Interaction of Prostaglandin E\(_2\) and Bradykinin in the Induction of Afferent Splanchnic Nerve Discharges in Cats

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Abstract—In anesthetized cats, the administration of either bradykinin (BK) (0.3–3 \(\mu\)g/kg) or prostaglandin E\(_2\) (PGE\(_2\)) (1–30 \(\mu\)g/kg) into the cranial mesenteric artery dose-dependently evoked the firing discharge in the proximal end of the afferent greater splanchnic nerves with a latency of about 10 sec, the pronounced contraction of the longitudinal muscle of the jejunum, and changes in blood pressure. PGE\(_2\) at the doses of 1–10 \(\mu\)g/kg i.a. potentiated the BK (1 \(\mu\)g/kg i.a.)-induced firing discharge of the afferent splanchnic nerves of which latency was also shortened, but did not alter the BK-induced jejunal contraction and blood pressure change. However, trimoprostil (30 \(\mu\)g/kg i.a.), a trimethyl PGE\(_2\) derivative, did not change all of these responses to BK. Aspirin at 50 mg/kg i.v. markedly prevented the BK-induced nerve discharges, but not the BK-induced jejunal contraction. These results taken together indicate that PGE\(_2\) may be involved in the facilitatory response of afferent splanchnic nerve discharges to BK, but not involved in the BK-induced jejunal contraction and blood pressure change. The findings on trimoprostil present the possibility that derivatization of PGE\(_2\) could modify its inherent ability to produce adverse side-effects.

Prostaglandins E\(_1\) and E\(_2\) (PGE\(_1\), PGE\(_2\)) and their synthetic derivatives were highly effective in inhibiting gastric acid secretion and on protecting the gastric mucosa. However, these prostaglandins are known to cause abdominal pain (1–3), diarrhea (1, 3, 4), nausea and emesis (3, 4) in human subjects.

In addition to the well known potentiating effects of the PGE series on the action of pain-inducing substances (5–8), their direct intradermal or intra-articular injection also causes hyperalgesia (9, 10). Nevertheless, the effects of PGE\(_2\) on the abdominal pain model of animals have not been fully elucidated. Trimoprostil (trimethyl PGE\(_2\)) is known to inhibit gastric hypersecretion and lesion more than PGE\(_2\) and to elicit diarrhea less than PGE\(_2\) (11).

The present experiment was designed to determine whether the local injection of PGE\(_2\) and trimoprostil might elicit the afferent splanchnic nerve discharge and jejunal contraction and interact with the effects of bradykinin (BK).

Materials and Methods

Eleven cats of either sex weighing 3.2–4.2 kg were subjected to experiments after a period of 18 hr-fasting. Under ether anesthesia, cannulae were implanted to the trachea (for artificial respiration), femoral artery (for blood pressure) and femoral vein (for maintenance of anesthesia). Animals were then anesthetized by pentobarbital sodium (20 mg/kg i.v. at first and 2.5 mg/kg i.v./hour infusion) and immobilized by alcuronium chloride (0.2 mg/kg i.v. and 0.2 mg/kg i.v./hour infusion) and ventilated artificially. A polyethylene tube (PE50, Clay Adams) was inserted into the cranial mesenteric artery from the caudal pancreaticoduodenal artery for the arterial injections of test drugs. For measurement of
intestinal motility, a strain-gauge arch (Nihon Koden, TH-612T) was sewn to the upper part of the jejunum in the area supplied by the cranial mesenteric artery. The left greater splanchnic nerve was exposed about 1.5 cm above the celiac ganglia, and the sheath was stripped off for the recording. The nerve was cut proximally, and the electrical activity was picked up by a bipolar platinum electrode and led into a dual beam oscilloscope (Nihon Koden, VC-9) and photographed on film with a polaroid camera. The signals were also stored on a TEAC R-410 data recorder for further analysis. The data were analyzed to determine the frequency of firing of units (spike/sec) during spontaneous activity and response to injections of bradykinin and prostaglandins with a Signal Processor (San-ei, 7S06-A) and plotted by an X-Y recorder.

Prostaglandin E2 (PGE2, Ono Pharmaceutical Co. Ltd.) and trimoprostil (11R,16,16-trimethyl-15R-hydroxy-9-oxoprostasis-5-trans-13-dienoic acid, Hoffmann-La Roche) were dissolved in absolute ethanol to make a 10 mg/ml stock solution and then diluted with saline to make final concentrations of 30, 100, 300 and 1000 µg/ml. Bradykinin (BK, Protein Research Foundation, Osaka) was dissolved in saline to concentrations 10, 30 and 100 µg/ml. These drugs (0.3–30 µg/kg) were administered into the cranial mesenteric artery. To determine the potentiating effects of PGE2 or trimoprostil on BK-induced splanchnic nerve discharge, PGE2 or trimoprostil (1, 3, 10 µg/kg) was given together with BK (1 µg/kg) via the same cannula. Aspirin suspended in 0.1% carboxymethylcellulose (CMC) solution was gradually administered into the femoral vein at 50 mg/kg.

The rate of increased firing integrated for each period of 30 sec was calculated from the difference between enhanced and control spike counts immediately before the intra-arterial injection. The latency of the agent effect was determined as the time interval from the immediate time after intra-arterial injection of the agent to the time when spike counts reached over 150% of the mean pre-administration value. The duration was determined as the time interval from the moment when firing increased to the time when spike counts returned to the pre-administration value.

Results

Effects of BK, PGE2 and trimoprostil on the activity of afferent splanchnic nerve and longitudinal jejunal contraction: It was examined whether BK, PGE2 and trimoprostil might produce visceral pain, the effects of these compounds on the electrical activity of the afferent greater splanchnic nerves.

The administration of 0.03 ml/kg saline or 10% ethanol in saline (vehicle for both prostaglandins at the maximal dose) into the cranial mesenteric artery caused no changes in the slight background activity of the splanchnic nerve. The injection of BK (3 µg/kg i.a.) significantly (P<0.01, n=7) evoked a discharge in the greater splanchnic nerve with the increased firing rate of 366±81% (integrated for the period of 30 sec), the latency of 10.4±1.2 sec and the duration of 38.7±2.8 sec; and it simultaneously induced the pronounced contraction of the upper part of the jejunal longitudinal muscle (Fig. 1, Table 1). BK (0.3–3.0 µg/kg i.a.) elicited a transient fall in femoral blood pressure followed by a 3 min rise in the blood pressure and heart rate.

The intra-arterial injection of PGE2 (30 µg/kg) also evoked the splanchnic nerve discharge (the rate of increased firing was 262±42%, P<0.01, n=4), the jejunal contraction and a fall of blood pressure accompanied with tachycardia (Fig. 2, Table 1). However, trimoprostil (3–30 µg/kg i.a.) caused no changes in the nerve activity and intestinal motility, while the prostanoid slightly and transiently elevated blood pressure (Fig. 3, Table 1).

Potentiating effects of PGE2 and trimoprostil on the BK-induced afferent splanchnic nerve discharges: PGE2 (1 and 3 µg/kg i.a.) did not produce any appreciable changes in the splanchnic nerve activities and cardiovascular parameters (Table 1). However, the higher dose of 10 µg/kg i.a. of PGE2 slightly increased the firing rate (44.8±13.7%, P<0.1, n=4) and jejunal motility, and evoked a fall of blood pressure with tachycardia (Fig. 4, Table 1). These doses of PGE2 produced a potentiation of the
Table 1. Effects of bradykinin, prostaglandin E₂ and trimoprostil on spontaneous discharges of the afferent splanchnic nerves

<table>
<thead>
<tr>
<th>Drugs</th>
<th>No. of animals</th>
<th>Splanchnic nerve discharge</th>
<th>Jejunal contraction</th>
<th>Blood pressure (mmHg)</th>
<th>Heart rate (beats/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(μg/kg i.a.)</td>
<td>increase in firing rate&lt;sup&gt;a&lt;/sup&gt; (%</td>
<td>latency&lt;sup&gt;b&lt;/sup&gt; (sec)</td>
<td>duration&lt;sup&gt;c&lt;/sup&gt; (sec)</td>
<td>primary</td>
</tr>
<tr>
<td>Bradykinin</td>
<td>(0.3)</td>
<td>0</td>
<td>—</td>
<td>—</td>
<td>1.76±0.44</td>
</tr>
<tr>
<td></td>
<td>(1.0)</td>
<td>65.2±14.9&lt;sup&gt;†&lt;/sup&gt;</td>
<td>9.38±0.80</td>
<td>32.3±2.1</td>
<td>4.71±0.72&lt;sup&gt;***&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>(3.0)</td>
<td>366 ±81.4&lt;sup&gt;***&lt;/sup&gt;</td>
<td>10.4 ±1.19</td>
<td>38.7±2.8</td>
<td>9.24±2.55*</td>
</tr>
<tr>
<td>Prostaglandin E₂</td>
<td>(1)</td>
<td>20.0±15.0</td>
<td>—</td>
<td>—</td>
<td>3.74±2.86</td>
</tr>
<tr>
<td></td>
<td>(3)</td>
<td>29.0±13.6</td>
<td>9.00±1.00</td>
<td>30.0±0</td>
<td>4.25±3.03</td>
</tr>
<tr>
<td></td>
<td>(10)</td>
<td>44.8±13.7&lt;sup&gt;†&lt;/sup&gt;</td>
<td>9.67±0.33</td>
<td>36.7±3.5</td>
<td>7.57±3.30†</td>
</tr>
<tr>
<td></td>
<td>(30)</td>
<td>262 ±42.2&lt;sup&gt;**&lt;/sup&gt;</td>
<td>9.75±1.18</td>
<td>48.0±6.6</td>
<td>8.93±3.71†</td>
</tr>
<tr>
<td>Trimoprostil</td>
<td>(3)</td>
<td>0</td>
<td>—</td>
<td>0</td>
<td>-6.5 / -6.0</td>
</tr>
<tr>
<td></td>
<td>(10)</td>
<td>0</td>
<td>—</td>
<td>0</td>
<td>-7.0 / -8.5</td>
</tr>
<tr>
<td></td>
<td>(30)</td>
<td>18.0±12.7</td>
<td>—</td>
<td>1.32±0.78</td>
<td>-6.0 / -5.5</td>
</tr>
</tbody>
</table>

All drugs were administered into the cranial mesenteric artery in lightly anesthetized cats. Data are means±S.E.M. or means obtained from the number of animals shown in the Table. <sup>a</sup>: The initial firing rate (means±S.E.M.) was 2.23±0.21 spikes/sec for 39 preparations. The rate of increased firing integrated for each period of 30 sec was calculated from the difference between the enhanced spike counts and control spike counts immediately before the intravenous injection. <sup>b</sup>: The latency of the drug effect was determined as the time interval from the immediate time after injection of the drug to the time when spike counts reached over 150% of the mean pre-administration value. <sup>c</sup>: The duration was determined as the time interval from the immediate time when firing increased to the time when spike counts returned to the pre-administration value, provided it was over 3 sec at least. <sup>†</sup>P<0.1, <sup>‡</sup>P<0.05, <sup>***</sup>P<0.01, <sup>****</sup>P<0.001. Paired Student's t-test was used to compare the values of pre-drugs and of post-drugs.
### Table 2. Enhancing effects of prostaglandin E₂ on bradykinin-induced splanchnic nerve discharges

<table>
<thead>
<tr>
<th>Drugs</th>
<th>No. of animals</th>
<th>Splanchnic nerve discharge</th>
<th>Jejunal contraction</th>
<th>Blood pressure (mmHg)</th>
<th>Heart rate (beats/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>increase in firing rate (%)</td>
<td>latency (sec)</td>
<td>duration (sec)</td>
<td>primary/secondary</td>
</tr>
<tr>
<td>Bradykinin+Saline</td>
<td>(1) 9</td>
<td>65.2±14.9</td>
<td>9.4±0.8</td>
<td>32.3±2.1</td>
<td>-14.6/-13.9/+11.0/+18.0</td>
</tr>
<tr>
<td>+Prostaglandin E₂</td>
<td>(1) 7</td>
<td>115.1±31.7*</td>
<td>9.5±0.99</td>
<td>38.5±4.0</td>
<td>-12.1/-10.3*/+8.3/+17.3</td>
</tr>
<tr>
<td></td>
<td>(3) 7</td>
<td>197.3±48.5*</td>
<td>6.6±1.5*</td>
<td>38.3±3.9</td>
<td>-13.1/-12.7/+6.8/+17.2</td>
</tr>
<tr>
<td></td>
<td>(10) 6</td>
<td>247.7±60.9*</td>
<td>5.0±0.8**</td>
<td>39.2±4.1</td>
<td>-11.2/-10.0/+10.6/+18.8</td>
</tr>
<tr>
<td>Trimoprostil</td>
<td>(30) 3</td>
<td>65.3±23.2</td>
<td>8.3±0.9</td>
<td>32.0±2.0</td>
<td>-12.7/-10.7/+10.3/+10.0</td>
</tr>
</tbody>
</table>

All drugs were administered into the cranial mesenteric artery. Data are means±S.E.M. or means obtained from the number of animals shown in the Table. a: The initial firing rate (mean±S.E.M.) was 2.11±0.24 spikes/sec for 32 preparations. The rate of increased firing integrated for each period of 30 sec was calculated from the difference between enhanced spike counts and control spike counts immediately before the intra-arterial injection. b: The latency of the drug effect was determined as the time interval from the immediate time after injection of the drug to the time when spike counts reached over 150% of the mean pre-administration value. c: The duration was determined as the time interval from the immediate time when firing increased to the time when spike counts returned to the pre-administration value, provided it was over 3 sec at least. *P<0.05, **P<0.01. Paired Student's t-test was used to compare response of the control group (bradykinin 1 µg/kg+saline i.a.) and the prostaglandins treated group (1 µg/kg bradykinin+prostaglandin E₂ or trimoprostil i.a.)
BK (1 μg/kg i.a.)-induced excitatory action on the firing rate of the splanchnic nerve by 65.2±14.9% (P<0.1, n=9) (Fig. 4, Table 2). The potentiating action of PGE₂ was dose-dependent (1–10 μg/kg i.a.). The mean control value for the latency of the nerve response to BK was 9.4±0.8 sec, while after the simultaneous administration of PGE₂ at doses of 3 and 10 μg/kg i.a., latencies were significantly shortened to 6.6±1.5 sec (P<0.05, n=7) and 5.0±0.8 sec (P<0.01, n=6), respectively. Little change in the duration of the response to BK was found (Table 2). The BK-induced cardiovascular changes and jejunal motility were not significantly affected with PGE₂. On the other hand, the simultaneous administration of trimoprostil (30 μg/kg i.a.) did not change the BK-induced activation of the afferent splanchnic nerve discharge (Fig. 4, Table 2).

Effects of aspirin on BK-induced splanchnic nerve discharges and jejunal contraction: The effect of aspirin was examined on the BK-induced splanchnic nerve discharges and longitudinal jejunal contraction. In 6 anesthetized cats, the pretreatment with aspirin (50 mg/kg i.v.) consistently decreased the splanchnic nerve discharge produced by the intraarterial administration of BK at 30, 60 and 120 min later (Fig. 5, Table 3), but did not alter the jejunal contraction (Fig. 6).
Fig. 2. Effect of the administration of prostaglandin E₂ into the cranial mesenteric artery on spontaneous discharges of the afferent splanchnic nerve in an anesthetized cat (No. 123).

Table 3. Inhibitory effects of aspirin on bradykinin-induced increase in the splanchnic nerve discharges in anesthetized dogs

<table>
<thead>
<tr>
<th>Time (min) after an intravenous administration</th>
<th>Aspirin 50 mg/kg i.v. (6)</th>
<th>Jejunal contraction (Jg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Increase in firing rate[(%)</td>
<td>latency (sec)</td>
</tr>
<tr>
<td>Bradykinin</td>
<td>311±79₁</td>
<td>10.33±1.91</td>
</tr>
<tr>
<td>+ Aspirin 30 min</td>
<td>83±30*</td>
<td>10.30±2.75</td>
</tr>
<tr>
<td>+ Aspirin 60 min</td>
<td>108±20*</td>
<td>13.00±3.87</td>
</tr>
<tr>
<td>+ Aspirin 90 min</td>
<td>116±34*</td>
<td>10.25±0.75</td>
</tr>
<tr>
<td>+ Aspirin 120 min</td>
<td>169±63</td>
<td>9.17±1.30</td>
</tr>
</tbody>
</table>

Bradykinin at the dose of 3 μg/kg was administered into the cranial mesenteric artery. Data are means ±S.E.M. obtained from 6 animals. *: The initial firing rate was 2.20±0.42 spikes/sec (6), while bradykinin i.a. increased the rate to the level of 8.70±1.90 spikes/sec (6). *: % increase in firing rates as compared to untreated-initial controls. *: P<0.05 compared with bradykinin controls before the intravenous administration of 50 mg/kg aspirin.
**Disc**ussion

BK has been known to possess strong algesic properties in the peripheral sites (12, 13). Nociceptive visceral messages induced by the injection of algogenic substances (BK, ACh) into the inferior mesenteric artery converge on dorsal horn cells receiving noxious cutaneous afferents in spinal cats (14). The splanchnic nerve includes the thin afferent fibers belonging to the Aδ and C groups which conduct the splanchnic pain to the dorsal horn (15).

PGE₂ causes abdominal pain in humans (2, 3). Many investigators have emphasized the involvement of prostaglandins in the production of pain, and the inhibition of their synthesis has been suggested as the mechanism by which aspirin produces analgesia (6, 7, 16, 17). PGE₁ and PGE₂ are known to enhance nociceptive responses by BK at the peripheral site (5, 6, 8, 18). The present investigation reveals that PGE₂ at the large doses evokes multi-fiber activity in the afferent splanchnic nerves. The non-effective small doses of PGE₂ were found to enhance the BK-induced responses. However, PGE₁ and PGE₂ are reported to have no effect on the central activity of nociceptive dorsal horn neurons (17) and on the excitatory nociceptive cardiac reflex (8, 16). Therefore, the role of prostaglandins in the production of pain may be the sensitization of receptors to the effects of algesic agents.
rather than stimulating the receptors themselves. The facilitatory response of the large doses of PGE$_2$ may be due to the hypersensitization of receptors to the effects of all endogenous algogenic substances such as BK, ACh, histamine, 5-hydroxytryptamine, and substance P.

It is also reported that BK facilitates the
release of PGE-like substances, and this release is suppressed by aspirin-like drugs (8, 16, 19). Furthermore, systemic administration of aspirin abolished the BK-induced responses (5, 20). It is of interest in the present finding that BK-induced afferent splanchnic nerve discharges were markedly decreased by the pretreatment with aspirin. These results indicate that the BK-induced abdominal pain may be mediated at least partly by prostaglandins. Lim et al. (20) reported that the injection of BK into the splenic artery elicited splanchnic nerve discharges in the spinal cat and dog and vocalization in conscious dogs, which were blocked by aspirin and not by morphine. These findings taken together suggest that PGE2 is involved in these actions of BK.

Trimoprostil, which reportedly inhibited gastric acid secretion (21–23) and gastric lesion (11), did not evoke the afferent splanchnic nerve discharges and did not
potentiate the BK-induced facilitation.

As for the activation of longitudinal jejunal contractility with PGE\textsubscript{2} or BK, it is quite interesting to note that PGE\textsubscript{2} does not potentiate the BK-induced contractility. Aspirin had no appreciable effect on the BK-induced jejunal contraction. These findings suggest that prostaglandins are not involved in the jejunal contraction caused with BK. PGE\textsubscript{2} dose-dependently (1–30 \(\mu\)g/kg i.a.) increased the amplitude of jejunal motility, whereas trimoprostil did not alter the jejunal motility. Indeed, PGE\textsubscript{2} given p.o. to mice enhanced their intestinal transport of a food mixture and produced diarrhea more extensively than trimoprostil (11). The PGE series was reported to increase the jejunal pressure in vivo (24) and contraction of the longitudinal muscle (25–28). These effects of PGE\textsubscript{2} may be causally related to the
incidence of diarrhea.

In conclusion, PGE2 may be involved in the BK-elicited discharges of the afferent splanchnic nerves. The present findings on trimoprostil show the possibility that derivatization of PGE2 could modify its inherent ability to evoke adverse side-effects.

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