In vitro contraction of the pupillary sphincter by substance P and its stable analogs

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The effects of substance P and substance P analogs have been studied quantitatively by use of an isolated preparation of bovine pupillary sphincter muscle. Substance P contracts the pupillary sphincter in a dose-dependent manner with a value for median effective dose (ED50) of 1.0 x 10^-6 M. The effects of substance P result from its interaction with a specific receptor in the pupillary sphincter. These results strengthen the view that substance P is involved in the oculopupillary reflex.

Key words: substance P, bovine pupillary sphincter, oculopupillary reflex

Otricker1 in 1876, Bayliss2 in 1901, and Langley3 in 1923 have shown that the stimulation of the distal ends of severed cutaneous sensory nerves causes an arteriolar dilatation. This is the well-known phenomenon of "antidromic vasodilatation," which has been explained on the basis of the release of a "neurohumor" from the stimulated sensory nerve endings. The "neurohumor" released still must be identified, but a growing body of evidence suggests it is substance P (SP), a putative sensory neurotransmitter4 with vasodilatory and smooth-muscle-contracting properties.5 In the eye, the electrical or mechanical stimulation of the trigeminal nerve causes an increase in intraocular pressure, miosis, and signs of inflammation.6,7 Similar effects are observed upon irritation of the cornea, conjunctiva, or lids by chemical agents.8 These phenomena are due in part to an axon reflex9,10 of the trigeminal nerve. During the stimulation of the nerve endings, the generated action potentials travel both orthodromically via the gasserian ganglion to the trigeminal nucleus and antidromically into the collateral branches of the dendrites of the trigeminal nerve in the pupillary sphincter and on blood capillaries in the iris.

Substance P has been found to be highly concentrated in primary sensory terminals in the spinal trigeminal nucleus, from which it is released upon stimulation.11 Since SP is present all along small-caliber sensory fibers and since it is found in both peripheral and central endings of these fibers,12 it is possible that SP is released in the periphery during antidromic stimulation or following an axon...
Material and methods

Substance P, pGlu-Phe-Phe-Gly-Leu-Met-NH₂ (pGlu-pentapeptide), and Boc-Gln-Gln-Phe-Phe-Gly-Leu-Met-NH₂ (Boc-heptapeptide) were synthesized according to the method of Bergmann et al. The purity of the peptides was checked by thin-layer chromatography (silica, n-butanol/acetic acid/water, 4:1:1 or 2:1:1). The compounds were characterized by their optical rotation, elemental analysis (C, H, N, S), and amino acid analysis. Synthetic SP from Sigma Chemical Co. was used also and displayed the same properties as our preparation of SP. Atropine was a product from Assia Chemical Laboratories, propranolol (Deraline) was from Abic Ltd., phenotolamine (Regitine) from CIBA-Geigy, diphenhydramine (Benadryl) from Sigma, flufenamic acid from Parke, Davis & Co., and tetrodotoxin from Sigma.

In vitro constriction of the isolated pupillary sphincter. Bovine eyes were obtained from a local slaughterhouse and kept at room temperature in Tyrode’s solution bubbled with O₂ until use, not more than 4 hr after enucleation. The Tyrode solution contained 137 mM NaCl, 2.7 mM KCl, 1.8 mM CaCl₂, 1.1 mM MgCl₂, 0.4 mM NaH₂PO₄, 11.9 mM NaHCO₃, 5.5 mM glucose, 4.4 mM fumarate, 3.9 mM pyruvate, and 4.2 mM glutamate.

The pupillary sphincter was dissected from a cow’s eye in the following way. First, the cornea is removed by cutting circularly around the limbus. Then the pupillary sphincter is cut off at its base and prepared as a ring. This ring is placed between two hooks, one at the bottom of a 10 ml glass vessel filled with Tyrode’s solution at 37° C and the other attached via a nylon thread to the lever of an isotonic smooth-muscle transducer from Harvard Apparatus. The tension on the pupillary sphincter muscle is about 200 mg.

The organ bath was thermostated at 37° C and bubbled with air. The pupillary sphincter was allowed to recuperate for 30 min before the addition of drugs. Isotonic contractions were measured under a constant load of 200 mg. Dose-response curves were obtained by adding the peptide to the bath and washing with Tyrode’s solution as soon as a steady response was obtained. The time between peptide addition and washing was about 1 min and between two consecutive additions of peptide about 2 min. Each peptide was characterized by a value for median effective dose (ED₅₀) corresponding to a peptide concentration that causes 50% of the maximal response. Two or three dose-response curves were obtained with a given sphincter reflex.
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Results

We have found the isolated preparation of cow pupillary sphincter muscle to be suitable for the investigation of the miotic properties of SP. This muscle, once isolated, keeps its tonus for about 1 hr if it is maintained in an aerated Tyrode solution that contains fumarate, pyruvate, and glutamate. When incubated for more than 1 hr, the muscle begins to contract and cannot be made to relax.

When bath-applied to the isolated cow pupillary sphincter muscle, SP causes a dose-dependent contraction of the pupil. Fig. 1 illustrates a series of isotonic contractions obtained with SP (panel A) and pGlu-pentapeptide (panels B and C). In both cases, the peptide-induced contraction does not take place immediately upon addition of the peptide but reaches its full extent in about 1 min. Washing out the peptide by adding fresh Tyrode’s solution produces a relaxation of the sphincter within 15 to 20 sec. A comparison of the time needed for a relaxation of the sphincter following SP or pGlu-pentapeptide contractions indicates an apparently slower washout of SP. However, a contraction obtained with pGlu-pentapeptide on a sphincter preparation different (panel C) from that used in panel B shows that the relaxation time does not depend on the structure of the peptide but varies with the preparation. The contraction of the sphincter by SP and its analogs is not affected by the previous exposure of the preparation or by the concomitant presence in the bath of a muscarinic antagonist (10^{-6}M atropine), an α-adrenergic antagonist (3.5 × 10^{-6}M phenolamine), a β-adrenergic antagonist (3.8 × 10^{-6}M propranolol), a histamine antagonist (4 × 10^{-7}M diphenhydramine), an inhibitor of prostaglandin biosynthesis (3.5 × 10^{-6}M flufenamic acid), or a blocker of action potentials (10^{-7}M tetrodotoxin).

Fig. 2 illustrates the dose-response curves obtained with SP and with two of its stable analogs, pGlu-pentapeptide and Boc-heptapeptide. The p(ED_{50}) value for SP is 6.0 ± 0.2 S.D. (five determinations) whereas the p(ED_{50}) value of its two analogs is 7.4 ± 0.2 S.D. (seven determinations).

Discussion

In this article, we show that SP and its analogs are capable of contracting the pupillary sphincter muscle in vitro. These effects of SP cannot be prevented by cholinergic, adrenergic, or histaminergic drugs, by agents that block nerve conduction, or by a blocker of prostaglandin biosynthesis. The effects of SP are therefore mediated by the direct interaction of this peptide with specific receptors in the muscle fibers.

From the dose-response curves obtained on the isolated cow pupillary sphincter, it appears that SP is a less potent miotic than its analogs, Boc-heptapeptide and pGlu-pentapeptide. However, it is almost as potent as carbamylcholine, which displays an ED_{50} value of 6 × 10^{-7}M (manuscript in preparation). Although the ED_{50} values of SP and of its analogs, Boc-heptapeptide and pGlu-pentapeptide, are identical when they are measured on the guinea pig ileum or on the rat ileum, they are consistently different in the cow pupillary sphincter. Such difference was also observed on the guinea pig urinary bladder. The reasons for this difference are not yet clear, but it is possible that different types of SP receptors are involved.

The potent miotic and vasodilatory properties of SP and of its analogs raise several
questions relevant to the physiology and pathology of the iris.

In the eye, the sensory outputs from the whole eyeball are carried out by the ophthalmic branch of the trigeminal nerve. Although the mechanical or electrical stimulation of this nerve produces pain, it also triggers an oculopupillary reflex which causes both a dilatation of the capillaries of the iris and a constriction of the pupillary sphincter.\(^6\)-\(^8\) 13\(^\mathbf{15}\)

According to Dale's hypothesis,\(^18\) the neurotransmitter of the trigeminal nerve should be released at both its central and peripheral branches and should produce a noxious response on the one hand a vasodilatory and smooth-muscle-constricting effect on the other. Of all the putative sensory neurotransmitters, only SP displays such properties. Although it is not fully established that SP is indeed the neurotransmitter of the ophthalmic division of the trigeminal nerve, several pieces of evidence are strongly in support of such assignment.

1. SP-containing nerve fibers have been localized by immunofluorescence in the gasserian ganglion and in the iris.\(^12\) The destruction of the gasserian ganglion is accompanied by a significant decrease of SP levels in the iris and in the ciliary body.\(^19\)

2. A release of SP-like immunoreactivity into the aqueous humor has been observed during intracranial stimulations of the trigeminal nerve.\(^13\)

3. Stimulation of the spinal trigeminal nucleus causes a release of SP.\(^11\)

4. Topical application of nitrogen mustard to the eye causes a rapid rise in intraocular pressure which can be blocked by topical, retrobulbar, or intracranial administration of capsaicin,\(^20\) a chemical known to cause a depletion of SP in primary sensory neurons.\(^21\)

The miotic properties of histamine have been evoked in relation to the oculopupillary reflex, as described in the literature.\(^15\) This is, however, not in contradiction with the assignment of SP as the transmitter released during the axon reflex. Indeed, SP is capable of releasing histamine from mast cells,\(^22\) and such a release could well amplify both the miosis and the vasodilatation. This idea is in line with the findings\(^23\) that the antidromic vasodilatation and neurogenic plasma extravasation induced by the antidromic stimulation of the saphenous nerve are almost abolished by pretreatment with capsaicin and are markedly affected by pretreatment with histamine antagonists. Prostaglandins are also involved in ocular inflammations\(^24\), \(^25\) but probably not at early stages.\(^20\)

The possible role of SP in the oculopupillary reflex can lead to some speculations concerning the pathophysiology of the iris. For instance, a dull and swollen iris and an irregular and miotic pupil, even in the presence of atropine, are common signs of iritis. A participation of SP in these phenomena should perhaps not be ruled out. Should the sensory fibers present in the iris be stimulated during the iritis, a sustained release of SP could take place, leading to a persistent miosis.

Our finding that SP is capable of constricting an atropinized pupil is indeed in line with this proposition.

REFERENCES