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## INVOLVEMENT OF HINDBRAIN AND PERIPHERAL PROSTANOIDS IN GASTRIC MOTOR AND CARDIOVASCULAR RESPONSES TO DELTA-9-TETRAHYDROCANNABINOL IN THE RAT

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We previously reported that delta-9-tetrahydrocannabinol (delta-9-THC), the primary psychoactive constituent of *Cannabis sativa*, inhibited gastric motor activity and evoked bradycardia and hypotension upon its parenteral administration in the rat. As prostanoids are important mediators of the actions of cannabinoids, we hypothesized that the inhibitory gastric motor and cardiovascular effects of delta-9-THC could depend on cyclooxygenase (COX) activation in the hindbrain and/or in the periphery. To test this hypothesis, vehicle or delta-9-THC (0.2 mg/kg, i.v.) were administered before and 15-min after the COX inhibitor tolmetin (50 mg/kg, i.v.) or 15 min after topical application of tolmetin to the surface of the dorsal medulla (0.5 mg/rat) in chloralose-anesthetized rats. Delta-9-THC-evoked gastric motor inhibition and bradycardia were abolished by parenteral and were attenuated by hindbrain administration of tolmetin. Moreover, administration of delta-9-THC after parenteral tolmetin evoked marked and long-lasting hypertension. We concluded that the inhibitory gastric motor and cardiovascular effects of systemically administered delta-9-THC depend on the hindbrain and peripheral activation of COX.

**Key words:** *antral contractile activity, blood pressure, brainstem, cyclooxygenase, heart rate, intragastric pressure, delta-9-tetrahydrocannabinol, tolmetin*

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### INTRODUCTION

Due to their anti-inflammatory and analgesic properties, *Cannabis sativa* preparations have been used for thousands of years to treat pain and other ailments (1). Endogenous cannabinoids (or endocannabinoids), such as anandamide, were identified and are structurally and physiologically similar with phytocannabinoids, e.g. delta-9-tetrahydrocannabinol (delta-9-THC) (2). Two cannabinoid receptors have been identified and cloned, namely CB1 and CB2 (3). CB1 receptors are predominantly expressed in the central nervous system (4), but are also present in virtually all peripheral tissues, including the cardiovascular and enteric nervous system, albeit at much lower levels (5-7). In the brain, CB1 receptors have been identified in the hypothalamus (8), the dorsal vagal complex (DVC) of the hindbrain and the neighboring area postrema (9, 10). The peripheral CB2 receptor is associated with cells of the immune system, the spleen, the lymph nodes and bone marrow, as well as with adipocytes, keratinocytes and pancreatic islet cells (11). CB2 receptors have also been identified in primed microglia (12) and brainstem neurons (13).

Cannabinoids inhibit gastric motor function in the rat when given peripherally (15, 16) and provide gastric mucosal protection against ethanol- and water immersion and restrain stress (WRS)-induced lesions by the activation of CB1 receptors (16, 17).

Similarly, cannabinoid agonists inhibited gastrointestinal transit in the mice after both central and peripheral routes of administration (18). Accordingly, the synthetic cannabinoid dronabinol is used in the treatment of nausea and vomiting in cancer and AIDS patients (19).

In anesthetized rodents, delta-9-THC, anandamide and synthetic cannabinoid ligands evoke CB1 receptor-mediated hypotension and bradycardia, and depress cardiac contractility (15, 20, 21). However, novel findings indicate that the hypotensive effect of anandamide in anesthetized rats is complex, and is mediated *via* CB1, vanilloid TRPV1 and non-CB1 endothelial cannabinoid receptors, as well as *via* degradation products (22, 23).

It has been reported that endocannabinoids, structurally similar to arachidonic acid, can function as substrates for cyclooxygenase (COX) enzymes, resulting in the production of prostaglandin (PG) ethanolamides and PG glycerol esters (24). It is also clear that cannabinoids inhibit COX enzymes, but in a higher concentration range, as compared to anti-inflammatory drugs (25). Moreover, cannabinoids have been shown to affect the potency of non-steroidal anti-inflammatory drugs *via* modulation of the COX pathway (26). Delta-1-THC, another psychoactive ingredient of *Cannabis sativa*, when administered peripherally, was reported to increase brain PG E<sub>2</sub> (PGE<sub>2</sub>) levels which could be suppressed by the systemic administration of

PGE<sub>2</sub>-antiserum (27). Similarly, the intraperitoneal (i.p.) administration of delta-8-THC inhibited the lever-pressing behavior, which was significantly antagonized by both the selective cannabinoid CB1 receptor antagonist SR141716A (rimonabant) and the COX inhibitor diclofenac (28).

COX, which exists in at least two isoforms: constitutive COX-1 and inducible COX-2, catalyzes the first key step in the synthesis of all PGs by converting arachidonic acid into PGH<sub>2</sub>. In most tissues, COX-1 is expressed constitutively but can also be over-expressed, for instance by shear stress (29), while COX-2 is often induced at the site of inflammation (30). However, COX-2 is also expressed constitutively in several organs and cell types (31). For example, vascular endothelial cells constitutively express both COX isoforms (32, 33). COX-immunoreactivity is not only present in the periphery but also in the brain, including the DVC (34). Consequently, we reported that COX inhibition in the DVC evokes vagally-mediated increases in intragastric pressure and gastric smooth muscle contractile activity (35). It has also been demonstrated that PGE<sub>2</sub> receptors are located on vagal afferents (36) and that vagal sensory neurons in the nodose ganglion synthesize mRNA encoding receptors for PGE<sub>2</sub> (37). Additionally, peripherally synthesized PGs evoke *c-fos* expression in the hindbrain (38). Therefore, the main purpose of this study was to test the hypothesis that the inhibitory gastric motor and CV effects of delta-9-THC depend on COX activation not only in the periphery but also in the hindbrain.

## MATERIAL AND METHODS

Male Sprague-Dawley rats (210–340 g; Charles River Laboratories, Wilmington, MA) were used in the study and all procedures performed were with the approval of the LSUHSC Institutional Animal Care and Use Committee. Food was withheld 12 hours before experiments but the animals had free access to tap water.

The animals were initially anesthetized with a ketamine and xylazine mixture (50 and 5 mg/kg i.m., respectively) and separate cannulae were placed in the left femoral artery and vein. Afterwards,  $\alpha$ -chloralose (80 mg/kg) was administered i.v. and a tracheotomy was performed to ease respiration. Next, an intraluminal latex balloon was inserted into the stomach through an incision in the fundus for the continuous recording of intragastric pressure (IGP). A small gauge was sutured to the surface of the stomach to monitor circular smooth muscle contractile activity in the distal antrum (DACA). Heart rate (HR) was monitored by a tachograph triggered by the arterial pressure pulse (model 7P4H, Grass Instrument Co., Quincy, MA). Rectal temperature was maintained between 37.0 and 37.5°C.

The experiments were initiated at least 1.5 h after the completion of surgical instrumentation. No further manipulations were performed until stable baseline gastric and cardiovascular recordings were obtained. This was generally 2.5 h after the initial anesthetic dose.

In total, eight animals were then placed in a stereotaxic frame and, after a limited occipital craniotomy, the caudal floor of the fourth ventricle and the surrounding structures of the dorsal medulla oblongata were exposed by an incision of the dura mater and the arachnoid membrane. The opening was covered with a small gauze pad soaked in saline. Under visual control using a 3× stereoscopic eyeglass magnifier (Stereomax, A. Siebert, Wetzlar, Germany), 5  $\mu$ l aliquots of either vehicle (0.15M NaCl) or tolmetin (0.5 mg/rat) were directly applied, using a 10  $\mu$ l Hamilton micro syringe, to a cut-to-shape small piece of filtration paper positioned on the surface of the dorsomedial medulla (the presence of the piece of paper did not affect gastric motor and/or cardiovascular function); the paper

was left in place for the next 5 min. After that, it was carefully removed and the opening was once again covered with a small gauze pad soaked in saline. Delta-9-THC was administered i.v. 15 minutes after tolmetin had been applied to the surface of the dorsal medulla. The experiments were terminated after the responses to subsequently administered delta-9-THC returned to baseline. Gastric motor function, MAP and HR were continuously measured and recorded throughout the experiment.

In a separate group of animals, delta-9-THC (0.2 mg/kg) or its vehicle (see below) were administered as bolus i.v. injections through the catheter positioned in the femoral vein in a volume of 1 ml/kg. Tolmetin (50 mg/kg) was administered through the same catheter after cardiovascular and gastric motor function returned to baseline, following the first i.v. injection of delta-9-THC. Delta-9-THC was administered i.v. 15 min after tolmetin. In our experience, repeat administration of delta-9-THC at the dose of 0.2 mg/kg in the same animal more than two hours apart, produces cardiovascular and gastric motor responses of a comparable magnitude (data not shown).

Tolmetin sodium dihydrate (5-(p-toluoyl)-1-methyl-2-pyrrolyacetic acid; Sigma, St. Louis, MO) was dissolved in 0.15M NaCl. This vehicle was previously shown to not affect gastric motor and cardiovascular function when applied to the surface of the dorsal medulla (35). The dose of tolmetin applied to the dorsal surface of the medulla was chosen on the basis of our previous study (35), whereas the i.v. dose of the COX inhibitor was taken from available literature (39). Delta-9-THC, obtained from the National Institute on Drug Abuse, was dissolved in 1:1:18 (emulphor:ethanol:saline). Emulphor (Alkamuls EL-620L), a polyoxyethylated vegetable oil, was purchased from Rhone-Poulenc (Princeton, NJ). The dose of delta-9-THC was chosen to produce marked but transient cardiovascular and gastric motor responses on the basis of our previous study using a range of doses (15).

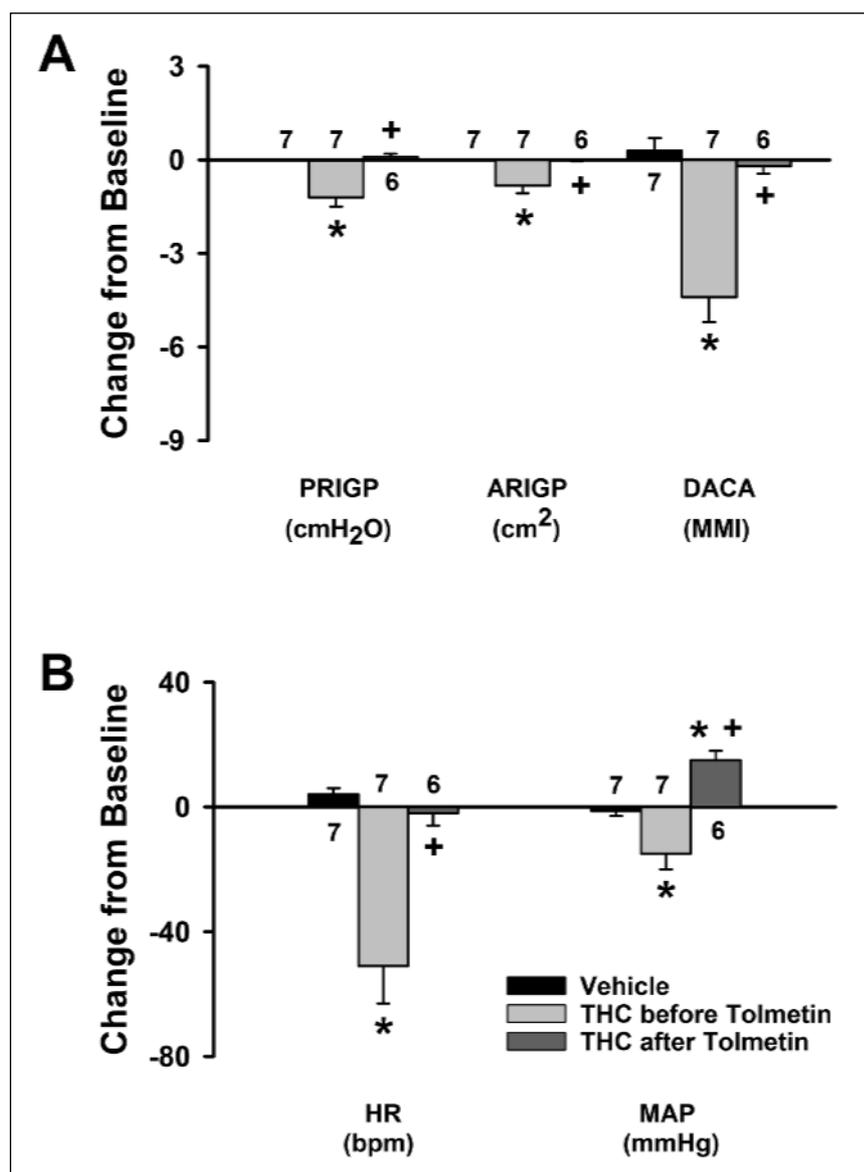
Mean arterial pressure (MAP) was calculated as a sum of one-third of the difference between systolic and diastolic pressure and the diastolic pressure. The peak change in IGP (PRIGP) was calculated as a maximum difference from baseline after drug application and the area of the response in IGP (ARIGP) for each treatment was calculated using a computerized imaging system, as reported previously (15). Distal antral contractile activity (DACA) was calculated as minute motility index (MMI) (15).

The statistical significance of differences between various treatments/groups was assessed using a paired *t*-test (cardiovascular and gastric motor effects of tolmetin) or a one-way repeated measures analysis of variance followed by the Student-Newman-Keuls test. Values of *P* < 0.05 were considered statistically significant.

## RESULTS

### *Effect of systemic COX blockade on gastric motor and cardiovascular effects of delta-9-THC*

The effects of vehicle and delta-9-THC (0.2 mg/kg, i.v.) on gastric motor activity and cardiovascular function, before and after i.v. administration of tolmetin (50 mg/kg), are shown in *Fig. 1*. The administration of delta-9-THC before tolmetin resulted in significant decreases in IGP (both PRIGP and ARIGP) and DACA (*Fig. 1A*) as well as HR and MAP (*Fig. 1B*) when compared with the effect of vehicle. However, when delta-9-THC was administered 15 min after tolmetin, inhibitory gastric motor responses and bradycardia were abolished. Moreover, a marked hypertension was observed when delta-9-THC was administered after tolmetin.



*Fig. 1.* Cardiovascular and gastric motor effects of vehicle or delta-9-THC at a dose of 0.2 mg/kg i.v. before and 15 min after tolmetin (50 mg/kg; i.v.). (A) Illustrates compiled data showing effects of delta-9-THC on intragastric pressure (peak response; PRIGP and area of the response; ARIGP) and distal antral contractile activity (DACA). DACA values reflect changes which occurred within 3 min after injections. (B) Illustrates changes in heart rate (HR) and mean arterial pressure (MAP) responses. The number of animals is indicated below or above each bar. \*Statistically significant when compared with the effect of vehicle. †Statistically significant when compared with the effect of delta-9-THC before tolmetin.

Tolmetin alone (at a dose of 50 mg/kg, i.v.), evoked gastric motor excitation and tachycardia when compared with the effect of vehicle but did not affect blood pressure in the same animals (Table 1).

*Effect of COX blockade in the lower brainstem on gastric motor and cardiovascular effects of delta-9-THC*

The effects of i.v. injection of vehicle and delta-9-THC (0.2 mg/kg) on gastric motor activity and cardiovascular function before and after application of tolmetin to the surface of the dorsal medulla (0.5 mg/rat), are shown in Fig. 2. Administration of delta-9-THC before tolmetin resulted in decreases in IGP (both PRIGP and ARIGP) and DACA (Fig. 2A) as well as in bradycardia and hypotension (Fig. 2B) when compared with the effect of vehicle. However, when delta-9-THC was administered 15 minutes after tolmetin had been applied to the surface of the dorsal medulla, the effects of the cannabinoid on intragastric pressure, DACA and HR were significantly lower when compared with the effects of delta-9-THC before tolmetin. Tolmetin applied to the surface of the dorsal medulla did not affect delta-9-THC-evoked decrease in MAP.

Tolmetin alone (0.5 mg/rat), applied to the surface of the dorsal medulla, evoked gastric motor excitation when compared with the effects of vehicle but did not affect cardiovascular function (Table 2). The gastric motor excitation in response to tolmetin returned to baseline within 10 minutes after its topical application.

## DISCUSSION

Our experiments demonstrated that both peripheral and hindbrain inhibition of COX affects gastric motor and bradycardic responses to systemically administered delta-9-THC.

There are numerous reports in the literature linking the effects of delta-9-THC action to PG formation. Burstein *et al.* (40) were the first to report that aspirin was able to inhibit delta-1-THC-induced hypotension. Later on, anandamide and delta-9-THC-evoked dilation of rabbit cerebral arterioles was found to be blocked by indomethacin, a non-selective COX inhibitor, suggesting that both endo- and phytocannabinoids enhance the release and metabolism of endogenous arachidonic acid (41). Delta-9-THC was also reported to increase PGE<sub>2</sub> in human

*Table 1.* Effects of i.v. injection of vehicle and tolmetin (50 mg/kg) on intragastric pressure (peak response; PRIGP and area of the response; ARIGP) and distal antral contractile activity (DACA) as well as on heart rate (HR) and mean arterial pressure (MAP) maximum responses. Values are means  $\pm$ S.E. for the number (n) of animals.

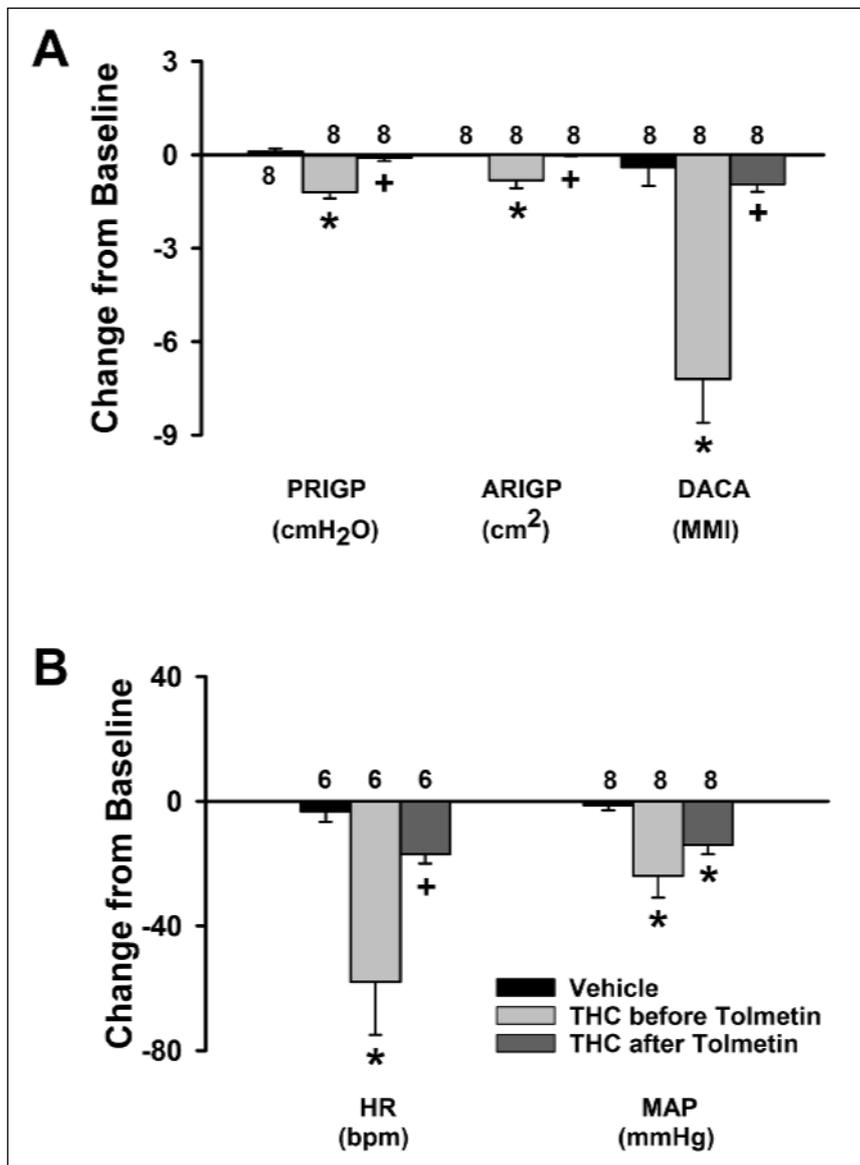
Treatment	PRIGP (cmH <sub>2</sub> O)	ARIGP (cm <sup>2</sup> )	DACA (MMI)	HR (bpm)	MAP (mmHg)
Vehicle	0.0 $\pm$ 0.0 (6)	0.1 $\pm$ 0.1 (6)	0.4 $\pm$ 0.3 (6)	-1 $\pm$ 3 (5)	4 $\pm$ 2 (5)
Tolmetin	1.3 $\pm$ 0.5 <sup>a</sup> (6)	1.8 $\pm$ 0.6 <sup>a</sup> (6)	3.0 $\pm$ 1.0 <sup>a</sup> (6)	10 $\pm$ 3 <sup>a</sup> (6)	2 $\pm$ 1 (6)

<sup>a</sup>Statistically significant when compared with corresponding mean for vehicle injection.

*Table 2.* Effects of vehicle and tolmetin (0.5 mg/rat) applied to the surface of the dorsal medulla on intragastric pressure (peak response; PRIGP and area of the response; ARIGP) and distal antral contractile activity (DACA) as well as on heart rate (HR) and mean arterial pressure (MAP) maximum responses. Values are means  $\pm$ S.E. for the number (n) of animals.

Treatment	PRIGP (cmH <sub>2</sub> O)	ARIGP (cm <sup>2</sup> )	DACA (MMI)	HR (bpm)	MAP (mmHg)
Vehicle	0.1 $\pm$ 0.2 (6)	0.0 $\pm$ 0.0 (6)	0.1 $\pm$ 0.1 (5)	2 $\pm$ 1 (6)	1 $\pm$ 2 (6)
Tolmetin	1.8 $\pm$ 0.6 <sup>a</sup> (6)	0.4 $\pm$ 0.1 <sup>a</sup> (6)	2.0 $\pm$ 0.7 <sup>a</sup> (6)	1 $\pm$ 2 (5)	2 $\pm$ 2 (5)

<sup>a</sup>Statistically significant when compared with corresponding mean for vehicle injection.



*Fig. 2.* Cardiovascular and gastric motor effects of vehicle or delta-9-THC at a dose of 0.2 mg/kg i.v. before and 15 min after tolmetin (0.5 mg/rat) applied to the surface of the dorsal medulla. (A) Illustrates compiled data showing effects of delta-9-THC on intragastric pressure (peak response; PRIGP and area of the response; ARIGP) and distal antral contractile activity (DACA). DACA values reflect changes which occurred within 3 min after injections. (B) Illustrates changes in heart rate (HR) and mean arterial pressure (MAP) responses. The number of animals is indicated below or above each bar. \*Statistically significant when compared with the effect of vehicle. +Statistically significant when compared with the effect of delta-9-THC before tolmetin.

plasma (42). Since prostanoids, especially PGE<sub>2</sub>, are known to inhibit gastric motor activity (43), it is not surprising that peripherally administered tolmetin blocked gastric motor inhibition evoked by delta-9-THC in our experiments.

Our previous study showed that the prolonged depressor and bradycardic responses to i.v. delta-9-THC were attenuated by SR141716A, indicating that SR141716A-sensitive CB1 receptors are involved in these responses (15). So far, the mechanisms by which cannabinoids elicit the depressor effect have not been well defined. CB1 receptors are abundantly expressed in the central and peripheral nervous system, suggesting that cannabinoids may influence cardiovascular function *via* modulating autonomic outflow in both the central and peripheral nervous systems. Accumulated evidence has demonstrated that presynaptic CB1 receptors on sympathetic nerve terminals inhibit norepinephrine release (44, 45). In addition, CB1 receptors expressed in the vasculature and myocardium directly mediate vasodilation and negative inotropy (46). All of these actions may contribute to the depressor and bradycardic effects of delta-9-THC.

However, the question remained whether the inhibitory gastric motor and/or cardiovascular effects of delta-9-THC were mediated through PGs released by the cannabinoid in the periphery, centrally or both. This was because peripherally administered delta-9-THC was shown to evoke PGs increases in the rat's brain (47). Moreover, PGs of a peripheral origin evoke *c-fos* expression in the hindbrain following the noxious stimulation (38) and both PGE<sub>2</sub> and iloprost (a stable prostacyclin analogue) receptors were found in both the area postrema (a circumventricular organ allowing the blood-borne action of circulating agents) and neighboring nucleus of the solitary tract (NTS) (36, 48), and PGE<sub>2</sub> is known to modulate synaptic transmission in the NTS (49). In this study, we did not examine specific types of PGs that may be involved in gastric motor and cardiovascular responses to delta-9-THC; it can only be concluded that in general, the formation of PGs by COX is required for the effects of delta-9-THC.

Central administration of PGE<sub>2</sub> evokes inhibition of gastrointestinal motility in rats and dogs (50). Indeed, we observed vagally-mediated increases in intragastric pressure and gastric smooth muscle contractile activity after microinjection of tolmetin directly into the DVC (35). If circulating delta-9-THC releases PGE<sub>2</sub> from perivascular cells in the medulla, as shown for interleukin-1 (IL-1) (51), it may explain why tolmetin, applied directly to the surface of the dorsal medulla, is able to attenuate inhibitory gastric motor and bradycardic effects of peripherally administered delta-9-THC. Since the central dose of tolmetin used in the current study was 20–30 times lower than the peripheral one and it was kept in place by the use of a piece of filtration paper, it is highly unlikely that the COX inhibitor could also work in the periphery. In fact, application of tolmetin directly to the surface of the dorsal medulla evoked gastric motor excitation but, unlike its peripheral administration, was unable to affect cardiovascular function. Based on our earlier study (35), the neighboring DVC is the most likely site of action for tolmetin in the medulla.

Tolmetin was initially reported to produce its analgesic action mainly by a peripheral mechanism with no effect on the central nervous system (52, 53). However, according to other investigators, tolmetin does cross the blood-brain barrier but its concentration in the brain after i.p. administration is much lower than that in the i.p. exudates (54). Indeed, the peripheral administration of tolmetin was recently reported to result in a reduction of quinolinic acid-induced spatial reference memory deficits (55) and in the alteration of brain neurotransmitter levels (56). Therefore, it is highly possible that the effects of peripherally administered tolmetin in the present study could have been mediated both peripherally and, at least in part,

centrally. However, it is unclear which brain structures, besides the DVC, could have been affected.

Since PGs facilitate the release of the excitatory amino acid L-glutamate in the brain (57), we speculate that the inhibitory gastric motor effects of peripherally administered delta-9-THC may be also mediated through L-glutamate released by PGE<sub>2</sub> in the NTS. This is because L-glutamate is a neuromodulator in the vagus nerve sensory afferents terminating in the NTS (58) and, when microinjected into the NTS, the amino acid inhibits gastric motility (unpublished observations). This premise is supported by the recent demonstration of the stimulatory effect of L-glutamate on COX (59). Additional experiments are needed to test this hypothesis.

The CB1 receptors are located within the enteric nervous system (6) and nodose ganglion (60) as well as within the central nervous system (61). The i.p. administration of delta-8-THC, an isomer of the naturally occurring delta-9-THC, was shown to stimulate PGE<sub>2</sub> synthesis in the brain (28). Therefore, one could speculate that delta-9-THC-evoked gastric motor and cardiovascular inhibition is caused by enhanced PG synthesis in the DVC in response to the activation of cannabinoid receptors which have been localized at this site (60, 62). However, the topical application of delta-9-THC to the surface of the dorsomedial medulla, which is supposed to predominantly affect neurons in the DVC, produces only transient and moderate, albeit significant, inhibition of gastric motor and cardiovascular function (15). Intracerebroventricular administration of the synthetic cannabinoid agonist WIN 55,212-2 was reported to decrease gastrointestinal transit in mice and ED<sub>50</sub> values after central administration were significantly lower than the corresponding ED<sub>50</sub> values after systemic administration (18). However, the gastrointestinal effects of i.p. injected WIN 55,212-2 were not affected by the ganglionic blocker hexamethonium and, therefore, were most likely mediated by peripheral CB1 receptors (14).

There exists a strong correlation between gastric motility and stress-induced gastric ulcer formation (63). The recent study of Warzecha *et al.* (17) investigating the gastroprotective effect of peripherally administered anandamide in WRS-induced gastric lesions, revealed that anandamide reduced the WRS-evoked increase in serum level of the pro-inflammatory cytokine IL-1 $\beta$ . Consequently, a selective CB1 receptor antagonist increased gastric ulcer areas in rats with intact sensory nerves and abolished the protective effect of anandamide (17). One important action of IL-1 $\beta$  is to liberate arachidonic acid and induce the production of PGs *via* COX (64). Indeed, circulating IL-1 $\beta$ , which has low diffusion across the blood brain barrier, has been shown to upregulate COX activity within brain vascular endothelium causing the release of PGs into brain parenchyma (65). IL-1 $\beta$  within brain parenchyma can also cause the release of PGs from brain glial cells (66). Thus, PGs are in a position to mediate some of the effects of IL-1 $\beta$  within the brain.

The contribution of PGs in vagal afferent transmission was demonstrated in several studies. For example, sensory neurons in the nodose ganglion express receptors to PGE<sub>2</sub> (36, 37) and PGs exhibit multifaceted regulatory actions on vagal afferents transmitting cardiac, baroreceptor, and pulmonary sensory information (67, 68). Intra-left cardiac injection of PGE<sub>2</sub> was demonstrated to activate central autonomic neuronal circuitries originating in the NTS (a well characterized primary target of viscerosensory information transmitted *via* the vagal and glossopharyngeal nerves) (69). It has also been reported that intact sensory nerves are needed for peripherally administered anandamide to exert its gastroprotective effect in rats exposed to WRS (17). Specifically, ablation of sensory nerves by capsaicin only partly attenuated the gastroprotective effect of peripherally administered high doses (1.5 and 3.0  $\mu$ mol/kg) of anandamide.

On the other hand, anandamide given at a low dose of 0.3  $\mu\text{mol/kg}$  was ineffective in protecting gastric mucosa against WRS in rats with ablated sensory nerves but was still effective in rats with intact sensory nerves (17). Therefore, it is highly possible that vagal afferent transmission is also involved in the cardiovascular and gastric motor responses to PGs released by peripherally administered delta-9-THC. Additional experiments are needed in order to prove this hypothesis.

In summary, inhibitory gastric motor and bradycardic effects of parenterally administered delta-9-THC in the rat depend on COX activation in the hindbrain and in the periphery. The mechanisms of the opposite hypertensive effects of delta-9-THC after peripheral COX blockade require further investigation.

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