Pharmacological Analysis of Receptors Involved in the Late, Tachykininergic Contractile Response to Electrical Transmural Stimulation in Isolated Rabbit Iris Sphincter Muscle

Masaru Kunitomo, Noriko Imaizumi, Emiko Sameshima and Motohatsu Fujiwara

Department of Pharmacology, Faculty of Pharmaceutical Sciences, Mukogawa Women's University, 11-68 Koshien Kyuban-cho, Nishinomiya 663, Japan

Received February 10, 1993 Accepted April 13, 1993

ABSTRACT—We characterized the pharmacological nature of the tachykinin receptor subtype mediating the contractile response to electrical transmural stimulation (ETS) in the isolated rabbit iris sphincter muscle preparation by using selective NK₁-receptor antagonists, spantide and L-668,169, and a selective NK₂-receptor antagonist, L-659,877. ETS caused a biphasic contraction in this preparation: a rapidly developing cholinergic component followed by a slowly decaying tachykininergic component. The tachykininergic contractile response to ETS was effectively attenuated by spantide and L-668,169, but only slightly by L-659,877, indicating that the tachykinin receptors mediating ETS-induced contraction are of the NK₁ type. In the same preparation, the contractile activity of substance P (SP) was slightly more potent than that of neurokinin A (NKA). Unlike in other tissues rich in NK₁-receptor subtypes, spantide and L-668,169 antagonized the contractile response to NKA more effectively than that to SP, and the reverse was observed for L-659,877. These results strongly suggest that the tachykininergic contraction induced by ETS in the rabbit iris sphincter preparation is mediated by NK₁-receptors which are activated by endogenously released NKA.

Keywords: Iris (rabbit), NK₁-receptor, Neurokinin A, Substance P, Tachykinin antagonist (selective)

It has been reported that the rabbit iris sphincter muscle is innervated by substance P (SP)-containing sensory fibers from the trigeminal nerve (1–3) and that specific and high-affinity binding sites for SP are localized therein (4–6). It has also been demonstrated that other members of the tachykinin family such as neurokinin A (NKA) and neurokinin B (NKB) are present in the iris sphincter muscle of rabbits (7, 8). Electrical transmural stimulation (ETS) of the isolated rabbit iris sphincter muscle produces a noncholinergic, nonadrenergic contraction that is inhibited by tetrodotoxin and tachykinin antagonists (2, 9–12). On the basis of pharmacological analysis, Ueda et al. (11) and Muramatsu et al. (12) have proposed that NKA rather than SP is a possible transmitter for mediating such a contraction. Beding-Barnekow et al. (8) have suggested that ETS of rabbit iris sphincter muscle results in simultaneous release of SP and NKA.

On the other hand, the tachykinin receptor subtypes in the rabbit iris sphincter are heterogeneous because of a striking discrepancy between the relative potencies of tachykinin agonists and agonist-selective antagonist potencies in this preparation (4, 13, 14). Recently, in experiments employing selective agonist ligands, Hall et al. (15) have suggested the presence of both NK₁- and NK₂-receptor subtypes in this tissue.

In the present study, we tried to characterize the pharmacological nature of tachykinin receptors involved in the contractile response of the isolated rabbit sphincter muscle to endogenous tachykinins released during ETS by using selective NK₁-receptor antagonists, spantide and L-668,169 (16), and a selective NK₂-receptor antagonist, L-659,877 (17, 18). Some of this work has been presented at the International Symposium on “SP and Related Peptides”, held in Shizuoka, Japan, in November, 1992.

MATERIALS AND METHODS

Male albino rabbits, weighing 2.0 to 3.0 kg, were exsanguinated under pentobarbital sodium anesthesia, and the eyes were immediately enucleated after death. Two strips
of iris sphincter muscle were prepared from each eye. Each strip was then mounted in a 10-ml tissue bath (37°C) containing Krebs-Henseleit solution, which was continuously aerated with a gas mixture of 95% O2 and 5% CO2, maintaining a pH of 7.4. The composition of Krebs-Henseleit solution was as follows: 118.4 mM NaCl, 4.7 mM KCl, 2.5 mM CaCl2, 1.2 mM KH2PO4, 1.2 mM MgCl2, 25.0 mM NaHCO3 and 10.0 mM glucose. The preparation was connected vertically to a force-displacement transducer under an initial resting tension of 150 mg. All preparations were allowed to equilibrate for about 90 min in the Krebs-Henseleit solution before the start of the experiments, after which the maximal response to carbachol was established in the individual preparations. Changes in isometric tension produced by ETS or by the addition of compounds to be tested were recorded on a pen recorder. ETS with square-wave pulses (10 Hz, 50 V intensity, 0.3-msec duration, pulse train of 10 sec) was applied by means of a pair of platinum electrodes connected to an electric stimulator (MSE-3R, Nihon Kohden, Tokyo). Cumulative concentration-response curves to NKA and SP were also obtained in each preparation by increasing the concentrations of agents as soon as a steady response to the previous administration had been achieved. The antagonist was added 10 min before recording each concentration-response curve. Since the prolonged washout time of responses to SP was required and the subsequent responses to this agonist became smaller, the concentration-response curve to SP was determined at the end of the experiments, and experiments were carried out in parallel in the absence or presence of each antagonist. All EC50 values (the molar concentration that induces 50% of the maximal response) were calculated by linear regression analysis to the steep part of each concentration-response curve, and results were presented in terms of the pEC50: the negative logarithm of EC50.

The following agents were used: NKA, SP and spantide (Peptide Institute, Inc., Osaka), atropine sulfate (Wako Pure Chemical Ind. Ltd., Osaka) and carbachol (Sigma Chemical Co., St. Louis, MO, U.S.A.). L-668,169 and L-659,877 were gifts from Merck Sharp & Dohme Research Laboratories, Essex, U.K. All values obtained were expressed as the mean ± S.E.M. Statistical analyses were performed by Student’s t-test for paired or unpaired data.

RESULTS

Effects of selective tachykinin antagonists on the responses to ETS

ETS caused a reproducible, neurogenic contraction of the isolated rabbit iris sphincter muscle preparation (Fig. 1A); the rapidly developing cholinergic component was followed by a slowly decaying tachykininergic component, as evidenced by the attenuation of such a biphasic response in the presence of atropine and a tachykinin antagonist, respectively, confirming the earlier results reported by Ueda et al. (9) and Muramatsu et al. (12). As shown in Fig. 1 (A and B), the late, slowly decaying tachykininergic contractile response to ETS was effectively attenuated by a selective NK1-receptor antagonist, spantide or L-668,169, but only slightly attenuated by a selective NK2-receptor antagonist, L-659,877. In the presence of 10^{-6} M atropine, the late, slow contraction evoked by ETS was reduced to 21.8±1.7% (n=6), 31.9±2.4% (n=6) and 87.9±1.0% (n=8) of the control level at 10^{-6} M L-668,169, spantide and L-659,877, respectively.

Effects of selective tachykinin antagonists on tachykinin-induced responses

SP and NKA consistently contracted the isolated rabbit iris sphincter muscle preparation in a concentration-dependent manner, and yet both maximal effects were comparable: 0.98 and 1.03 of the maximal response to carbachol, respectively. The contractile activity of SP was more than that of NKA, as indicated by the pEC50 values (control values of SP vs. NKA in Table 1; P<0.01). The contractile response to SP was markedly inhibited by L-659,877, but not significantly affected by spantide or L-668,169. In contrast, the contractile response to NKA was inhibited by spantide or L-668,169 more effectively than by L-659,877 (Table 1).

<table>
<thead>
<tr>
<th>Antagonist</th>
<th>pEC50 SP</th>
<th>pEC50 NKA</th>
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</thead>
<tbody>
<tr>
<td>NK1-Selective</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>8.82±0.14</td>
<td>8.34±0.04</td>
</tr>
<tr>
<td>+Spantide</td>
<td>8.57±0.16</td>
<td>7.68±0.04*</td>
</tr>
<tr>
<td>Control</td>
<td>8.77±0.08</td>
<td>8.33±0.11</td>
</tr>
<tr>
<td>+L-668,169</td>
<td>8.53±0.11</td>
<td>7.40±0.17*</td>
</tr>
<tr>
<td>NK2-Selective</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>8.80±0.08</td>
<td>8.22±0.07</td>
</tr>
<tr>
<td>+L-659,877</td>
<td>8.34±0.07*</td>
<td>8.12±0.06</td>
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</tbody>
</table>

Numbers in parentheses indicate the number of tissue preparations used. pEC50 values were obtained from cumulative concentration-response curves to SP or NKA in the absence (control) or presence of a 10^{-5} M tachykinin antagonist. Each value is the mean±S.E.M. Significant difference from the control, *P<0.01.
DISCUSSION

In the present studies, we demonstrated that in the isolated rabbit iris sphincter muscle, ETS-induced tachykininergic contraction was markedly inhibited by selective NK₁-antagonists, spantide or L-668,169, but only slightly inhibited by a selective NK₂-antagonist, L-659,877. This implies that the tachykinin receptors involved in the ETS-induced contraction are mainly of the NK₁ type.

It is well known that SP and NKA possess the highest affinity for NK₁- and NK₂-receptor types, respectively (19). In this preparation, however, it may be difficult to establish the presence of a single tachykinin receptor sub-
type on the basis of the difference between the contractile responses to agonists such as SP and NKA, since exogenously administered SP was only slightly more potent than NKA in contractile activity, in accordance with earlier results (4, 11–13). Studies using more highly selective agonist ligands suggested the presence of both NK1- and NK3-receptors (15).

We also demonstrated that in the isolated rabbit iris sphincter muscle preparation, spantide and L-668,169 attenuated the contractile response to exogenous NKA more effectively than that to SP, and that the antagonistic effects of L-659,877 were the reverse. On the basis of experiments with the SP-antagonist [D-Arg\(^1\), D-Pro\(^2\), D-Trp\(^7,9\), Leu\(^11\)]-SP, other workers reported a heterogeneous receptor population in this tissue, where the same antagonist was effective against NKA, but essentially inactive against SP and NKB (4, 12–14). Therefore, it is apparent that in contrast to tissues rich in the NK3-receptor subtype such as the guinea pig ileum and vas deferens, the NK3-selective antagonist preferentially antagonizes the contractile response to NKA rather than SP in the rabbit iris sphincter muscle. A similar unexpected behavior has been noted in neonatal rat spinal motoneurones (20) and hamster trachea (21) to which NKA and spantide or L-668,169 bind competitively with a high affinity. Ueda et al. (11) have reported that in the isolated rabbit iris sphincter preparation, the contractile response to SP is more prolonged not only in the time to reach the maximal response but also in washout time required to restore the original level as compared to the case of the NKA response, and yet the contractile response declines gradually upon repetitive application of SP in contrast to no development of such tachyphylaxis to NKA. Furthermore, preceding exposure to SP reduces the subsequent responses to ETS as well as NKA (11). Thus, the present observations together with our previous data let us suppose that NKA is released concomitantly with SP by ETS in the rabbit iris sphincter, and that the former plays an important role in mediating the ETS-induced contraction by preferentially activating NK1-receptors.

Note added in proof

After completing the present experiment, a paper indicating that the NK1-selective antagonist preferentially antagonizes the contractile response to exogenous SP, but essentially inactive against NKA, and the NK1-selective antagonist preferentially antagonizes the contractile response to NKA rather than SP in the rabbit iris sphincter muscle. A similar unexpected behavior has been noted in neonatal rat spinal motoneurones (20) and hamster trachea (21) to which NKA and spantide or L-668,169 bind competitively with a high affinity. Ueda et al. (11) have reported that in the isolated rabbit iris sphincter preparation, the contractile response to SP is more prolonged not only in the time to reach the maximal response but also in washout time required to restore the original level as compared to the case of the NKA response, and yet the contractile response declines gradually upon repetitive application of SP in contrast to no development of such tachyphylaxis to NKA. Furthermore, preceding exposure to SP reduces the subsequent responses to ETS as well as NKA (11). Thus, the present observations together with our previous data let us suppose that NKA is released concomitantly with SP by ETS in the rabbit iris sphincter, and that the former plays an important role in mediating the ETS-induced contraction by preferentially activating NK1-receptors.

REFERENCES


