (ANOVA) was conducted. Sphericity was evaluated using Mauchly's test, and if it was not assumed, the Greenhouse Geisser correction was performed. If statistical differences were found, a Bonferroni test was applied.

A one-way ANOVA followed by Bonferroni or T2 Tamhane as *post hoc* tests were used to evaluate changes in cardiorespiratory (HR, *f*R, arterial pressures and T) and acid-base variables (pH, $PaCO_2$ and PaO_2) between the different MAC periods in each treatment. The pair comparison MACb1 and MACb2 and between MACpostdrug1 and MACpostdrug2, were analysed in each treatment.

A p value ≤ 0.05 was set to indicate statistical significance. Data with a normal distribution are presented as mean ± standard deviation, and non-parametric variables are presented as median (25-75th percentile).

Results

A total of 4 males and 5 females (n = 9) adult beagle dogs completed the study. They were 5 years old and weighed 14.01 ± 1.3 (R treatment) and 13.91 ± 0.9 (S treatment). The time needed for the different MAC determinations are shown in Table 1. The median time of remifentanil infusion administration was 187 (166.5–190) minutes.

MAC

MAC values are expressed in Table 2 and represented in Figure 2. The overall mean value for MACb1 for both treatments was 2.8 ± 0.4%. After administration of a remifentanil CRI of 0.3 μg kg^{-1} minute^{-1}, the sevoflurane MAC was reduced by 43.7% No significant differences were found between MACpostdrug1 and

MACpostdrug2 within each treatment, the R treatment (p = 0.78) or the S treatment (p = 0.62). Likewise, there were no significant differences between MACb1 and MACb2 within each treatment, the R treatment (p = 0.99) or the S treatment (p = 0.99). There was a significant difference between MACpostdrug2 and MACb1 within the S treatment (p = 0.031). Mild inflammation of the stimulated area was evident 24–48 hours after the experiment.

MNT data

No significant differences were observed between MNTb and MNT3 in the R treatment (p = 0.99) or the S treatment (p = 0.99), MNTb and MNT7 in the R treatment (p = 0.99) or the S treatment (p = 0.37) or MNT3 and MNT7 in the R treatment (p = 0.99) or the S treatment (p = 0.25) (Table 3).

Cardiovascular and acid-base variables

Cardiovascular and acid-base variables were compared between MACb1 and MACb2 and between MACpostdrug1 and MACpostdrug2 within the R and S treatments to confirm that MAC determination was performed under the same conditions. No significant differences were found for HR, SAP, DAP, MAP, *f*R, PE´CO_2, SpO_2, pH, $PaCO_2$, PaO_2 or T.

Discussion

The present study does not provide evidence of acute tolerance development or hyperalgesia induced by the administration of remifentanil in the experimental conditions investigated. The reduction in sevoflurane MAC seen with remifentanil CRI did not change significantly throughout administration. Sevoflurane MAC did not change significantly from baseline when it was determined 1 week after remifentanil administration. Likewise, the mechanical nociceptive threshold did not change 3 or 7 days after remifentanil administration.

AOT and OIH have been described in animal models and humans after the administration of remifentanil (Cabañero et al. 2009; Gómez de Segura et al. 2009; Aguado et al. 2011; Kim et al. 2014). However, studies in mice (Ishii et al. 2013) and humans (Gustorff et al. 2002; Angst et al. 2009; Treskatsch et al. 2014) did not identify AOT or OIH after remifentanil administration, suggesting that there is insufficient information to justify the reduced use of remifentanil (Kim et al. 2014).

Few studies have evaluated the effect of AOT or OIH in dogs. Hall et al. (1987) described a significant decline in the degree of MAC reduction in dogs, while steady, low sufentanil plasma levels were maintained for several hours during enflurane anaesthesia. More recently, Freye and Levy (2010) demonstrated that remifentanil and sufentanil induced a small degree of hyperalgesia in conscious dogs. No other studies in dogs have found AOT or OIH based on changes in MAC over time. Tolerance to the effects of fentanyl was described in dogs but based on the depressant effects of fentanyl on somatocardiovascular reflexes (Askitopoulou et al. 1985). Monteiro et al. (2010) reported that the remifentanil-induced isoflurane MAC sparing effect remained constant throughout a 6-hour remifentanil infusion. In the same way, MAC reduction of sevoflurane was not diminished by fentanyl CRI (Reilly et al. 2013) or remifentanil CRI (Murahata et al. 2018) administration in dogs. The results of our study concur with these earlier results.

Monteiro et al. (2010) did not investigate OIH, rather they focused on the isoflurane MAC-sparing effect of remifentanil using different infusion rates. Their results suggest that tolerance did not occur, since isoflurane MAC did not change throughout the 6 hours of infusion. Interaction between inhalational and injectable anaesthetic drugs was reported by Reilly et al. (2013). Therefore, the object of the present study was to assess AOT or OIH development as result of the interaction of remifentanil CRI during sevoflurane anaesthesia.

The influence of dose and duration of treatment on AOT and OIH is controversial. Some observations provide support for the hypothesis that OIH is caused by chronic opioid exposure (Lee et al. 2011). Data about AOT onset in dogs is lacking. Previous studies in humans have demonstrated AOT after 60-90 minutes of a remifentanil infusion (Vinik & Kissin 1998) and in rabbits

AOT was seen after 90 minutes of a remifentanil infusion (Hayashida et al. 2003). Therefore, it was assumed that the administration time in this study of 187 (166.5–190) minutes was sufficient to detect AOT. Kim et al. (2014) suggested that the immediate postoperative period might not be optimal to detect OIH, since OIH and AOT might be confused. Abreu et al. (2015b) assessed OIH 1 day after remifentanil administration to rats, which persisted for 21 days. Therefore, in our study, dogs were tested 3 and 7 days after remifentanil administration using a mechanical nociceptive stimulus and the sevoflurane MAC was determined 1 week later.

Cohen et al. (2008) demonstrated that both dose and duration of treatment are directly correlated with OIH, but Cabañero et al. (2009) showed that pronociceptive effects of remifentanil in mice are determined by the dose rather than by the duration of infusion. In humans, an intraoperative remifentanil CRI above 0.25 $\mu g\ kg^{-1}\ minute^{-1}$ is associated with tolerance, since it was associated with higher postoperative opioid consumption (Guignard et al. 2000). In addition, doses greater than 0.1 $\mu g\ kg^{-1}\ minute^{-1}$ were be expected to produce hyperalgesia in humans (Kim et al. 2014). Therefore, it could be anticipated that an infusion rate of 0.3 $\mu g\ kg^{-1}\ minute^{-1}$ would be sufficient to cause hyperalgesia or tolerance in dogs, since a ceiling effect was apparent at higher infusion rates (Monteiro et al. 2010). Comelon et al. (2016) showed that a gradual withdrawal of remifentanil infusion may prevent OIH after 30 minutes of CRI administration, so remifentanil infusion was abruptly stopped to facilitate AOT and OIH determination in our study. In view of the results of this research, more studies investigating remifentanil AOT or OIH development under different conditions should be performed in dogs.

MAC is an indirect, although clinically valuable method to determine the analgesic potency of opioids in the intraoperative period (Gómez de Segura et al. 2009). Multiple factors may influence MAC, including circadian rhythm, age, the methodology for MAC assessment, hypercapnia, hypothermia, hypoxaemia, acidaemia or alkalaemia (Aranake et al. 2013). In the present study, potentially confounding factors were controlled to minimise differences between MAC determination periods: the experiments were started at 8 am, the

study population was homogeneous, the method used for MAC determination (Valverde et al. 2003) was validated, and the anaesthesia protocol and monitoring were standardised. Temperature, cardiopulmonary and acid-base variables were statistically similar between the MAC periods compared within each treatment. The use of sevoflurane might be considered controversial, since it has been suggested that it produces an inhibitory effect at N-methyl-D-aspartate (NMDA) receptors, antagonising remifentanil stimulation at these receptors, thereby avoiding AOT development (Fodale et al. 2006). Nevertheless, a minimal inhibitory effect on NMDA receptors by sevoflurane has been described (Solt et al. 2006) and AOT and OIH under sevoflurane anaesthesia have been previously described (Gómez de Segura et al. 2009; Abreu et al. 2015a). In addition, the common clinical use of sevoflurane in veterinary anaesthesia justifies the selection of this inhaled anaesthetic for the present study.

In humans electrical stimulation has been reported to cause either no hyperalgesia or less pronounced hyperalgesia (Angst & Clark 2006). However it has been found to act as a supramaximal nociceptive stimulus in dogs and rabbits, with no difference when compared to clamping techniques (Valverde et al. 2003). The MACpostdrug2 value was statistically higher than the MACb1 in the saline treatment probably owing to the repeated application of the nociceptive stimulus without analgesic administration. This suggests that the repeated application of the stimulus over time could influence the MAC value determination in the 0.9% saline treatment. No references to tissue damage due to electrical stimulation had been described previously, but inflammation of the stimulated area was evident 24–48 hours after the experiment, as described by Petrenko et al. (2012).

A limitation of this study is that the plasma concentration of remifentanil was not determined. Remifentanil is rapidly eliminated in dogs and its accumulation in plasma as result of prolonged CRI is unlikely to occur in humans and dogs (Chism & Rickert 1996; Hoke et al. 1997).Chism & Rickert (1996) reported a remifentanil half-life of 3–5 minutes in dogs and Hoke et al. (1997) reported a remifentanil terminal half-life of 6 minutes with a brain-blood equilibration half-life between 2.3 and 5.2 minutes in dogs. Based on these

findings, a steady-state remifentanil plasma concentration should be reached within 20 minutes of commencing a remifentanil infusion. We started MACpostdrug1 determination 20 minutes after remifentanil CRI started in order to allow enough time to achieve a steady-state plasma concentration before determination.

Conclusion

In conclusion, the administration of a remifentanil CRI of 0.3 μg kg^{-1} $minute^{-1}$ did not decrease the sparing effect of sevoflurane MAC in dogs, indicating that acute tolerance to remifentanil is not observed under these conditions in dogs. No changes were found in MNT 3 or 7 days after remifentanil administration or changes in the sevoflurane MAC a week after infusion, indicating that hyperalgesia was not shown under these conditions in dogs. Development of AOT or OIH following remifentanil administration in dogs is not supported by the findings of this study.

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3.1. Tablas y figuras del estudio III

Table 1 Time for Minimum Alveolar Concentration (MAC) determinations

MACb1: baseline MAC; MACpostdrug1S: MAC determined 20 minutes after 0.9% saline constant rate infusion (CRI) started; MACpostdrug1R: MAC determined 20 minutes after remifentanil CRI started; MACpostdrug2S: MAC determined 30 minutes after MACpostdrug1S determination; MACpostdrug2R: MAC determined 30 minutes after MACpostdrug1R determination; MACb2S: baseline MAC determined one week after 0.9% saline CRI administration; MACb2R: baseline MAC determined one week after remifentanil CRI administration. Data are expressed as median (25-75th percentile). No statistical difference was found between treatments.

MAC	Time (minutes)
MACb1	84 (73.5-93.5)
MACpostdrug1S	57 (46.5-63.5)
MACpostdrug1R	67 (49-75.5)
MACpostdrug2S	61 (53-80)
MACpostdrug2R	54 (50-79.5)
MACb2S	94 (70.5-98)
MACb2R	80 (62.5-89)

Table 2 Minimum Alveolar Concentration (MAC) values determined in remifentanil (R) and 0.9% saline (S) treatments

S treatment: 0.9% saline treatment; R treatment: remifentanil treatment; MACb1: baseline MAC; MACpostdrug1: MAC determined 20 minutes after constant rate infusion (remifentanil or 0.9% saline) started; MACpostdrug2: MAC determined 30 minutes after MACpostdrug1 was determined (remifentanil or 0.9% saline); MACb2: MAC determined 1 week after the constant rate infusion (remifentanil or 0.9% saline). *Significant difference with respect to MACb1 in R treatment: MACpostdrug1 (1.23%: 95% CI 0.819 to 1.65%; p = 0.001) and MACpostdrug2 (1.07%: 95% CI 0.56 to 1.57%; p = 0.001). † Significant difference with respect to MACb1 in S treatment: MACpostdrug2 (0.26%: 95% CI 0.023 to 0.49%; p = 0.031). Data are expressed as mean ± standard deviation.

Treatment	R treatment	S treatment
MACb1 (%)	2.8 ± 0.4	2.8 ± 0.4
MACpostdrug1 (%)	1.6 ± 0.2*	2.9 ± 0.3
MACpostdrug2 (%)	1.7 ± 0.4*	3 ± 0.3 †
MACb2 (%)	2.9 ± 0.4	2.9 ±0.4

Table 3 Mechanical nociceptive threshold (MNT) values determined in remifentanil (R) and 0.9% saline (S) treatments

S treatment: 0.9% saline treatment; R treatment: remifentanil treatment; MNTb: MNT at baseline before the first anaesthesia; MNT3: MNT 3 days after remifentanil or 0.9% saline constant rate infusion (CRI) administration; MNT7: MNT 7 days after remifentanil or 0.9% saline CRI administration. Data are expressed as mean ± standard deviation. No statistical difference was found between treatments.

Treatment	R treatment	S treatment
MNTb (N)	25 ± 8	23 ± 4
MNT3 (N)	24 ± 5	23 ± 4
MNT7 (N)	23 ± 4	26 ± 6

Figure 1 Minimum Alveolar Concentration (MAC) determination flow chart. Schematic view of the experimental design for MAC determination

MACb1: baseline MAC; MACpostdrug1: MAC determined 20 minutes after constant rate infusion (remifentanil or 0.9% saline) started; MACpostdrug2: MAC determined 30 minutes after MACpostdrug1 was determined (remifentanil or 0.9% saline); MACb2: MAC determined one week after the constant rate infusion (remifentanil or 0.9% saline)

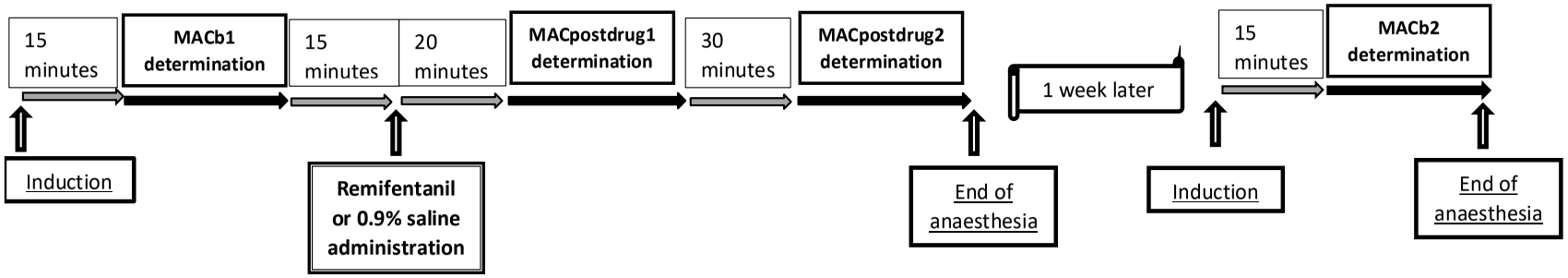
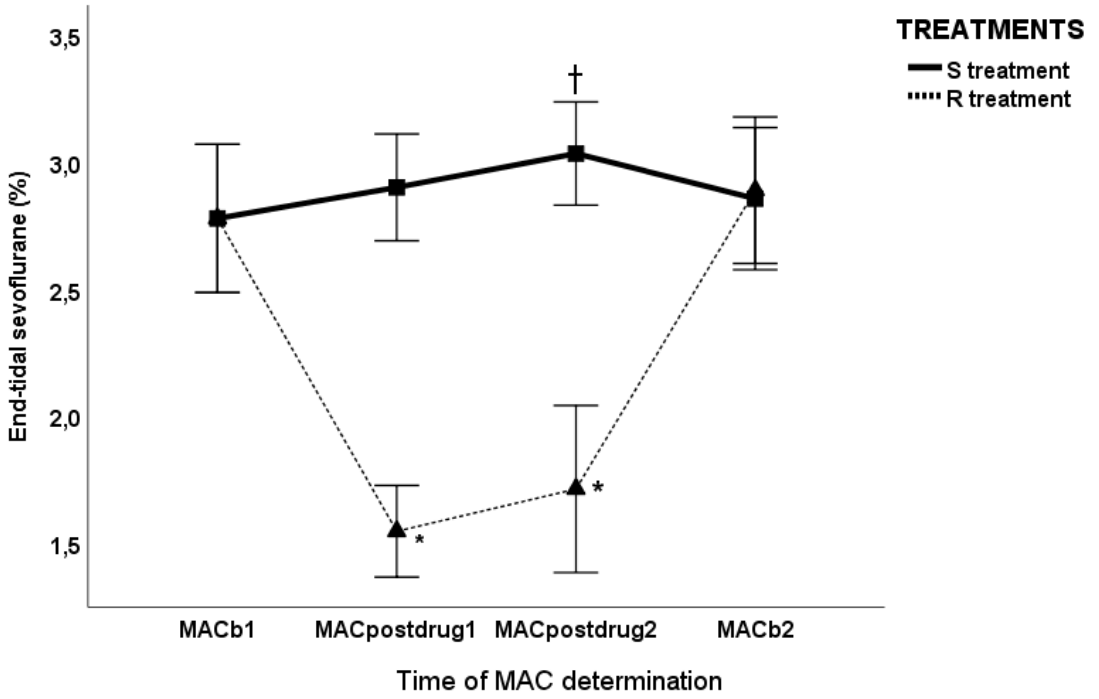

15 minutes
MACb1 determination
15 minutes
20 minutes
MACpostdrug1 determination
30 minutes
MACpostdrug2 determination
1 week later
15 minutes
MACb2 determination
Induction
Remifentanil or 0.9% saline administration
End of anaesthesia
Induction
End of anaesthesia

Figure 2 Graphical representation of Minimum Alveolar Concentration (MAC) values in remifentanil (R) and 0.9% saline (S) treatments

S treatment: 0.9% saline treatment; R treatment: remifentanil treatment. The x ASIS MACb1: baseline MAC; MACpostdrug1: MAC determined 20 minutes after constant rate infusion (remifentanil or 0.9% saline) started; MACpostdrug2: MAC determined 30 minutes after MACpostdrug1 was determined (remifentanil or 0.9% saline); MACb2: MAC determined 1 week after the constant rate infusion (remifentanil or 0.9% saline). *Significant difference from MACb1 in R treatment: MACpostdrug1 (1.23%: 95% CI 0.819 to 1.65%; *p*= 0.001) and MACpostdrug2 (1.07%: 95% CI 0.56 to 1.57%; *p* = 0.001). † Significant difference from MACb1 in S treatment: MACpostdrug2 (0.26%: 95% CI 0.023 to 0.49%; *p* = 0.031). Data are expressed as mean ± standard deviation.



4. Conclusiones

The hypothesis about remifentanil CRI causing a decrease of the sparing effect of sevoflurane MAC as consequence of acute tolerance or a decrease in the mechanical threshold or increase in the sevoflurane MAC one week after its administration as consequence of hyperalgesia development was rejected.

The next conclusions were obtained:

1. The administration of a remifentanil CRI of 0.3 μg kg^{-1} $minute^{-1}$ did not decrease the sparing effect of sevoflurane MAC in dogs, indicating that acute tolerance to remifentanil is not observed under these conditions in dogs.
2. No changes were found in MNT 3 or 7 days after remifentanil administration or changes in the sevoflurane MAC a week after infusion, indicating that hyperalgesia was not shown under these conditions in dogs.

Development of AOT or OIH following remifentanil administration in dogs is not supported by the findings of the study performed.

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Producción científica derivada de esta Tesis Doctoral

Como se detalla a continuación, el desarrollo de esta Tesis Doctoral ha dado lugar, de forma directa, a una serie de trabajos científicos, dos de ellos publicados online y en impresión en la revista *Veterinary Anaesthesia and Analgesia* indexada en el Journal Citation Report (JCR) y comunicaciones a congresos nacionales e internacionales. Algunas de estas comunicaciones han sido también publicadas. Además, otros dos trabajos científicos se han enviado a la revista *Veterinary Anaesthesia and Analgesia.*

Producción científica:

Artículos científicos:

- **Ruíz-López, P**; Domínguez, JM; Granados, MM. Intraoperative nociception-antinociception monitors: a review from the veterinary perspective. Veterinary Anaesthesia and Analgesia (2019). DOI: 10.1016/j.vaa.2019.09.006

- **Ruiz-López, P**; Navarrete-Calvo, R; Morgaz, J; Domínguez, JM; Quirós-Carmona, S; Muñoz-Rascón, P; Gómez-Villamandos, RJ; Fernández-Sarmiento, JA; Granados, MM. Determination of acute tolerance and hyperalgesia to remifentanil constant rate infusion in dogs undergoing sevoflurane anaesthesia. Veterinary Anaesthesia and Analgesia (2019). DOI: 10.1016/j.vaa.2019.09.005

- **Ruiz-López, P**; Domínguez, JM; Morgaz, J; Quirós-Carmona, S; Navarrete-Calvo, R; Gómez-Villamandos, RJ; Fernández-Sarmiento, ja; Granados, MM. Evaluation of the parasympathetic tone activity (PTA) index and its dynamic variation (ΔPTA) in dogs undergoing laparoscopic ovariectomy. Veterinary Anaesthesia and Analgesia Journal. Enviado.

- **Ruiz-López, P**; Morgaz, J; Quirós-Carmona, S; Navarrete-Calvo, R; Domínguez, JM; Gómez-Villamandos, RJ; Fernández-Sarmiento, JA; Granados, MM. Assessment of autonomic nervous system activity by monitoring parasympathetic tone activity (PTA) in horses after a nociceptive stimulus and subsequent administration of morphine,

ketamine and dobutamine. Veterinary Anaesthesia and Analgesia. Enviado.

Comunicaciones científicas a congresos internacionales:

- Comunicación oral: **Ruiz-López, P**; Quirós-Carmona, S; Morgaz, J; Navarrete-Calvo, R; Domínguez, JM; Gómez-Villamandos, R; Granados, MM (2019) Evaluation of the Parasympathetic Tone Activity (PTA) monitor in horses undergoing xylazine-ketamine-isoflurane anaesthesia. Preliminary study. Spring Meeting of the Association of Veterinary Anaesthetists. Association of Veterinary Anaesthetists (AVA). Bristol, Reino Unido.
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- Comunicación oral: Ruiz-López, P; Domínguez-Pérez, JM; Morgaz-Rodríguez, J; Quirós-Carmona, S; Navarrete-Calvo, R; Fernández-Castañer, J; Gómez-Villamandos, RJ; Granados-Machuca, MM (2017) Intraoperative evaluation of the parasympathetic tone activity (PTA) in dogs undergoing laparoscopic ovariectomy. Autumn Meeting of the Association of Veterinary Anaesthetists (AVA) Berlin. Berlín, Alemania.
    - Publicado como "**Abstracts presented at the autumn meeting of the association of the Veterinary Anaesthetists, Berlin, Gerrmany-9-11th November, 2017**". Veterinary Anaesthesia and Analgesia 2018,45:584-588. ISSN/ISBN:1467-2987

- Comunicación escrita: Granados, MM; **Ruiz, P**; Funes, FJ; Dominguez, JM; Navarrete, R; Gómez-Villamandos, RJ; Aguado, D; Muñóz-Rascón, P; Fernández-Sarmiento, JA; López, I; Morgaz, J (2014). Short term administration under sevoflurane anesthesia does not induce hyperalgesia

or tolerance in dogs. The 13th European Veterinary Emergency and Critical Care Society Congress. Praga, República Checa.

Comunicaciones a congresos nacionales:

- Comunicación oral: **Ruíz López, P**; Quirós Carmona, S; Morgaz J; Navarrete Calvo, R; Caravaca Paredes ME; Granados Machuca, MM (2019) Evaluación intraoperatoria del monitor "Actividad del Tono Parasimpático" (PTA) en caballos anestesiados con xilacina-ketamina-isoflurano. XV Congreso Nacional Sociedad Española de Anestesia y Analgesia Veterinaria. Toledo, España.

- Comunicación oral: **Ruiz López, P**; Morgaz Rodríguez, J; Navarrete Calvo, R; Quirós Carmona, S; Sánchez de Medina Baena, A; Granados Machuca, MM (2018) Evaluación intraoperatoria del monitor Parasympathetic Tone Activity (PTA) en el caballo. Estudio preliminar. XIV Congreso Nacional Sociedad Española de Anestesia y Analgesia Veterinaria. Pamplona, España.

- Comunicación escrita: **Ruiz López, P**; Granados Machuca, MM (2016) Evaluación de hiperalgesia y tolerancia tras la administración de una infusión continua de remifentanilo en el perro. V Congreso Científico de Investigadores en Formación de la Universidad de Córdoba. Córdoba, España

    - Publicado como "**Creando redes doctorales. Volumen V**" Universidad de Córdoba 2016, 5:321-324. ISBN: 978-84-9927-271-9

- Comunicación oral: **Ruiz López, P**; Domínguez Pérez, JM; Quirós Carmona, S; Morgaz Rodríguez, J; Mengual Riera, C; Navarrete Calvo, R; Fernández Castañer, J; Aguilar García, D; Muñoz Rascón, P; Granados

Machuca, MM (2016) Evaluación intraoperatoria del monitor PTA (Parasympathetic Tone Activity) en perras sometidas a cirugía laparoscópica. XII Congreso Nacional Sociedad Española de Anestesia y Analgesia Veterinaria. Sevilla, España.

- Premio a la mejor comunicación oral “Ignacio Cruz” de la Sociedad Española de Anestesia y Analgesia (SEAAV) 2016.

Fe de erratas

- En los dos manuscritos enviados (Estudio I y II) había una errata en cuanto a la descripción técnica de como mide el monitor Parasympathetic Tone Activity de la que nos hemos dado cuenta tras el envío de ambos manuscritos, por lo que nos pusimos en contacto con el proveedor.

  La descripción correcta, que ha sido corregida en el manuscrito de Tesis Doctoral es la siguiente:

  "The PTA monitor displays two PTA values updated every second, the instantaneous PTA calculated on the 54 previous PTA values and the average PTA calculated on the 176 previous values."

- Además, en el estudio II, en la conclusión se ha encontrado la siguiente errata: "Ketamine decreased the sympathetic activity…" y se ha corregido en el manuscrito de Tesis Doctoral por "Ketamine increased the sympathetic activity…".

- También han sido corregidos pequeños defectos de forma de la bibliografía de los manuscritos enviados (Estudio I y II)

Estas erratas serán corregidas durante las revisiones de los manuscritos con la revista, así como las oportunas correcciones que sean requeridas.

Artículos producidos