Effect of cyclooxygenase-2 inhibitors at therapeutic doses on body temperature during anesthesia in healthy dogs administered with amino acids

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ABSTRACT. In healthy dogs, amino acid infusion significantly attenuates the decrease in body temperature during anesthesia by facilitating insulin secretion, suggesting that such an increase in insulin secretion is related to increased heat production. In dogs, selective cyclooxygenase-2 (COX-2) inhibitors, which are used for pain relief in veterinary medicine, possess anti-pyretic action. And, in mice and humans, selective COX-2 inhibitors increase insulin secretion and sensitivity. Therefore, treatment with COX-2 inhibitors may negate or accelerate the attenuating effect on decreased body temperature during anesthesia by amino acid infusion. In the present study, influences on insulin secretion and body temperature by treatment with meloxicam or robenacoxib at therapeutic dose were evaluated in healthy dogs. Treatment with meloxicam or robenacoxib did not affect insulin secretion in the unanesthetized and anesthetized dogs, and did not affect body temperature and heart rate under the anesthetized condition with amino acid infusion. In conclusion, COX-2 inhibitors at therapeutic doses did not affect body temperature during anesthesia in dogs administered amino acids.

KEY WORDS: amino acid infusion, body temperature, insulin secretion, dog

Intraoperative hypothermia in dogs, defined as a temperature of <36.7°C [10], prolongs the recovery time from anesthesia [17] and the time to extubation [18]. We previously demonstrated that amino acid infusion at 1.2 g/kg/hr in healthy dogs significantly attenuates the decrease in body temperature during anesthesia by the facilitation of insulin secretion [21]. Since protein synthesis in skeletal muscle is stimulated by exogenously administered insulin [4, 5, 7], we interpreted the outcome of our previous study to be the result of the production of heat from muscle protein synthesis induced by insulin secretion. Conversely, since the thermic effect induced by amino acid infusion was not altered in patients with type II diabetes [22], insulin resistance may not affect heat production by amino acid infusion. Moreover, in humans, amino acid infusion increases the metabolic rate and resting core temperature [15]; therefore, heat production by amino acid infusion is thought to occur via various mechanisms, including those still unknown.

During general anesthesia in healthy dogs, cyclooxygenase (COX) inhibitors are used to prevent acute inflammation and pain during the perioperative period [16]. The COX family of enzymes consists of two isoforms: COX-1 and COX-2. COX-1 is thought to have cytoprotective effects. Although COX-1 is detected in most normal tissues, COX-2 is not present; however, expression of COX-2 is induced by stimuli such as proinflammatory cytokines (IL-1b and TNF-α), lipopolysaccharides, growth factors (fibroblast growth factor, platelet-derived growth factor, and epidermal growth factor), hormones (luteinizing hormone), and disorders of water-electrolyte hemostasis, resulting in increased synthesis of prostaglandins in inflamed and neoplastic tissues [12, 24]. Therefore, this inducible isozyme has been implicated in pathological processes such as inflammation and cancer. COX-2 causes the production of prostaglandin E_2 (PGE_2), which evokes and maintains inflammation and pain. Selective COX-2 inhibitors exert analgesic and anti-inflammatory actions via the inhibition of PGE_2 production. While their effects have been recognized in dogs [8, 9, 19, 23], meloxicam [8] and cimicoxib [9] also have shown anti-pyretic action. And, in mice and humans, selective COX-2 inhibitors increase insulin secretion and sensitivity [3, 6, 13]. Therefore, treatment with COX-2 inhibitors may negate or accelerate the attenuating effect of decreased body temperature during anesthesia by amino acid infusion. In veterinary medicine, meloxicam and robenacoxib are widely used for pain relief, and are presumed to use with amino acid infusion during the perioperative period. It was necessary to elucidate the effect of the combined use of COX-2 inhibitor and amino acid infusion

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on insulin secretion. In the present study, as a first, we evaluated the influence of the administration of meloxicam and robenacoxib at therapeutic doses on the insulin secretion by intravenous glucose tolerance test in unanesthetized dogs. Subsequently, we determined whether the treatment of meloxicam and robenacoxib attenuated effect of decreased body temperature by amino acid infusion at 1.2 g/kg/hr during anesthesia.

MATERIALS AND METHODS

Animals

All procedures involving the study dogs were performed at Gifu University and were approved by the Animal Care and Use Committee for Animal Experimentation of Gifu University (approval number: 12001). In the intravenous glucose tolerance test, five mature beagle dogs were used (one intact male and four castrated males, 3.8 ± 0.8 years old, 13.7 ± 1.3 kg body weight, 5.6 ± 0.5 body condition score of nine-point scale). And, in the amino acid infusion during anesthesia with administration of selective COX-2 inhibitors, six mature beagle dogs were used (four castrated males and two spayed females, 4.7 ± 1.2 years old, 12.6 ± 1.9 kg body weight, 5.3 ± 0.6 body condition score). All dogs were assessed as healthy based on the results of a physical examination, complete blood count, and biochemical profile performed prior to each experiment. Food was withheld from the dogs for the 12 hr preceding the experiment, but free access to water was allowed. In both experiments, each dog was used all three treatments separated by a 1-week washout period.

Administration of selective COX-2 inhibitors

In both experiments, each dog underwent three different administrations: non-administration, subcutaneous administration of meloxicam (Metacam, 5 mg/m*l*, Boehringer-Ingelheim Vetmedica Japan, Tokyo, Japan, 0.2 mg/kg) or robenacoxib (Onsior, 20 mg/m*l*, Novartis Animal Health, Camberley, U.K., 2.0 mg/kg). Selective COX-2 inhibitors were administered 1 hr prior to intravenous glucose tolerance test or anesthesia induction, since blood concentrations of meloxicam and robenacoxib administered subcutaneously at these doses are elevated after 1 hr [1, 19].

Intravenous glucose tolerance test with administration of selective COX-2 inhibitors

Each dog underwent three treatments (non-administration, meloxicam: MEL, and robenacoxib: ROB) separated by a 1-week washout period in random order. After 1 hr from administration of selective COX-2 inhibitors, glucose solution (50%, w/v, 1 g/kg body weight) was administered intravenously in a bolus. Blood samples were collected at just before glucose injection, 5, 7.5, 10, 15, 30, and 60 min post-glucose injection from cephalic vein.

Amino acid infusion during anesthesia with administration of selective COX-2 inhibitors

In the present study, only one amino acid infusion rate was used, since our previous study [21] had revealed an optimal infusion rate of 1.2 g/kg/hr for the attenuation of hypothermia during anesthesia. A 10% amino acid solution (Amiparen, Otsuka Pharmaceutical Factory, Inc., Tokushima, Japan) containing a mixture of 18 amino acids that provided 15.65 g nitrogen/l was used as the test solution. Each dog underwent three different treatments separated by a 1-week washout period in random order: infusion of amino acids only (AA), infusion of amino acids plus subcutaneous injection of meloxicam (AA + MEL), and infusion of amino acids plus subcutaneous injection of robenacoxib (AA + ROB). The dogs underwent general anesthesia for 120 min and were infused at 12 m*l*/kg/hr with the 10% amino acid solution for 60 min prior to the induction of anesthesia and during the first 60 min of anesthesia. Subsequently, the dogs were infused with acetated Ringer's solution (Solacet F, Terumo, Tokyo, Japan) at the same flow rate until the end of anesthesia.

Anesthesia was induced via i.v. injection of 6 mg/kg propofol (1% propofol for animals; Mylan, Inc., Tokyo, Japan). Following endotracheal intubation, the dogs were placed in lateral recumbency. Anesthesia was maintained with isoflurane in >90% oxygen under intermittent positive pressure ventilation that was delivered by a mechanical ventilator (KV-2N, Kimura Ikakiki Co., Tokyo, Japan) to maintain the end-tidal carbon dioxide tension at 35–45 mmHg. The end-tidal isoflurane concentration was adjusted to 1.3% using an animal biometric monitor (Bio-Scope AM120, Fukuda ME Kogyo Co., Tokyo, Japan), which was calibrated in accordance with the manufacturer's instructions. Isoflurane was discontinued 120 min after induction, and the dogs were allowed to recover from anesthesia, being extubated following recovery of the swallowing reflex. During the trial, the room temperature was maintained at 21–23°C and no warming devices were used. Exclusion criteria were movement of the body or recovery of spontaneous respiration during anesthesia.

Blood samples for the measurement of plasma insulin and glucose concentrations were obtained from the saphenous vein by percutaneous puncture at −60 min (immediately prior to subcutaneous administration) and 0, 60, and 120 min following the induction of anesthesia.

Rectal temperature (RT) was measured using an electronic thermometer (SureTemp Plus 690, Welch Allyn Japan, Tokyo, Japan) at the onset of infusion (at −60 min) prior to subcutaneous administration and at intervals of 10 min beginning 10 min after induction. Heart rate (HR) and noninvasive mean arterial pressure (MAP) were recorded using the animal biometric monitor at intervals of 10 min beginning 10 min after induction. For the measurement of arterial pressure, an oscillometric cuff of appropriate size (No. 3 disposable cuff, Fukuda ME Kogyo Co.) was placed on the forelimb distal to the elbow. The time to extubation following the discontinuation of anesthesia was recorded.

Blood analysis

In both experiments, corrected blood samples were placed in chilled polyethylene terephthalate tubes containing EDTA-2Na and aprotinin (NT-EA0205, Nipro, Tokyo, Japan) to prevent the deterioration of insulin activity. Plasma was separated by centrifugation (4°C, 1,000 g, 15 min) and stored at −80°C until assayed. Plasma insulin concentrations were determined using a validated sandwich ELISA kit (Mercodia Canine Insulin ELISA; Mercodia, Inc., Uppsala, Sweden), which can determine canine insulin concentrations from 0.02 to 1.5 ng/ml. Plasma glucose concentrations were determined with dry-slide technology using a multilayered analytical film (Fuji DRI-CHEM, Fujifilm, Tokyo, Japan).

Statistical analysis

Statistical analysis was performed using Excel 2010 (Microsoft, Redmond, WA, U.S.A.) with the add-on software Statcel 3 (OMS Publishing, Saitama, Japan). All data are represented as the mean ± standard deviation. In the intravenous glucose tolerance test, mean values at just before glucose administration were used as the baseline. In the amino acid infusion, mean values at −60 min (immediately prior to infusion) were used as the baseline. Normality of data was confirmed by the chi-square goodness-of-fit test. Differences in RT and insulin and glucose concentrations between the baseline and each time point within each treatment and between control (non-administration or AA) and administrated group (MEL or ROB, or AA + MEL and AA + ROB) within each time point were analyzed by one-way repeated-measures analysis of variance and Holm's *post-hoc* test. During anesthesia, differences in HR and MAP and the time to extubation between dogs treated with AA and those treated with AA + MEL or AA + ROB were analyzed by the Williams test. Statistical significance was defined as $P<0.05$.

RESULTS

Alterations in plasma insulin and glucose in glucose tolerance test with administration of selective COX-2 inhibitors

In all dogs, plasma insulin concentrations from 5 to 15 min after glucose tolerance was significantly higher than that at baseline, followed by a decline to baseline concentrations at 60 min (Table 1). Also, in all dogs, the plasma glucose concentrations increased immediately after the administration of glucose solution (Table 1). The highest glucose concentrations were observed at 5 min post-administration. The 5-, 7.5-, 10-, and 15 min values were significantly greater than the baseline concentrations, followed by a decline to baseline concentrations at 60 min. There were no significant differences in plasma insulin and glucose concentrations at any time point between dogs non-administrated and administrated MEL or ROB (Table 1).

Alterations in insulin and glucose concentrations by amino acid infusion

In dogs treated with AA or AA + MEL, the plasma insulin concentration at 0 min was significantly higher than that at baseline (Table 2). In all three treatments, the plasma insulin concentration at 60 min was significantly higher than that at baseline (Table 2). At 120 min, there were no significant differences in plasma insulin concentrations between the baseline and each time point of the three treatments (Table 2). There were no significant differences in plasma insulin concentration at any time point between dogs treated with AA and those treated with AA + MEL or AA + ROB (Table 2). There were no significant differences in the plasma

Table 1. Plasma insulin and glucose concentrations in six Beagle dogs submitted intravenous glucose tolerance test (50%, w/v, 1 g/kg)

Variable	Time points (min)	Treatments		
		Non-administration	MEL	ROB
Insulin (ng/ml)	Just before glucose tolerance	0.07 ± 0.03	0.07 ± 0.05	0.10 ± 0.06
	5	0.67 ± 0.28[a]	0.79 ± 0.10[a]	0.73 ± 0.25[a]
	7.5	0.73 ± 0.24[a]	0.87 ± 0.17[a]	0.66 ± 0.22[a]
	10	0.70 ± 0.25[a]	0.88 ± 0.24[a]	0.74 ± 0.26[a]
	15	0.80 ± 0.20[a]	0.94 ± 0.25[a]	0.83 ± 0.23[a]
	30	0.22 ± 0.21	0.25 ± 0.13	0.18 ± 0.14
	60	0.07 ± 0.05	0.04 ± 0.04	0.04 ± 0.03
Glucose (mg/dl)	Just before glucose tolerance	97.2 ± 9.7	107.2 ± 13.8	106.0 ± 6.4
	5	317.0 ± 28.5[a]	368.0 ± 27.0[a]	366.6 ± 23.5[a]
	7.5	294.6 ± 20.9[a]	340.6 ± 23.7[a]	333.4 ± 23.8[a]
	10	271.4 ± 31.3[a]	312.6 ± 18.8[a]	309.6 ± 26.8[a]
	15	236.4 ± 28.8[a]	252.6 ± 44.0[a]	261.0 ± 29.9[a]
	30	126.8 ± 44.8	140.4 ± 37.6	134.6 ± 30.7
	60	93.0 ± 4.9	101.6 ± 7.9	98.8 ± 8.6

Plasma samples were obtained just before and after the test (5, 7.5, 10, 15, 30, and 60 min). Before 1 hr prior to intravenous glucose tolerance, used dogs were subcutaneously non-administrated and administrated meloxicam (MEL) or robenacoxib (ROB). Value are means ± SD. Baseline was time point −60. a) Significantly different from baseline within treatments ($P<0.01$). There are no significant differences between Non-administration and MEL or ROB at the same time point.

Table 2. Plasma insulin and glucose concentrations in six Beagle dogs before treatment (−60, baseline), before induction of anaesthesia (0), at 60 min of anaesthesia and the end of treatment (60) and after a further 60 min of anaesthesia (120)

Variable	Time points (min)	Treatments		
		AA	AA + MEL	AA + ROB
Insulin (*ng/ml*)	−60	0.05 ± 0.02	0.06 ± 0.03	0.07 ± 0.05
	0	$0.39 \pm 0.30^{a)}$	$0.35 \pm 0.22^{a)}$	0.31 ± 0.18
	60	$0.51 \pm 0.23^{b)}$	$0.66 \pm 0.25^{b)}$	$0.56 \pm 0.33^{b)}$
	120	0.06 ± 0.06	0.05 ± 0.06	0.06 ± 0.05
Glucose (mg/d*l*)	−60	97.7 ± 12.3	103.3 ± 17.9	103.5 ± 2.4
	0	103.2 ± 15.2	104.5 ± 10.3	106.7 ± 9.7
	60	106.3 ± 15.4	102.0 ± 13.5	99.8 ± 6.4
	120	97.7 ± 10.3	98.5 ± 4.8	99.7 ± 6.1

Used dogs were intravenously infused amino acids at 1.2 g/kg/hr for 60 min before anaesthesia and for the first 60 min during anaesthesia, and were subcutaneously non-administrated (AA) or administrated meloxicam (AA+MEL) or robenacoxib (AA+ROB) just before infusion (just after time −60). Values are means ± SD. Baseline was time point −60. Significantly different in plasma insulin concentrations from baseline within treatments (a) $P<0.05$, b) $P<0.01$). In each treatments, there are no differences in plasma glucose concentrations between baseline and each time point. Within same time points, there are no significant differences in plasma insulin and glucose concentrations between AA and AA+MEL or AA+ROB.

Table 3. Rectal temperature (°C) in six Beagle dogs 60 min (times −60) before anaesthesia and every 10 min (times 10–120) during isoflurane anaesthesia with propofol

Time points (min)	Treatments		
	AA	AA+MEL	AA+ROB
−60	38.6 ± 0.5	38.3 ± 0.2	38.6 ± 0.5
10	$37.5 \pm 0.4^{a)}$	37.8 ± 0.4	37.7 ± 0.5
20	$37.6 \pm 0.3^{a)}$	$37.5 \pm 0.4^{a)}$	$37.5 \pm 0.7^{a)}$
30	$37.4 \pm 0.5^{a)}$	$37.2 \pm 0.4^{a)}$	$37.3 \pm 0.5^{a)}$
40	$37.0 \pm 0.4^{a)}$	$37.0 \pm 0.3^{a)}$	$37.1 \pm 0.6^{a)}$
50	$36.8 \pm 0.5^{a)}$	$36.9 \pm 0.4^{a)}$	$36.9 \pm 0.6^{a)}$
60	$36.7 \pm 0.6^{a)}$	$36.8 \pm 0.3^{a)}$	$36.7 \pm 0.6^{a)}$
70	$36.4 \pm 0.5^{a)}$	$36.6 \pm 0.4^{a)}$	$36.5 \pm 0.7^{a)}$
80	$36.3 \pm 0.4^{a)}$	$36.3 \pm 0.4^{a)}$	$36.2 \pm 0.7^{a)}$
90	$36.1 \pm 0.4^{a)}$	$36.1 \pm 0.4^{a)}$	$36.0 \pm 0.8^{a)}$
100	$35.9 \pm 0.4^{a)}$	$35.8 \pm 0.3^{a)}$	$35.9 \pm 0.6^{a)}$
110	$35.8 \pm 0.4^{a)}$	$35.6 \pm 0.4^{a)}$	$35.7 \pm 0.6^{a)}$
120	$35.5 \pm 0.5^{a)}$	$35.4 \pm 0.4^{a)}$	$35.5 \pm 0.5^{a)}$

Used dogs were intravenously infused amino acids at 1.2 g/kg/hr for 60 min before anaesthesia and for the first 60 min during anaesthesia, and were subcutaneously non-administrated (AA) or administrated meloxicam (AA+MEL) or robenacoxib (AA+ROB) just before infusion (just after time −60). Value are means ± SD. Baseline was time point −60. a) Significantly different from baseline within treatments ($P<0.01$). There are no significant differences between AA and AA+MEL or AA+ROB at the same time point.

glucose concentration between the baseline and each time point for each treatment and among treatments at each time point (Table 2).

Rectal temperature and time to extubation

Following the induction of anesthesia, all dogs showed decreases in RT, and hypothermia (<36.7°C) was induced after 60 min (Table 3). RT was significantly lower than baseline in dogs treated with AA from 10 to 120 min and in those treated with AA + MEL or AA + ROB from 20 to 120 min (Table 3). There were no significant differences in RT at each time point prior to and during anesthesia between dogs treated with AA and those treated with AA + MEL or AA + ROB (Table 3). There were no significant differences in the time to extubation between dogs treated with AA (7.5 ± 4.3 min) and those treated with AA + MEL (9.3 ± 5.2 min) or AA + ROB (8.0 ± 2.5 min).

Heart rate and MAP during anesthesia

Immediately prior to the induction of anesthesia, HR in dogs treated with AA + MEL (100.8 ± 23.8 beats/min) or AA + ROB (93.0 ± 11.0 beats/min) was not significantly different from that in dogs treated with AA (93.0 ± 8.4 beats/min). HR in dogs treated with AA + MEL or AA + ROB showed a similar variation to that in dogs treated with AA throughout anesthesia (Fig. 1a). At 70,
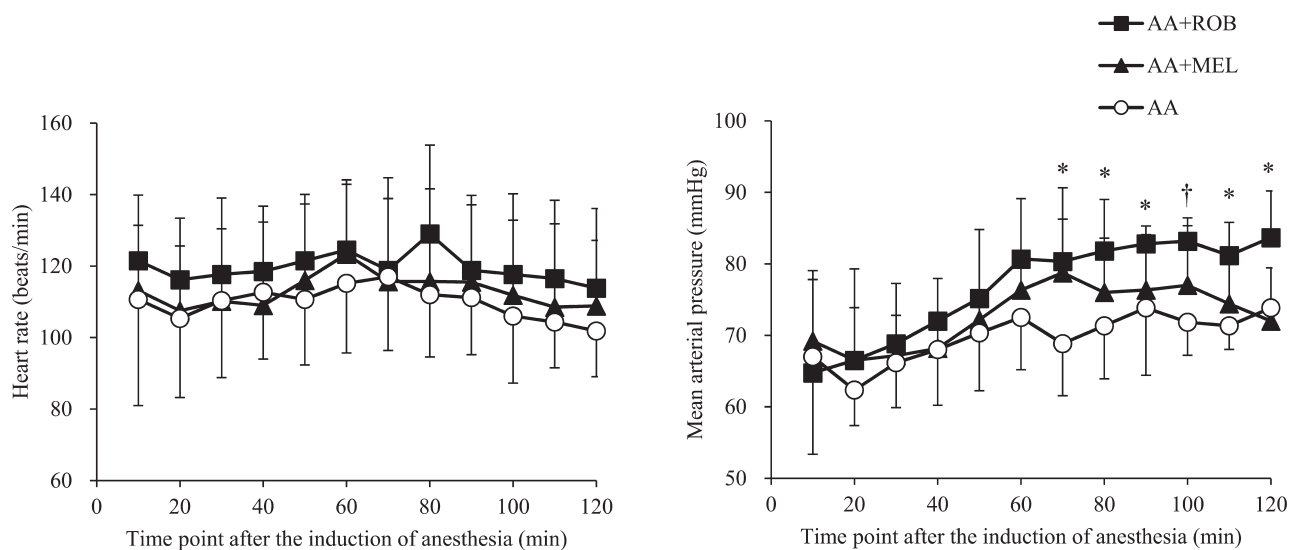
Fig. 1. Heart rate (a) and mean arterial pressure (b) during anesthesia in six beagle dogs infused with amino acids at 1.2 g/kg/hr for 60 min before anesthesia and for the first 60 min during anesthesia. Each dog underwent three different treatments: infusion of amino acids only (AA), infusion of amino acids plus subcutaneous injection of meloxicam (AA + MEL), and infusion of amino acids plus subcutaneous injection of robenacoxib (AA + ROB). Values are expressed as mean ± standard deviation. *$P<0.05$, †$P<0.01$, significantly different from AA at the same time point.

80, 90, 100, 110, and 120 min of anesthesia, MAP in dogs treated with AA + ROB was significantly higher than that in dogs treated with AA (Fig. 1b). There were no significant differences in MAP at each time point during anesthesia between dogs treated with AA and those treated with AA + MEL (Fig. 1b).

DISCUSSION

We previously demonstrated that amino acid infusion at 1.2 g/kg/hr significantly attenuated the decrease in body temperature during anesthesia in healthy dogs [21]. This finding was interpreted to be the result of the production of heat from muscle protein synthesis via an increase in plasma insulin concentration. In our previous study, the body temperature of dogs administered amino acids 120 min following anesthesia induction was 35.5 ± 0.4°C, declining 2.8°C from 38.3 ± 0.3°C at 60 min prior to induction [21]. Moreover, the body temperature of dogs administered acetated Ringer's solution 120 min following anesthesia induction was 34.3 ± 0.2°C, declining 3.9°C from 38.2 ± 0.3°C at 60 min prior to induction [21]. In the present study, the body temperature of dogs administered amino acids at 120 min following anesthesia induction was 35.5 ± 0.5°C, declining 3.1°C from 38.6°C ± 0.5°C 60 min prior to induction, which is comparable with that seen in our previous study. Therefore, although this study lacked a group of dogs administered acetated Ringer's solution as a negative control, amino acid infusion attenuated hypothermia during anesthesia.

Our present study demonstrated that subcutaneously administration of meloxicam and robenacoxib at therapeutic dose did not affect insulin secretion in unanesthetized dogs, and did not change in insulin and glucose levels following amino acid infusion. Moreover, in dogs administered meloxicam or robenacoxib, body temperature during anesthesia or the time to extubation were similar to those in dogs not administered selective COX-2 inhibitors. In dogs, meloxicam [8] and cimicoxib [9] have shown anti-pyretic action. In mice, selective COX-2 inhibitors increase insulin secretion and glucose uptake in body tissues [3, 13]. In healthy human subjects, celecoxib, which is a selective COX-2 inhibitor, increases insulin sensitivity [6]. Therefore, we predicted the probability that treatment with COX-2 inhibitors would negate or accelerate the attenuation of the decreased body temperature during anesthesia by amino acid infusion. However, this hypothesis was rejected in the present study. The reason why our hypothesis in dogs was rejected might be a difference in administration method of COX-2 inhibitors from previous reports in mouse and human. While dogs in our study were administrated COX-2 inhibitors in a single and subcutaneous dose, mouse and human in the previous studies were administrated in daily and oral dose. At least, in dogs during anesthesia, single and subcutaneous administration with meloxicam and robenacoxib at therapeutic doses could not negate the facilitation of insulin secretion by amino acid infusion.

In our previous study, amino acid infusion at 1.2 g/kg/hr significantly attenuated the reduction in HR and MAP following the induction of anesthesia [21]. Insulin has a stimulatory action on sympathetic nerves, which relates to nitric oxide in the vascular endothelium [14]. Although, in healthy dogs that are awake, it has been a report [2] that subcutaneous robenacoxib at therapeutic doses does not produce changes in HR or arterial blood pressure, the present study demonstrated that MAP in dogs treated with robenacoxib, but not meloxicam, was significantly higher throughout anesthesia than that in untreated dogs. In comparison with meloxicam, robenacoxib is highly potent against COX-2 and significantly inhibits PGE$_2$ synthesis [11]. In addition, robenacoxib,

which does not inhibit COX-1, has no impact on TXA_2 synthesis. Therefore, in anesthetized dogs, robenacoxib may elevate peripheral vascular resistance by inhibiting the production of PGE_2 and PGI_2, which act as peripheral vasodilators, without inhibition of TXA_2, which acts as a peripheral vasoconstrictor [20].

In conclusion, treatment with the selective COX-2 inhibitors, meloxicam and robenacoxib, at therapeutic doses did not affect body temperature during anesthesia in dogs administered amino acids. The present results ensure the safety of treatment with meloxicam and robenacoxib at therapeutic doses in anesthetized dogs infused amino acid.

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