NONCHOLINERGIC, NONADRENERGIC CONTRACTION AND SUBSTANCE P IN RABBIT IRIS SPHINCTER MUSCLE

Naoko UEDA*, Ikunobu MURAMATSU, Yoshihiko SAKAKIBARA and Motohatsu FUJIWARA

Departments of Pharmacology and *Ophthalmology, Kyoto University, Faculty of Medicine, Kyoto 606, Japan

Accepted September 3, 1981

Abstract—In the rabbit iris sphincter muscle, electrical transmural stimulation produced fast and slow components of contraction which were markedly attenuated by tetrodotoxin. The fast component was augmented by physostigmine and was abolished by atropine, while the slow component was little affected. Adrenergic and ganglionic blocking agents did not inhibit the slow component. Therefore, the fast component is probably cholinergic, while the slow one is noncholinergic, nonadrenergic in nature. Capsaicin did produce a considerable contractile response, but there was a gradual decline with repetitive application and a tachyphylaxis occurred. Under such conditions the slow but not the fast component was abolished. Substance P and acetylcholine produced the largest contraction, while ATP, histamine, serotonin and noradrenaline produced little or no response. In cold stored preparations, the responses to electrical transmural stimulation and capsaicin were either markedly attenuated or abolished, whereas substance P and acetylcholine produced considerable contractions. Baclofen and theophylline did not inhibit the slow response to electrical transmural stimulation or the response to substance P and capsaicin. Thus, electrical transmural stimulation produces cholinergic and noncholinergic, nonadrenergic contractions in the rabbit iris sphincter muscle and the latter response is considered to be mediated by substance P or a related peptide released from the neural components.

Stimulation of the fifth cranial nerve and chemical or mechanical irritation of the eye result in irritative ocular responses such as miosis, conjunctival and iridial hyperemia, breakdown of the blood-aqueous barrier and an increased intraocular pressure. The responses to mechanical irritation of the rabbit eye are reportedly mediated through prostaglandins (1-3), while the responses to chemical irritation or to the fifth nerve stimulation are considered to be of neurogenic origin (1-8). However, autonomic blocking agents did not inhibit these inflammatory responses (7).

Recent histological studies revealed the presence of substance P in the eye and that this polypeptide is released by primary sensory nerve stimulation (9-11). Substance P is also a potent contractile substance in the iris sphincter muscle (12, 13). Capsaicin reduces the substance P content in the primary sensory nerve (14, 15) and inhibits the neurogenic inflammatory response (16-18). Furthermore, capsaicin produces the hypertensive and miotic responses in the isolated rabbit eye with the intact sensory
nerve (19). Thus, substance P may possibly play important physiological roles in functions of the iris and perhaps even other parts of the eye.

We studied the pharmacological properties of the responses to electrical transmural stimulation and to administration of capsaicin in the rabbit iris sphincter muscle and our findings are reported herein.

MATERIALS AND METHODS

Albino rabbits of either sex and weighing 2.0 to 3.0 kg were exsanguinated and eyes were enucleated within 15 min after death. Most of the so-obtained eyes were used for experiments on the same day while others were refrigerated in Krebs solution (3 to 5°C) for 6 to 8 days.

One strip of iris sphincter was cut from each eye, according the method of Kern (20). The strip was then mounted in a bath of 20-ml capacity and which contained Krebs solution gassed with 95% O₂ and 5% CO₂. The temperature of the bath was maintained at 37.5±0.5°C. Composition of the Krebs solution was as follows (mM): NaCl, 120.7; KCl, 5.9; MgCl₂ 1.2; CaCl₂ 2.5; NaH₂PO₄ 1.2; NaHCO₃, 15.5 and glucose 11.5.

The free end of the preparation was connected by a light weight thread to a force-displacement transducer and a resting load of about 150 mg was maintained during the experiments. In preliminary experiments, acetylcholine in a dose of 10⁻³ M produced the maximum contraction. Isometric tension was recorded through a force-displacement transducer on an ink-writing oscillograph. Before start of experiments, preparations were allowed to equilibrate for about 90 min in control medium.

Electrical transmural stimulation was given every 10 min by means of a pair of platinum electrodes. Stimulus parameters were 0.3 msec duration, 80 V intensity (supramaximal voltage) and frequency of 30 Hz for 10 sec, unless otherwise stated.

Cumulative dose-response curves to substance P and acetylcholine were obtained by increasing the concentrations of drugs as soon as a steady response to the previous administration had been achieved.

For sympathetic denervation of the eye ten days previous to the experiments, three rabbits were anesthetized with pentobarbital sodium (30 mg/kg i.v.) and the superior cervical ganglia were removed. In three other rabbits, reserpine (1 mg/kg) was given i.v. twenty-four hours before sacrifice.

The following drugs were used: atropine sulfate, serotonin creatinine sulfate, capsaicin and physostigmine salicylate (Merck Sharp & Dohme, West Point, PA); tetrodotoxin (Sankyo Co. Ltd., Tokyo); hexamethonium chloride, theophylline, histamine dihydrochloride and d-tubocurarine chloride (Nakarai Chemicals Ltd., Kyoto); guanethidine sulfate (Tokyo-Kasei, Tokyo); phentolamine mesylate (Ciba, Basel), propranolol hydrochloride (Sumitomo Chemicals Osaka), baclofen (Ciba-Geigy, Tokyo), substance P (Protein Research Foundation, Osaka), indomethacin and 1-noradrenaline bitartrate (Sigma Chemical Co., St. Louis, MO), adenosine triphosphate (ATP; Kohjin Company Ltd., Tokyo).

RESULTS

Response to electrical transmural stimulation: Isolated rabbit iris sphincter muscle produced a fast contraction which developed rapidly and declined with cessation of the stimulation. Thereafter, the slow contraction developed and a second peak appeared. Figure 1 shows the representative response to electrical transmural stimulation at 30 Hz. The fast component of contraction was abolished by 10⁻⁶ M atropine and was augmented by 10⁻⁷ M physostigmine. However, the slow component was little affected by these drugs. Tetrodotoxin...
(10^{-7}\text{–}10^{-6} \text{ M}) all but completely inhibited both components of contraction.

Figure 2 shows the stimulus frequency-response curves of the fast and slow components of contraction. The slow component was evident after treatment with 10^{-6} \text{ M atropine and } 5 \times 10^{-6} \text{ M guanethidine. The curve of the fast component increased gradually with an increase in the stimulus frequency. Even at 50 Hz, the frequency-response curve did not reach a maximum. On the other hand, the curve of the late component reached a maximum at a frequency of 20 Hz. Therefore, the amplitude of the slow component was significantly smaller than that of the fast component at frequencies over 30 Hz. Moreover, under constant frequency (30 Hz) and duration (0.3 msec) of pulse, the thresholds of stimulus intensity for the fast and late components were 12–13 V and 15–17 V (n=4), respectively. These contractile responses were all but completely inhibited by 10^{-6} \text{ M tetrodotoxin. It is suggested that these two contractile components are mediated by two distinct neural components.}

Effects of various drugs on the responses to electrical transmural stimulation: The fast component of contraction was abolished by 10^{-6} \text{ M atropine and was augmented by } 10^{-7} \text{ M physostigmine (Fig. 1)}, while the slow component was not affected by these...
cholinergic agents. Furthermore, the slow component observed in the presence of atropine was little affected by hexamethonium (10^{-5} M, n=5), d-tubocurarine (10^{-5} M, n=5), guanethidine (5\times10^{-6} M, n=7), phentolamine (3\times10^{-6} M, n=5), propranolol (10^{-6} M, n=4), theophylline (3\times10^{-5} M, n=5) and baclofen (10^{-5} M, n=5) (Fig. 3).

Effects of capsaicin on the responses to electrical transmural stimulation: The response to electrical transmural stimulation was markedly attenuated by capsaicin. Figure 4 shows the representative result. 10^{-5} M capsaicin itself produced a considerable contraction in the rabbit iris sphincter muscle and the amplitude was larger than that induced by 10^{-3} M acetylcholine. This capsaicin-induced contraction declined gradually and about 40 min later, the tension returned to the original resting level. When electrical stimulation was applied at this time, only the fast component was produced; however, the amplitude was small as compared with the response before treatment with capsaicin. The response to acetylcholine was also reduced in the presence of capsaicin. After the wash out of capsaicin, the fast component induced by electrical transmural stimulation and the contraction induced by acetylcholine were to some extent recovered and the amplitude was approximately 70% of the control responses. The fast component observed after treatment with capsaicin was abolished by 10^{-6} M atropine.

In Fig. 5, the preparation was pretreated with 10^{-6} M atropine and were electrically stimulated at a frequency of 30 Hz. Hexamethonium (10^{-6} M), d-tubocurarine (10^{-6} M), guanethidine (5\times10^{-6} M), phentolamine (3\times10^{-6} M), propranolol (10^{-6} M), theophylline (3\times10^{-6} M), baclofen (10^{-6} M) or tetrodotoxin (10^{-8} M) was applied for at least 15 min. To examine the effect of capsaicin, the preparation was treated with 10^{-6} M capsaicin for 2 hr and then washed at 20-min intervals for 1 hr. Thereafter, electrical transmural stimulation was applied. The contraction before treatment with each drug was taken as 100%. Mean±S.E. of 4 to 7 experiments. The figure in parenthesis means the number of experiments.
with $10^{-6}$ M atropine and the effects of capsaicin on the slow component were examined. This component was abolished after treatment with $10^{-5}$ M capsaicin and did not recover following repetitive wash out of the drug.

Effects of reserpinization, sympathectomy and cold storage on the responses to electrical transmural stimulation: The iris sphincter muscle taken from reserpinized rabbits ($n=3$) responded to electrical transmural stimulation with fast and slow contractions, in a manner similar to that seen in preparations from the nontreated rabbits. Furthermore, the iris muscle isolated from the rabbit ($n=3$), in which both superior cervical ganglia had been removed 10 days previously, responded with fast and slow contractions to electrical transmural stimulation. On the other hand, the responses to electrical transmural stimulation were either markedly attenuated or abolished in the cold storage preparations (Fig. 6).

Responses to various agonists: Acetylcholine and substance P produced the largest contraction, among all the agonists tested. Figure 7 shows the dose-response curves in the case of these two compounds. As compared with acetylcholine, substance P
P was more effective at lower concentrations. The ED50 value of substance P was approximately ten thousand times lower than that of acetylcholine. Capsaicin also produced a considerable contraction. The minimum effective concentration was approximately 10^{-6} M. The contraction induced by 10^{-5} M capsaicin was 105 to 140% of the contraction induced by 10^{-3} M acetylcholine (Fig. 4). When capsaicin was applied at 1-hr intervals (by wash out as soon as the response reached the peak), there was a progressive decline in the amplitude of the contractile response. The higher the concentration of capsaicin, the more rapid was the decline. When the preparation was treated with 10^{-5} M capsaicin for 2 hr, there was no response to the second application of capsaicin, at the same concentration. Under conditions in which tachyphylaxis to capsaicin developed, the slow component was abolished and only the fast component was obtained upon electrical transmural stimulation.

The response to acetylcholine was inhibited by 10^{-6} M atropine and was augmented by 10^{-7} M physostigmine. However, the responses to substance P and capsaicin were not affected by such agents. Tetrodotoxin (10^{-6} M), hexamethonium (10^{-5} M), d-tubocurarine (10^{-5} M), guanethidine (5\times10^{-6} M), phentolamine (3\times10^{-6} M), propranolol (10^{-6} M), theophylline (3\times10^{-5} M) and baclofen (10^{-5} M) had no effect on the responses to substance P and acetylcholine. The contractile response to administration of acetylcholine or substance P was obtained in preparations which had been stored at 4°C for 6 to 8 days, while the response to capsaicin was markedly reduced or abolished by such treatment (Fig. 6).

Histamine (up to 10^{-4} M) and serotonin (up to 10^{-4} M) produced no response in the iris muscles. The responses to noradrenaline (10^{-4} M) and ATP (10^{-4} M) were slight (approximately 13% and 9% of the response to 10^{-3} M acetylcholine, respectively).

**DISCUSSION**

The iris is densely innervated by cholinergic and noradrenergic nerves (21, 22). Indeed, stimulation of the cholinergic nerve produces miosis in vivo and a contraction of the sphincter muscle of iris in vitro (23–25). In the present study, also, the isolated rabbit iris sphincter muscle produced a contractile response to electrical transmural stimulation and this response was composed of fast and slow components. The fast component was augmented by physostigmine and was abolished by atropine, while the slow component was not affected by such cholinergic agents. Furthermore, the slow component was little affected by adrenergic or ganglionic blocking agents. Both contractions were either markedly attenuated or were abolished by tetrodotoxin or cold storage of the preparation. These findings indicate that the fast component is cholinergic and the slow one noncholinergic, non-adrenergic in nature.

Noncholinergic, nonadrenergic responses have been detected in various tissues, and different substances are considered to be transmitter candidates. ATP is a classical candidate in the noncholinergic, non-adrenergic nerves of the guinea-pig taenia coli and the urinary bladder (26, 27). In the rabbit iris sphincter muscle, the response to ATP was small. The late contraction was not inhibited by theophylline, a purinergic P1 receptor antagonist (28). Furthermore, there is no histochemical evidence that the iris is innervated with purinergic components (27).

Among the various agonists tested, substance P and acetylcholine were the most potent contractile agents in this iris muscle and the sensitivity to substance P proved to be much higher. This suggested that substance P may be associated with the slow
contraction induced by electrical transmural stimulation, because this polypeptide is present in primary sensory nerves and is released upon the nerve stimulation (9–11, 29–31). We then examined the effects of capsaicin on the slow contractile response as this compound is known to release and deplete substance P from the nerves (14, 15, 32–35). Following treatment with capsaicin, the rabbit iris sphincter muscle produced only fast but not slow contractions in response to electrical transmural stimulation. The response to exogenously applied substance P remained intact following treatment with capsaicin. Thus the slow contraction may be related to substance P or to a related peptide released from the noncholinergic, nonadrenergic nerves.

Although capsaicin did produce a considerable contraction in the rabbit iris sphincter muscle, with repetitive application, the response to this drug gradually declined and a tachyphylaxis occurred. In the cold stored preparations, the response to capsaicin was markedly attenuated, whereas the responses to substance P and acetylcholine remained unchanged. Following prolonged cold storage, the action of the substances which act on the muscle fibers directly is retained, whereas the action of those which act indirectly is lost (36). The cold storage presumably causes functional disintegration or degeneration of nerve terminals in the tissue (37). These results obtained suggest that capsaicin releases substance P or a related peptide from certain nerves, as are the cases in the spinal cord (16, 33), and in sympathetic ganglia (34, 35) and contraction occurs. This result is consistent with the finding that capsaicin desensitizes selectively the neurogenic inflammation after initial stimulation (17–19), and supports the view that neurogenic inflammation is mediated through the release of substance P. However, it is still unsolved whether or not capsaicin is a specific depletor of substance P in tissues. In our experiments, the fast contraction induced by electrical transmural stimulation and the contraction induced by acetylcholine were somewhat reduced following the treatment with capsaicin. It is possible that capsaicin has an antimuscarinic or a nonspecific inhibitory action on the rabbit iris muscle at concentrations used. Further analyses are required to confirm such a possibility.

Additionally, there was another unexpected result that although the amplitude of fast contraction induced by electrical transmural stimulation was larger than that of late contraction, the contraction induced by acetylcholine was smaller than that by capsaicin. There are possible explanations, namely 1) exogenously applied acetylcholine is rapidly destroyed by the enzyme acetylcholine esterase (23), or the acetylcholine reaches less effectively to the receptor site in the tissue, and 2) a quantity of substance P or related peptide released by capsaicin is larger than that released by electrical transmural stimulation. At present, it is not determined which is more probable.

Baclofen has antagonistic activity on substance P in the rat spinal cord (38). However, the slow contraction was not inhibited by this muscle relaxant. As the responses to substance P and capsaicin were not affected by baclofen, this compound is not useful as a substance P antagonist in the rabbit iris sphincter muscle.

The treatment with reserpine or sympathectomy of both superior cervical ganglia did not abolish the slow contraction and the response to capsaicin, indicating that the substance P containing nerves are not sympathetic in origin. Immunohistochemical studies have demonstrated that substance P-like immunofluorescence is present in the primary sensory nerves, and this compound is considered to be released from the trigeminal
nerve (10, 39). Moreover, Butler et al. reported that miosis induced by capsaicin was abolished following the degeneration of trigeminal nerve after its section (19). All these findings taken together suggest that substance P released from the trigeminal nerve in the rabbit iris sphincter muscle induces the slow response to electrical transmural stimulation and the capsaicin-induced contraction.

Addendum: After submission of this manuscript, we learned that the noncholinergic, nonadrenergic contractile response of the rabbit iris sphincter muscle was selectively inhibited by a substance P antagonist, (D-Pro², D-Trp⁷,⁹)-SP (Rosell et al., Fourth Symposium on Vascular Neuroeffector Mechanisms, Kyoto, July 27–30, 1981).

Acknowledgments: This work was supported in part by a Grant-in-Aid for Special Project Research, from the Ministry of Education, Science and Culture, Japan (No. 56220016). We thank M. Ohara for comments on the manuscript.

REFERENCES
1) Cole, D.F. and Unger, W.G.: Prostaglandins as mediators for the responses of the eye to trauma. Exp. Eye Res. 17, 357–368 (1973)
2) Eakins, K.E.: Prostaglandin and non-prostaglandin mediated breakdown of the blood-aqueous barrier. Exp. Eye Res. Supp. 483–498 (1977)
3) Unger, W.G., Cole, D.F. and Bass, M.S.: Prostaglandin and neurogenically mediated ocular response to laser irradiation of the rabbit iris. Exp. Eye Res. 25, 209–220 (1977)
4) Bruce, A.N.: Vasodilator axon reflexes. Q. J. exp. Physiol. 6, 339–354 (1913)
5) Jampol, L.M., Neufeld, A.H. and Sears, M.L.: Pathways for the response of the eye to injury. Invest. Ophth. 14, 184–189 (1975)
6) Jampol, L.M., Axelrod, A. and Tessler, H.: Pathways of the eye's response to topical nitrogen mustard. Invest. Ophth. 15, 486–489 (1976)
7) Butler, J.M., Unger, W.G. and Hammond, B.R.: Sensory mediation of the ocular response to neutral formaldehyde. Exp. Eye Res. 28, 577–589 (1979)
8) Camras, C.B. and Bito, L.Z.: The pathophysiological effects of nitrogen mustard on the rabbit eye. I. The biphasic intracranial pressure response and the role of prostaglandins. Exp. Eye Res. 30, 41–52 (1980)
9) Hökfelt, T., Johansson, O., Keller, J.-O., Ljungdahl, A., Nilsson, G., Nygards, A. and Pernow, B.: Immunohistochemical distribution of substance P. Substance P. Edited by von Euler, U.S. and Pernow, B., p. 117, Raven Press, New York (1977)
10) Bill, A., Stjernschantz, J., Mandahl, A., Brodin, E. and Nilsson, G.: Substance P; Release on trigeminal nerve stimulation, effects in the eye. Acta physiol. scand. 106, 371–373 (1979)
11) Butler, J.M., Powell, D. and Unger, W.G.: Substance P levels in normal and sensory denervated rabbit eyes. Exp. Eye Res. 30, 311–313 (1980)
12) Cohen, S., Duzman, E., Sira, I.B., Teichberg, V.L. and Blumberg, S.: Isolated preparation of bovine pupillary sphincter: pharmacological characterization of the substance P receptor. Invest. Ophth. Vis. Sci. 19 (ARVO Supp.), 220 (1980)
13) Soloway, M.R., Stjernschantz, J. and Sears, M.: The miotic effect of substance P on the isolated rabbit iris. Invest. Ophth. Vis. Sci. 20, 47–52 (1981)
14) Jessell, T.M., Iversen, L.L. and Cuello, A.C.: Capsaicin-induced depletion of substance P from primary sensory neurones. Brain Research, 152, 183–188 (1978)
15) Theraulat, E., Otsuka, M. and Jessell, T.: Capsaicin-evoked release of substance P from primary sensory neurones. Brain Res. 170, 209–213 (1979)
16) Jancso, N.: Role of the nerve terminals in the mechanism of inflammatory reactions. Bull. Millard Fillmore Hosp., New York 7, 53–77 (1960)
17) Jancso, N., Jancso-Gabor, A. and Szolcsanyi, J.: Direct evidence for neurogenic inflammation and its prevention by denervation and by pretreatment with capsaicin. Brit. J. Pharmacol. Chemother. 31, 138–151 (1967)
18) Camras, C.B. and Bito, L.Z.: The pathophysiological effects of nitrogen mustard on the rabbit eye. II. The inhibition of the initial hypertensive phase by capsaicin and the apparent role of substance P. Invest. Ophth. Vis. Sci. 19, 423–428 (1980)
19) Butler, J.M. and Hammond, B.R.: The effects of sensory denervation on the responses of the
rabbit eye to prostaglandin E\textsubscript{1}, bradykinin and substance P. Brit. J. Pharmacol. 69, 495–502 (1980)

20) Kern, R.: Die adrenergischen Receptoren der intraoculären Muskeln des Menschen. Albrecht v. Graefes Arch. klin. exp. Ophthalmol. 180, 231–248 (1970)

21) Beatie, J.C. and Stilwell, D.L., Jr.: Innervation of the eye. Anat. Rec. 141, 45–61 (1961)

22) Malmfors, T.: The adrenergic innervation of the eye as demonstrated by fluorescence microscopy. Acta physiol. scand. 65, 259–267 (1965)

23) Yamauchi, D.N., DeSantis, L. and Patil, P.N.: Relative potency of cholinomimetic drugs on the bovine iris sphincter strips. Invest. Ophthalmol. 12, 80–83 (1973)

24) Gustafsson, L., Hedqvist, P. and Lagercrantz, H.: Potentiation by prostaglandins E\textsubscript{1}, E\textsubscript{2} and F\textsubscript{2α} of the contraction response to transmural stimulation in the bovine iris sphincter muscle. Acta physiol. scand. 95, 26–33 (1975)

25) Narita, S. and Watanabe, M.: Response of the isolated rat iris sphincter to cholinergic and adrenergic agents and electrical stimulation. Life Sci. 29, 285–292 (1981)

26) Burnstock, G.: Purinergic nerves. Pharmacol. Rev. 24, 509–581 (1972)

27) Burnstock, G., Cocks, T., Crowe, R. and Kasakov, L.: Purinergic innervation of the guinea-pig urinary bladder. Brit. J. Pharmacol. 63, 125–138 (1978)

28) Burnstock, G.: A basis for distinguishing two types of purinergic receptor. Cell Membrane Receptors for Drugs and Hormones: A Multi-disciplinary Approach. Edited by Straub, R.W. and Bolis, L., pp. 107–118, Raven Press, New York (1978)

29) Takahashi, T. and Otsuka, M.: Regional distribution of substance P in the spinal cord and nerve roots of the cat and the effect of dorsal root section. Brain Res. 87, 1–11 (1975)

30) Otsuka, M. and Konishi, S.: Release of substance P-like immunoreactivity from isolated spinal cord of newborn rat. Nature 264, 83–84 (1976)

31) Olgart, L., Gazelius, B., Brodin, E. and Nilsson, G.: Release of substance P-like immunoreactivity from the dental pulp. Acta physiol. scand. 101, 510–512 (1977)

32) Gamse, R., Holzer, P. and Lenbrock, F.: Decrease of substance P in primary afferent neurons and impairment of neurogenic plasma extravasation by capsaicin. Brit. J. Pharmacol. 68, 207–213 (1980)

33) Gamse, R., Molnar, A. and Lenbrock, F.: Substance P release from spinal cord slices by capsaicin. Life Science 25, 629–636 (1979)

34) Konishi, S., Tsuno, A., Yama hara, N. and Otsuka, M.: Peptidergic excitatory and inhibitory synapses in mammalian sympathetic ganglia: roles of substance P and enkephalin. Biomed. Res. 1, 528–536 (1980)

35) Gamse, R., Wax, A., Zigmund, R.E. and Leeman, S.E.: Immunoreactive substance P in sympathetic ganglia: distribution and sensitivity towards capsaicin. Neuroscience 6, 437–441 (1980)

36) Ambache, N.: Interaction of drugs and the effect of cooling on the isolated mammalian intestine. J. Physiol. 104, 266–287 (1946)

37) Shibata, S., Hattori, K., Sakurai, I., Mori, J. and Fujiwara, M.: Adrenergic innervation and cocaine-induced potentiation of adrenergic responses of aortic strips from young and old rabbits. J. Pharmacol. exp. Ther. 177, 621–632 (1971)

38) Saito, K., Konishi, S. and Otsuka, M.: Antagonism between lioresal and substance P in rat spinal cord. Brain Research 97, 177–180 (1975)

39) Brodin, E., Gazelius, B., Lundberg, J.M. and Olgart, L.: Substance P in trigeminal nerve endings: Occurrence and release. Acta physiol. scand. 111, 501–503 (1981)