EFFECTS OF INTRA-ARTERIAL BRADYKININ AND SUBSTANCE P ON ISOLATED, BLOOD-PERFUSED SMALL INTESTINE OF THE RAT

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Abstract—Experiments were conducted on rat isolated, small intestine perfused at a fixed flow rate through the superior mesenteric artery with arterial blood from a donor rat, to determine the responses of the ileum to different peptides. Drugs were closely injected into the superior mesenteric artery. Single injections of bradykinin produced monophasic fast contractions of the ileum preceded by an initial fall and a subsequent rise of tone. The fast contraction was abolished by tetrodotoxin (TTX), morphine and hexamethonium (C6), but was resistant to blockade by atropine or mepyramine. Changes in ileal tone induced by bradykinin remained evident even in the presence of these blocking agents, thereby suggesting a direct action on smooth muscle fibers of the ileum. The fast contraction in response to substance P was not influenced by either TTX, morphine, C6, atropine or mepyramine. The present results indicate that bradykinin induces the fast contraction of the ileum by excitation of myenteric neuronal elements involving cholinergic interneurons, while substance P produces the contraction by a direct stimulation of smooth muscle fibers of the ileal region.

Naturally occurring nonapeptide bradykinin and undecapeptide substance P are similar in their spectra of pharmacological activity (1-2). Extravascular smooth muscle is contracted by these peptides (1) (3-12). Most workers have used isolated smooth muscle preparations bathed in physiological salt solutions. In a previous study (13), a difference was noted in the mode of action of 5-hydroxytryptamine (5-HT) between the isolated blood-perfused small intestinal preparation of the rat and rat isolated ileal strips bathed in a physiological salt solution; the action of 5-HT given intra-arterially to the blood-perfused intestinal preparation was primarily one which stimulated the local neuronal elements, while that of 5-HT applied to isolated ileal strips bathed in physiological salt solution was exerted on ileal smooth muscle. The mode of action of bradykinin and substance P on the ileal region was studied herein using the blood-perfused small intestine of the rat.

MATERIALS AND METHODS

Male Sprague-Dawley rats were anesthetized with pentobarbital sodium (65 mg/kg i.p.). The operative and perfusion procedures were as previously described (13). Recipient rats (about 150 g) were deprived of food overnight before the experiment but water was allowed ad libitum. The animals were anesthetized, the abdomen was opened by a midline incision, and the intestine was gently exteriorized. Both ends of the small intestine were
ligated and cut off, proximally at the junction between the pylorus and duodenum, and
distally at the ileum about 10 cm above the caecum. The isolated small intestine was perfused
at a fixed flow rate through the superior mesenteric artery with heparinized blood (37°C)
from the carotid artery of a donor (550–700 g) by means of a peristaltic pump (Mitsumi
Science, SJ-1210). Flow rate was precalibrated and re-checked at the end of the experiment.
The donor was allowed to respire spontaneously. A square wave electromagnetic flowmeter
(Nihon Kohden, MF-25) was used for the measurement of the mesenteric blood inflow.
The blood pressure of the donor and the mean perfusion pressure were measured with
pressure transducers (Nihon Kohden, MPU-0.5). The venous outflow from the portal
vein was returned to the donor through a reservoir by gravity.

A small opening was made in the wall of the ileum about 20 cm apart from its end, and
through the opening a water-filled balloon made of thin rubber, 3–5 mm long, was inserted
into the lumen of the intestine in the direction of the duodenum. The amount of water
filled in the balloon was adjusted initially to give a resting intraluminal pressure ranging
from 2–7 cmH₂O. The pressure of the ileal region was measured by means of a pressure
transducer (Nihon Kohden, LPU-0.1). Recordings were made on an ink-writing rectigraph
(TOA Electronics, EPR-3T). The isolated intestine was covered with a sheet of cellophane
to prevent drying.

Drugs used were as follows: synthetic bradykinin and synthetic substance P (Protein
Research Foundation), tetrodotoxin (TTX) and morrhine hydrochloride (Sankyo), hexame-
thonium bromide (C₆, Yamanouchi), atropine sulfate (Takeda), carbachol chloride (Tokyo
Kasei), histamine dihydrochloride (Wako Junyaku) and mepyramine maleate (Merck,
Sharp & Dohme). All drugs were dissolved in or diluted with 0.9% saline. Drug solutions
in a volume of 0.01 ml were injected over a period of 4 sec into the superior mesenteric artery
using individual microsyringes (Jintan Terumo Co.).

Values in the text are means±S.E. (unless otherwise noted). Differences between
mean values were analyzed by Student’s t-test.

RESULTS

Basal values of main parameters under resting conditions: Forty-six preparations were
used in the present experiments. Mean perfusion pressure was set at a value slightly lower
than the mean systemic blood pressure of the donor at the onset of perfusion with a flow rate
of about 3.5 ml/min. Shortly after the start of perfusion the pressure rose slightly, but fell
subsequently to reach a new steady-state level. The pressure was then re-adjusted to about
100 mmHg and, thereafter, remained almost constant for about 3 hr. Thus, most of the
preparations became stable within 30 min after the onset of perfusion. At this stage, the
measured parameters were as follows: mean mesenteric blood inflow, 4.1±0.1 ml/min;
mean perfusion pressure, 86.2±1.1 mmHg; and intraluminal pressure, 5.2±0.1 cmH₂O.

Ileal responses to increasing doses of bradykinin or substance P: Single injections of
bradykinin or substance P were given at intervals of at least 5 min into the superior mesen-
teric artery. Bradykinin in doses of 0.01 to 10 μg produced monophasic fast contractions
of the ileum preceded by an initial fall and a subsequent rise of tone. The fast contraction occurred after a latency occasionally continuing for 30–60 sec. Substance P at doses of 0.001 to 0.3 μg, produced a fast contractile response immediately after the administration. Fig. 1 shows an example of the ileal responses to bradykinin and substance P. The main objective in this study was to determine the mechanism of induction of the fast contractile response to these peptides. As seen in Table 1, the dose-response relations for the amplitude of the fast contractile response were singular; once a response was elicited, an increase in dose of the peptides failed to produce a further increase in the amplitude of the contractile response. Thus, the dose-response relations for the amplitude of the response were flat. However, when the incidence of the response was plotted against the dose, the dose-incidence relation was sigmoid for bradykinin in doses of 0.01–0.1 μg and for substance P in doses of 0.001–0.01 μg. Tachyphylaxis did not develop with the stimulant action of either 3 μg of bradykinin or 0.1 μg of substance P upon 5 successive intra-arterial administrations at 5–10 min intervals.

Effects of various blocking agents on the ileal contractile responses to bradykinin and substance P: The ileal fast contraction in response to 3 μg of bradykinin was abolished by a single intra-arterial administration of 1 μg of tetrodotoxin (TTX), 100 μg of hexamethonium (C₆) or 50 μg of morphine, but the initial fall and subsequent rise of ileal tone remained unchanged even in the presence of these blocking agents. The blocking effect of TTX, C₆ or morphine on the fast contractile response to bradykinin lasted for about 30 min, then the contraction reappeared. When 1 μg of TTX and 50 μg of morphine were injected into the mesenteric artery, the resting ileal tone rose transiently (1–5 min) and slightly (1–3 cmH₂O). On the other hand, C₆ at a dose of 100 μg had almost no effect, or at the most, induced a slight decrease in the ileal tone.

The fast contraction and the changes in ileal tone caused by 3 μg of bradykinin were not significantly modified by 10 μg of atropine or 100 μg of mepyramine given intra-arterially. The dosages of atropine and mepyramine used were sufficient to abolish the ileal contraction for about 30 min in response to 1 μg of carbachol and 30 μg of histamine, respectively. The fast contractile response to 0.1 μg of substance P was not prevented by either 1 μg of TTX, 100 μg of C₆, 50 μg of morphine, 10 μg of atropine or 100 μg of mepyramine. Fig. 2A is an example of such experiments and Fig. 2B shows a summary of the data.
Table 1. Dose-response relation for the ileal fast contractile responses to increasing doses of bradykinin or substance P

<table>
<thead>
<tr>
<th>Dose (μg)</th>
<th>0.001</th>
<th>0.003</th>
<th>0.01</th>
<th>0.03</th>
<th>0.1</th>
<th>0.3</th>
<th>1</th>
<th>3</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amplitude of Contraction (cmH$_2$O)</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Bradykinin</td>
<td>21.2 (1/4)</td>
<td>29.0 (2/4)</td>
<td>26.1±5.0 (4/4)</td>
<td>26.3±1.4 (4/4)</td>
<td>26.9±2.7 (4/4)</td>
<td>25.0±2.4 (4/4)</td>
<td>27.4±1.9 (4/4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Substance P</td>
<td>20.2 (1/4)</td>
<td>19.4±5.9 (3/4)</td>
<td>21.6±3.8 (4/4)</td>
<td>25.6±2.3 (4/4)</td>
<td>25.5±3.0 (4/4)</td>
<td>26.3±2.4 (4/4)</td>
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</table>

Doses were increased by a factor of about 3, and given at intervals of at least 5 min. Denominators in parentheses are the number of preparations and numerators are the frequency of incidence of the ileal fast contraction in response to bradykinin and substance P. Values are means±S.E. (calculated only with numerators).
Fig. 2. Effects of various blocking agents on the fast contractile responses of the ileum to bradykinin (BK) and substance P (SP). ILP, intraluminal pressure. A) Original tracings with the effects of tetrodotoxin, hexamethonium or morphine on the responses to bradykinin and substance P. B) Summarized data. Vertical bars represent ± S.E., number of experiments is given in parentheses. Open columns, before treatment; hatched columns, after treatment. CB, carbachol; HT, histamine. NS, no significant difference before and after treatment with blocking agents.

DISCUSSION

Single administrations of either bradykinin or substance P produced a monophasic fast contraction of the ileum. The fast contractile response to bradykinin, unlike that to substance P, was preceded by an initial fall and a subsequent rise of ileal tone and occurred abruptly after a latency of up to 30-60 sec. Thus, the fast contractile response to intra-arterially administered bradykinin may be elicited by biologically active substances released from the donor, by bradykinin which escaped from the perfused intestine to reach the donor.
However, even when large doses of bradykinin were administered intravenously to the donor, no changes were seen in the intraluminal and the perfusion pressure of the isolated perfused intestine. In addition, the time required for a drug to recirculate was at least 4 min. Therefore, the above-mentioned possibility can be ruled out. It is more likely that the fast contraction caused by bradykinin is due to its local action in the ileal region of the perfused intestine.

The changes in ileal tone induced by bradykinin appear to be mediated through a direct action on smooth muscle fibers, since these changes remained unaltered by treatment with either TTX, morphine or C₆. In contrast, the fast contraction was abolished by TTX or morphine, indicating that the response is due to excitation of intramural neural elements. The bradykinin-induced fast contraction was also blocked by C₆, a typical nicotinic receptor antagonist. The question arises as to whether or not bradykinin might directly stimulate nicotinic receptors on neural elements or whether the blocking action of C₆ might not be specific in the present preparation. Bradykinin has a C₆-resistant stimulatory action on the superior cervical ganglion of cats (14), therefore this peptide probably does not have a direct stimulatory action on nicotinic receptors and the blocking action of C₆ was specific. It can be assumed that bradykinin would stimulate cholinergic neurons in the myenteric neural plexus and that C₆ would block transmission via nicotinic receptors on cholinceptive neurons synapsing cholinergic neurons stimulated by bradykinin.

The fast contractile response to bradykinin was resistant to the blocking action of atropine, mepyramine or methysergide (not shown). Lack of any blocking effects of these antagonists suggests that neurons subserving as the final common pathway to the ileal smooth muscle cells probably are not cholinergic, histaminergic or tryptaminergic. Also, catecholamines produce primarily only an ileal relaxation in the same sort of preparations (13). Experiments carried out on isolated muscle strip preparations of the ileum and the taenia coli of the guinea pig or the rat uterus have shown that bradykinin has only a direct action on smooth muscle (3–5, 15). Thus, the potent neural excitatory action of bradykinin as revealed by the use of the isolated, blood-perfused intestinal preparation of the rat was of great interest.

The contractile response to substance P was resistant to the blockade of TTX, C₆ or morphine, thus the possible involvement of intramural neural elements in the response to substance P can be ruled out. Substance P may induce the ileal fast contraction by a direct stimulatory action on the smooth muscle fibers. The findings in the present work lend support to the conclusions drawn by Bury and Mashford (7), Chipkin et al. (9) and Yau (12).

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REFERENCES


