Parasympathetic Tone Activity Evaluation to Discriminate Ketorolac and Ketorolac/Tramadol Analgesia Level in Swine

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BACKGROUND: Evaluation of nociceptive–antinociceptive balance during general anesthesia is still challenging and routinely based on clinical criteria. Analgesic drug delivered may be optimized with parasympathetic tone activity (PTA) monitor. This study compares ketorolac and ketorolac/tramadol balance analgesia using a PTA monitor.

METHODS: Pain intensity response was assessed using a 0–100 numerical state scale (PTA) after nociceptive stimuli in pigs under stable sevoflurane anesthesia. Bispectral index, heart rate, noninvasive blood pressure, and respiratory parameters were also measured. Animals were divided into 3 groups: without analgesia, ketorolac, and ketorolac/tramadol. Mean values or mean areas under the curve (AUC) in selected time periods were compared over time and between groups through a mixed-model repeated measures analysis of variance and nonparametric Kruskal-Wallis tests, followed by Bonferroni or Dunn's multiple comparisons.

RESULTS: It was observed a significant decrease in the PTA AUC mean value after application of the stimulus in animals treated without analgesia and only with ketorolac. The PTA AUC mean value in the control group was significantly lower than the corresponding mean in ketorolac group. The ketorolac/tramadol group showed the highest PTA AUC mean values, significantly different from those obtained for the other 2 groups, with no significant differences detected over time. Bispectral index means showed no statistically significant differences either over time periods or between different treatment groups. Heart rate showed only a statistically significant increase in AUC mean between without analgesia and ketorolac/tramadol group, in the time period after the stimulus application. Noninvasive blood pressure means showed no statistically significant differences over time and between treatment groups.

CONCLUSIONS: This study shows that a low dose combination of ketorolac and tramadol is sufficient to block the pain responses induced with a needle holder in pigs 20 minutes after its administration. The PTA monitor was able to clearly recognize the analgesic level between treatments and may be used to optimize analgesic drug delivered. (Anesth Analg XXX;XXX:00–00)

KEY POINTS

- **Question:** How is the intraoperative analgesia monitoring performed?
- **Findings:** The parasympathetic tone activity monitor was able to clearly recognize the analgesic level between treatments and may be used to optimize analgesic drug delivered.
- **Meaning:** Evaluation of nociceptive–antinociceptive balance during general anesthesia is still challenging and routinely based on clinical criteria.

Anesthetic protocols are usually composed of a combination of sedatives, hypnotics, and analgesics drugs.[1] Preventive analgesia, before the surgical procedure, is used to reduce pain before, during, and after surgery.[2] It allows to decrease the amount of analgesic drugs used when compared to what would be needed if given solely after the pain stimulus.[3]

Opioids have become prominent choices for analgesic adjuncts in a patient's anesthetic management.[4] Tramadol is a centrally acting analgesic, which has low affinity for opioid receptors. It is a synthetic analog of codeine. The main action of tramadol is by the inhibition of the neuronal uptake of norepinephrine and serotonin at synapses in the descending inhibitory pain pathways.

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Ketorolac belongs to the pyrrolo-pyrrole group of nonsteroidal anti-inflammatory drugs. It has anti-inflammatory, analgesic, and antipyretic activities. Ketorolac primarily inhibits the cyclooxygenase enzyme that metabolizes the arachidonic acid to endoperoxide intermediates and prostaglandins that promotes pain.[5]

Analgesics, most commonly opioids, are administrated based on clinical experience and on the assessment of somatic or autonomic responses like arterial hypertension and tachycardia. Detection of intraoperative nociception is a fundamental goal for the anesthetists.[6]

The parasympathetic tone activity (PTA) monitor may be used for recognition of intraoperative nociception balance. It displays an index that is similar to the analgesia nociception index (ANI) used in human.[6–8] This index is based on the analysis of heart rate variability and reflects the relative parasympathetic tone and the sympathovagal balance of the animal. High value indicates a high parasympathetic tone and the absence of nociception. A low value reflects a low parasympathetic tone, which consequently indicates a high sympathetic tone value, representing a potential nociception.

No studies to date have used the PTA index to compare analgesic balance of different drug protocols. This study compares ketorolac and ketorolac/tramadol (K/T) combination analgesia efficacy during nociceptive stimulation measured with the PTA monitor in pigs during stable anesthesia.

METHODS

This prospective study was performed at the Minimally Invasive Surgery Centre Jesús Usón in Caceres, Spain. All procedures were approved by the local ethical committee and animal welfare body.

Animals

Twenty-seven large white healthy pigs, between 85 and 90 days of age with mean weight 49 (standard deviation, 5) kg, were used. Animals were housed 5–10 days before the study in individual indoors parks in a climate and light-controlled facility. Food was withheld 12 hours before the experiments, with free access to water.

Anesthetic and Monitoring Protocol

The anesthetic protocol was standardized. Animals were premedicated intramuscularly with a combination of ketamine 15 mg/kg (Ketaset 100 mg/mL; Zoetis Spain SL, Madrid, Spain) and diazepam 0.25 mg/kg (Valium 10 mg/2 mL; Roche Farma SA, Madrid, Spain). Ten minutes after premedication, pigs were transferred to the operating room. The right auricular vein was catheterized with a 18–20 gauge cannula for administration of fluids and drugs. Twenty minutes after premedication, the animals were induced with thiopental 6 mg/kg IV bolus administration (Tiobarbital 1 g; B Braun Medical SA, Barcelona, Spain). Pigs were intubated with 8.0- to 8.5-mm endotracheal tube. The tube was connected to a circular breathing circuit and mechanical ventilator with a Maquet Flow-i C20 (Maquet Critical Care AB, Solna, Sweden) anesthesia machine and monitor. The anesthesia was maintained to an end-tidal sevoflurane (Sevorane, Abbott Laboratórios, Barcelona, Spain) concentration between 3.0% and 3.2% and an end-tidal CO_2 tension maintained between 35 and 45 mm Hg. All animals were placed in left lateral recumbency.

Noninvasive blood pressure (NIBP) was automatically measured in the anterior carpus using 5-minute intervals. Cuff width was chosen to be approximately 40% of circumference of the limb. Electrocardiogram (ECG) was monitored by 3 ECG electrodes placed according to Academy of Veterinary Cardiology Committee. Oxygen saturation of hemoglobin was continuously monitored. Cardiovascular data were recorded using a Philips intelliVue MX450 Patient Monitor (Philips Medizin Systeme, Boeblingen, Germany).

The electroencephalography, electromyography, and bispectral index (BIS) were continuously monitored using the A-2000 Xp BIS monitor (Aspect Medical System, Natick, MA). Data were collected by a BIS Quatro sensor (Aspect Medical System) composed of 4 numbered electrodes. The first electrode was placed in the midline of the eyes, at the top of the skull. The second electrode was placed to the left of the first. The third electrode was positioned over the left temple and the fourth between the second and third, between the lateral corner of the left eye and the temporal bone. The depth of anesthesia was clinically assessed using the BIS value.

The PTA was continuously measured using PhysioDoloris (Mdoloris Medical Systems, Lille, France). This monitor records the ECG signal (lead II) using a 3-lead system with flatted crocodile clips attached to the skin. Clips were moistened with electrode gel to maintain electrical contact. The red clip and yellow clip were placed cranial to the right and left scapulohumeral joint, respectively. The black clip was placed in the right side of abdominal straight muscle about 20 cm caudal to the elbow.

PTA Measurement

The PTA index uses the ECG signal to evaluate heart rate variability, a noninvasive method to evaluate autonomic nervous system activity. Heart rate variability is defined as changes in the duration of consecutive cardiac cycles (heartbeats) and it is measured as the duration of the variation between R-R interval peaks in the QRS complexes between 2 consecutive cardiac cycles in an ECG wave. Heart rate variability has 2 main components: low frequency (0.004–0.15 Hz), which reflects the activation of sympathetic and parasympathetic systems, and the high frequency (0.15–0.5 Hz), mainly associated to the parasympathetic activity, and which are mainly influenced by respiratory sinus arrhythmia. Like ANI, PTA values are scored between 0 and 100: a value of 100 corresponds to a maximum parasympathetic tone that may correlate with an absence of nociception; conversely, a value of 0 corresponds to a decreased parasympathetic tone/increased sympathetic tone that may be associated with inadequate analgesia and nociception. An animal is considered to be in the analgesic comfort zone when PTA values are in the 50–85 range. Values <50 indicate that there is no analgesic balance and analgesic therapy should be increased. PTA values >85 indicate that the animal is in an analgesic overdose and the analgesic therapy should be decreased. According to the literature, a PTA

dynamic variation of at least 18 units represents biological significant differences in PTA scores and analgesia status.[9]

Study Design

After anesthetic induction, the animals were randomly allocated into 3 groups of 9 pigs, to assign 1 of the 3 analgesic treatments, routinely used in our laboratory in pigs. The 3 groups were: without analgesic drugs (WA), with ketorolac (K) 1.5 mg/kg (Ketorolaco trometamol Normon 30 mg/mL; Norman SA Labs, Madrid, Spain), and ketorolac (1 mg/kg) combined with tramadol (1 mg/kg) (Tramadol Normon 100 mg/2 mL; Norman SA Labs).

Twenty minutes after analgesic's intravenous administration and at the end of 10 minutes of stable general anesthesia, the animals were subjected to a noxious stimulus with a 18 cm Mayo Hegar needle holder (Art Cl-1413-Mian, Italy) until the last degree of strength. The stimulus was applied in the apex of left ear 1 cm from the margin, in animals.

The researcher was blinded to treatment groups and 3 mL of saline was given in the WA group. Animal monitoring was performed with an interval of 1 minute for PTA, HR, and BIS and was made with an interval of 5 minutes for NIBP by the same investigator. Supplemental Digital Content 1, Figure 1, http://links.lww.com/AA/C456, shows the project design study along the time.

Statistical Analysis

Statistical analysis was performed with SPSS V.25 (IBM Corporation, Armonk, NY) with significance level set at .05.

After a preliminary statistical data analysis conducted for each variable in each time instant, using the trapezoidal method, the data collected from 0 to 20 minutes for the PTA, BIS, and HR variables were converted into areas under the curve (AUC) in consecutive 5-minute time periods: 0–5 minutes ([0,5] time period 1), 5–10 minutes ([5,10] time period 2), 10–15 minutes ([10,15] time period 3), and 15–20 minutes ([15,20] time period 4); the NIBP variable was analyzed from original data observed in minutes 5, 10, 15, and 20 (4 time instants).

Mean values or AUC mean values differences between the 4 time instants or periods (as adequate) and between independent groups of 9 animals treated WA, ketorolac (K), and K/T, along with possible interactions, were explored using a mixed-model repeated measures analysis of variance (ANOVA), where the time is a within-subject factor and the analgesic treatment is a between-subject factor. The underlying normality repeated measures-ANOVA (RM-ANOVA) assumptions were verified using Shapiro-Wilk's test. Mauchly's test of sphericity was performed to inspect the variance homogeneity of the differences between all possible pairs of within-subject groups. Whenever Mauchly's test was significant, depending on the degree of sphericity violation, Greenhouse-Geisser (when $\varepsilon < 0.75$) or Huynh-Feldt (when $\varepsilon > 0.75$) corrections were applied to properly correct the degrees of freedom of the RM-ANOVA F-distribution, so that valid F ratios were obtained. Levene's test was conducted to inspect the homogeneity of variances between treatment groups. Whenever homogeneity of variances was violated, Kruskal-Wallis (nonparametric ANOVA) test was conducted. Finally, in the cases revealing significant effects of time, treatments, or interaction, Bonferroni or Dunn's post hoc pairwise comparisons (as adequate) were provided.

The sample size was computed a priori using G Power 3.1.9.2[10] based on the SPSS mixed RM-ANOVA results of a pilot sample of 9 animals (3 in each group). The pilot sample showed PTA mean dynamic variations higher than 18 units, which are considered differences with biological importance.[7,9] To have high probability of detecting clinically relevant effects, for each variable under analysis, a priori sample size computation was conducted assuming a statistical power of 80% (to avoid type II error) and a significance α level of .05 (to avoid type I error). The effect size considered in each test (between, within, and within-between factors) was determined directly from the partial η^2 estimates reported in SPSS for the PTA, BIS, HR, and NIBP variables in the pilot sample. To attain 80% of chance to correctly detect significant differences between analgesic treatments, across time, and significant time–treatment interaction effect, the power analysis required, respectively, minimum sample sizes of 9, 6, and 6 animals for PTA; 36, 27, and 18 animals for BIS; 15, 12, and 12 animals for HR; and 45, 33, and 30 animals for NIBP. Due to the number of available pigs with similar characteristics, the experiment was conducted in 27 animals.

RESULTS

For all the variables in each combination of time and treatment, Shapiro-Wilk's test statistics were nonsignificant ($P > .05$), allowing to assume the normality distribution. Sphericity was assumed for the PTA AUC values on results of Mauchly's test ($P = .06$), while for the remaining variables, Mauchly's statistics were significant ($P < .05$), so that a Huynh-Feldt correction was undertaken.

According to the Levene's test, the homogeneity of variances between treatment groups was only violated for BIS AUC and NIBP in the third time period and the fourth time instant, respectively ($P < .05$). Therefore, the nonparametric Kruskal-Wallis test was used to compare the BIS AUC and NIBP distributions between groups of treatments, in each time period or time instant.

The mixed RM-ANOVA revealed significant interaction effects between time and analgesic ($P = 7.4e\text{-}19$) on the PTA AUC mean values and significant main effects of the analgesic treatment ($P = 6.4e\text{-}15$). Both tests presented maximum power (1) and large effect sizes ($\eta_p^2 > 0.7$). PTA AUC mean profile is shown in Figure A and Tables 1 and 2. Tables 3 and 4 present, respectively, the mean differences of PTA AUC values between time periods (by treatment) and between treatments (by time period), and the respective 95% CI provided by Bonferroni test.

For K/T group, no statistically significant changes were detected in PTA AUC mean values between each of the 4 time periods. In contrast, for WA and K treatments, the PTA AUC mean value in the third time period (minutes after the stimulus application) was significantly smaller than those observed in any other time periods; no other PTA AUC means between time periods showed significant differences ($P > .05$). Results also showed that the analgesic treatment has clear implications in the PTA AUC mean values

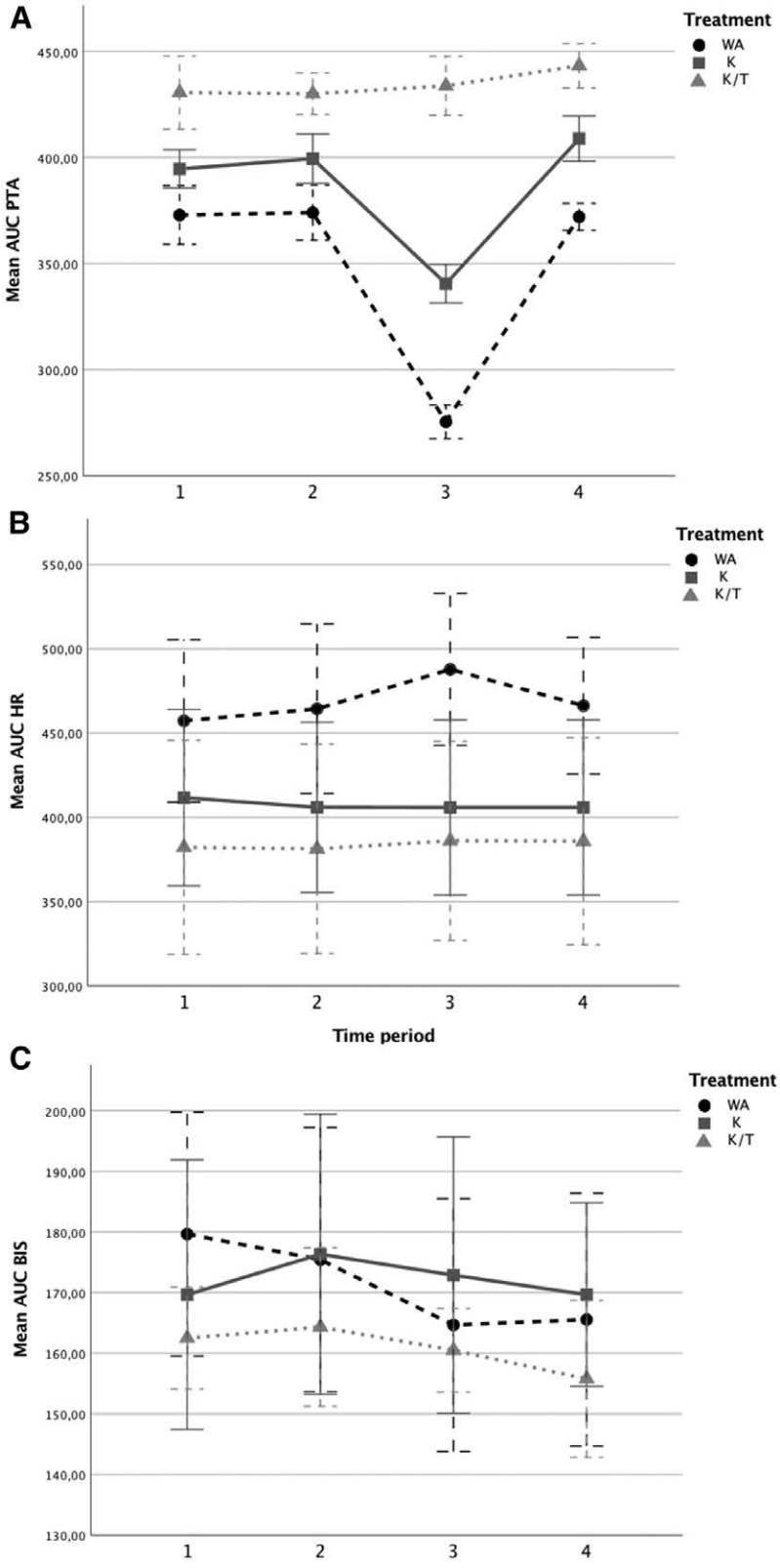

Figure. Mean area under the curve (AUC) observed during the independent 4 time periods and analgesic treatment for parasympathetic tone activity (PTA) (A), heart rate (HR) (B), and bispectral index (BIS) (C) variables. K indicates ketorolac; K/T, ketorolac/tramadol group; WA, without analgesia group.

changes. In fact, except for first time period, in which no significant differences were detected between PTA AUC means for WA and K treatments ($P > .05$), all other PTA AUC mean pairwise comparisons between treatment groups were significantly different ($P < .05$). Among the 3 treatments considered, the results showed that WA group is the one experiencing significantly lower PTA AUC mean value, in contrast with K/T group, that showed significantly higher PTA AUC mean values and K group that showed intermediate mean values. Results showed that the lowest PTA AUC

Table 1. Mean AUC and Standard Error of PTA and HR by Analgesic Treatment

	Analgesic Treatment		
Variable	**WA (n = 9)**	**K (n = 9)**	**K/T (n = 9)**
PTA AUC (0–100 numerical rate scale)	348.6 (3.3)	385.9 (3.3)	434.4 (3.3)
HR AUC (beats per minute)	468.9 (23.1)	407.3; (23.1)	383.9 (23.1)

Abbreviations: AUC, area under the curve; HR, heart rate; K, ketorolac group; K/T, ketorolac/tramadol group; PTA, parasympathetic tone activity; WA, without analgesia group.

Table 2. Time-by-Treatment Mean AUC and Standard Deviation of PTA and HR

	Time Period			
	TP1	**TP2**	**TP3**	**TP4**
Variable				
PTA AUC (0–100 numerical rate scale)				
Analgesic treatment				
WA (n = 9)	372.9 (18.0)	374.0 (16.8)	275.4 (10.3)	372.0 (8.3)
K (n = 9)	394.6 (11.8)	399.4 (15.2)	340.5 (11.8)	408.9 (13.8)
K/T (n = 9)	430.6 (22.4)	430.1 (12.7)	433.8 (18.1)	443.2 (13.6)
HR AUC (beats per minute)				
Analgesic treatment				
WA (n = 9)	457.3 (62.8)	464.4 (65.5)	487.8 (58.8)	466.2 (52.9)
K (n = 9)	411.7 (68.1)	405.9 (65.7)	405.8 (67.7)	405.8 (67.7)
K/T (n = 9)	382.2 (82.6)	381.3 (80.9)	386.1 (76.9)	385.8 (79.9)

TP1 and TP2: baseline periods; TP3 and TP4: periods after the pain stimulus.
Abbreviations: AUC, area under the curve; HR, heart rate; K, ketorolac group; K/T, ketorolac/tramadol group; PTA, parasympathetic tone activity; TP*i*, time period *i*, *i* = 1, 2, 3, 4; WA, without analgesia group.

mean values arise during the third time period, especially in animals within WA group. Supplemental Digital Content 2, Table 1, http://links.lww.com/AA/C457, presents the mean and standard deviation values for the original data of the variable PTA along the third time period. In WA and K groups, the PTA enabled to detect pain responses to the stimuli at 11 and 12 minutes.

For HR, the Huynh-Feldt–corrected mixed RM-ANOVA results revealed statistically significant interaction effects between time and analgesic (P = 8.9e-8) and main effects of the analgesic (P = .04) on the HR AUC mean values. The HR AUC mean profile plot is presented in Figure B and in Tables 1 and 2. Bonferroni test results are summarized in Tables 3 and 4. For K and K/T groups, Bonferroni results showed no statistically significant differences in HR AUC mean values over time periods (P > .05). In turn, for WA group, the HR AUC mean of the third time period revealed to be significantly higher than those of the other time periods. In the third time period, a significant increase in the WA group HR AUC mean was detected when compared with the K/T group (P < .05) and no differences were detected when comparing WA with K groups and K with K/T groups. No other significant differences were found between HR AUC mean values in WA, K, and K/T groups, for the remaining time periods considered.

The Huynh-Feldt–corrected mixed RM-ANOVA within-subjects results for BIS AUC and NIBP showed no statistically significant interaction effects between time and analgesic (P = .4 and .6, respectively).

The BIS AUC and NIBP mean profile plots are shown in Figure C and Supplemental Digital Content 3, Figure 2, http://links.lww.com/AA/C458, respectively. The single AUC for BIS and NIBP, in the time intervals [0,20] and [5,20], respectively, were thus considered to compare the analgesic effects. For both variables, Kruskal-Wallis test revealed no significant differences in the corresponding distributions between the 3 analgesic groups (P = .6 and .9, respectively).

DISCUSSION

In this prospective study, the effectiveness of analgesia during nociceptive stimulation was analyzed using the PTA, BIS, and HR monitor values, to evaluate nociceptive-antinociception balance.

With this study, we were able to conclude that PTA AUC mean values were statistically significantly different between the analgesic treatments within any period of time. The results showed the PTA AUC mean lowest values arise in animals in which analgesia was not given. Similar results were observed in healthy dogs.[9] The observed results also confirm that animals premedicated with analgesic drugs (ketorolac or K/T) have a greater activity of the parasympathetic tone causing higher PTA AUC mean values. Thus, this indicates a correct nociceptive–antinociceptive balance like it was seen in humans during sevoflurane-remifentanil anesthesia measured by ANI.[6]

Animals with ketorolac showed intermediated PTA AUC mean values. Ketorolac is a nonsteroidal anti-inflammatory drug with peripherally acting analgesic properties that will inhibit the cyclooxygenase enzyme that metabolizes arachidonic acid to endoperoxide intermediates and prostaglandins that promote pain. Oral and parenteral ketorolac has been shown to be effective for pain management.[5,11] Such ketorolac properties prevented a PTA AUC mean value sharp decrease after the stimuli as it was observed in animals WA.

In K/T group, the results showed that there were no changes in PTA AUC mean values after nociceptive stimulus. These results are in accordance with the previous study that showed the analgesic interaction between ketorolac and tramadol in rat[5] and in humans.[12] These studies showed that the association of ketorolac and tramadol has a synergic

Table 3. PTA/HR Mean Differences and Respective 95% CI for the Pairwise Comparisons of AUC Between Time Periods for Each Analgesic Treatment

	Time Period		
	TP2	TP3	TP4
Time period			
TP1			
Variable			
PTA AUC			
Treatment			
WA (n = 9)	−1.11 (−16.55 to 14.33)	97.44[a] (76.46–118.43)	0.89 (−20.1 to 21.88)
K (n = 9)	−4.83 (−20.28 to 10.61)	54.11[a] (33.13–75.09)	−14.28 (−35.26 to 6.71)
K/T (n = 9)	0.56 (−14.89 to 16)	−3.17 (−24.15 to 17.81)	−12.61 (−33.6 to 8.38)
HR AUC			
Treatment			
WA (n = 9)	−7.11 (−14.86 to 0.64)	−30.56[a] (−42 to −19.11)	−8.94 (−20.38 to 2.49)
K (n = 9)	5.72 (−2.03 to 13.48)	5.83 (−5.62 to 17.28)	5.83 (−5.6 to 17.27)
K/T (n = 9)	0.89 (−6.86 to 8.64)	−3.89 (−15.34 to 7.56)	−3.61 (−15.05 to 7.83)
TP2			
Variable			
PTA AUC			
Treatment			
WA (n = 9)		98.56[a] (83.37–113.74)	2.0 (−15.14 to 19.14)
K (n = 9)		58.94[a] (43.76–74.13)	−9.44 (−26.58 to 7.69)
K/T (n = 9)		−3.72 (−18.91 to 11.46)	−13.17 (−30.31 to 3.97)
HR AUC			
Treatment			
WA (n = 9)		−23.44[a] (−32.17 to −14.72)	−1.83 (−12.6 to 8.94)
K (n = 9)		0.11 (−8.61 to 8.84)	0.11 (−10.66 to 10.88)
K/T (n = 9)		−4.78 (−13.5 to 3.95)	−4.5 (−15.27 to 6.27)
TP3			
Variable			
PTA AUC			
Treatment			
WA (n = 9)			−96.56[a] (−110.06 to −83.05)
K (n = 9)			−68.39[a] (−81.89 to −54.89)
K/T (n = 9)			−9.44 (−22.95 to 4.06)
HR AUC			
Treatment			
WA (n = 9)			21.61[a] (14.11–29.11)
K (n = 9)			5.68e-14 (−7.5 to 7.5)
K/T (n = 9)			0.28 (−7.22 to 7.78)

95% CI for $\mu_{\text{treatment}\times\text{TPi}} - \mu_{\text{treatment}\times\text{TPj}}$. TP1 and TP2: baseline periods; TP3 and TP4: periods after the pain stimulus.
Abbreviations: AUC, area under the curve; HR, heart rate, in beats per minute; K, ketorolac group; K/T, ketorolac/tramadol group; PTA, parasympathetic tone activity, 0–100 numerical rate scale; TP*i*, time period *i*, *i* = 1, 2, 3, 4; WA, without analgesia group.
[a]$P < .05$, significant differences between $\mu_{\text{treatment}\times\text{TPi}}$ and $\mu_{\text{treatment}\times\text{TPj}}$.

effect, lower doses from these 2 drugs allowed, a higher degree of analgesia and fewer side effects.

We note that the continuous monitoring of PTA allows the detection of painful responses that hardly would have been noticed without this equipment. These painful responses were generally detected at 11 and 12 minutes and could be justified by the computerized data processing system, which may delay the PTA values displayed in response to the pain stimuli applied at the tenth minute.

BIS is a depth of anesthesia monitor that analyses the electroencephalogram and displays a dimensionless number between 0 (total suppression of electrical activity cortical) and 100 (awake), with 40–60 being suitable for surgical anesthesia.[13,14] BIS results showed that there are no differences in AUC mean values along the time or between different treatment groups. This lack of BIS fluctuation between groups showed that all animals were at equivalent depths of anesthesia. These results are in agreement with a previous BIS review.[14]

Heart rate results showed no significant differences between time period the first, second, and fourth time periods for all groups. During the third time period, there was only a significant increase in HR mean values in WA group when compared with the K/T group. Heart rate is the conventional method of analgesic monitoring. During surgical procedures, when there is an increase in HR, the clinician infers that the animal is in analgesic discomfort and reestablishes the analgesic balance. However, our results revealed that HR AUC mean values only showed differences between with or without analgesia. No significant differences were found in HR AUC mean values between K and K/T groups. As our results revealed statistically significant differences in PTA AUC mean values between the analgesic treatments, in any period of time, we may conclude that PTA presents a greater sensitivity in the differentiation of analgesic levels when compared to a conventional method like HR. These results are in accordance with the previous study in dogs[9] and in humans[7,15,16] which reported that, when using PTA

Table 4. PTA/HR Mean Differences and Respective 95% CI for the Pairwise Comparisons of AUC Between Analgesic Treatments for Each Time Period

	Treatment	
	K (n = 9)	K/T (n = 9)
Treatment		
WA (n = 9)		
Variable		
PTA AUC		
Time period		
TP1	−21.72 (−43.49 to 0.05)	−57.72[a] (−79.49 to −35.96)
TP2	−25.44[a] (−43.65 to −7.24)	−56.06[a] (−74.26 to −37.85)
TP3	−65.06[a] (−81.86 to −48.25)	−158.33[a] (−175.14 to −141.53)
TP4	−36.89[a] (−51.68 to −22.11)	−71.22[a] (−86 to −56.44)
HR AUC		
Time period		
TP1	45.61 (−41.3 to 132.52)	75.06 (−11.85 to 161.96)
TP2	58.44 (−27.79 to 144.68)	83.06 (−3.18 to 169.29)
TP3	82 (−0.74 to 164.74)	101.72[a] (18.98–184.46)
TP4	60.39 (−21.78 to 142.56)	80.39 (−1.78 to −162.56)
K (n = 9)		
Variable		
PTA AUC		
Time period		
TP1		−36[a] (−57.77 to −14.23)
TP2		−30.61[a] (−48.81 to −12.41)
TP3		−93.28[a] (−110.08 to −76.48)
TP4		−34.33[a] (−49.11 to −19.56)
HR AUC		
Time period		
TP1		29.44 (−57.46 to 116.35)
TP2		24.61 (−61.62 to 110.85)
TP3		19.72 (−63.02 to 102.46)
TP4		20 (−62.17 to 102.17)

95% CI for $\mu_{Ti\times\text{time period}} - \mu_{Tj\times\text{time period}}$. TP1 and TP2: baseline periods; TP3 and TP4: periods after the pain stimulus.

Abbreviations: AUC, area under the curve; HR, heart rate, in beats per minute; K, ketorolac group; K/T, ketorolac/tramadol group; PTA, parasympathetic tone activity, 0–100 numerical rate scale; TP*i*, time period *i*, *i* = 1, 2, 3, 4; WA, without analgesia group.

[a]$P < .05$, significant differences between $\mu_{Ti\times\text{time period}}$ and $\mu_{Tj\times\text{time period}}$, with *Ti*–treatment *i*, *i* = WA, K, and K/T.

or ANI monitors, it is possible to predict hemodynamic changes before they arise.

NIBP revealed no significant differences between treatment groups during all periods of time. This result is in accordance with the previous study in dogs[9] and in humans.[8,17,18] These studies concluded that PTA index is the best way to predict a hemodynamic reactivity triggered by a pain stimulus. These conclusions (as those drawn for AUC BIS) should be viewed with some caution and require further investigation. In future study, a larger sample should be considered to increase the power of the tests.

This study allows to conclude that a combination of low ketorolac analgesic doses (1 mg/kg) combined with tramadol (1 mg/kg) is sufficient to block the pain responses induced with a needle holder in pigs 20 minutes after its administration. This study also demonstrates that the PTA monitor was the best way to distinguish the analgesic level between treatments and that in the absence of PTA monitoring this painful moment hardly would be noticed using routine anesthesia monitoring. It is also very important to emphasize that the PTA monitor is a technological advance in pain management, analgesia balance quantification during anesthesia, and a potential tool in pain research translational sciences between species.

The present study has some limitations. The results of this study may not be generalized to animals on β-blockers, other drugs with influence on heart rate variability (ephedrine, phenylephrine, neostigmine, or atropine), or with chronic pain.[17] Ketamine seems to have no impact on PTA (or ANI) values.[19] The effects of other analgesic agents used during general anesthesia are still unknown. Therefore, factors that impact the autonomous tone, such as arrhythmia, intravascular volume, posture, medication, and consciousness level by itself, disturb the actual nociceptive input.[7] Consequently, PTA result interpretation may be limited by a large interindividual variability, but in all cases, the final clinical evaluation should be complemented with information provided by nociceptive/antinociceptive balance monitoring.

At the end of this study, the main idea to retain is that analgesic monitoring during intraoperative procedures remains a major challenge for clinicians. Our results showed that the use of equipment such as PTA may optimize the administration of analgesics, to ensure that the animal is in analgesic comfort, avoiding overdoses or underdosing. Although the PTA has some limitations, it should be seen as an added value in monitoring and therefore an additional equipment and not an equipment that will replace others.

Future research may include the validation of PTA with multicentric studies, its use in the context of balanced analgesia, and its predictability to detect animal's pain during procedures.

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