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Trigeminal antidromic vasodilatation and plasma extravasation in the rat: effects of acetylcholine antagonists and cholinesterase inhibitors

Réjean Couture, A. Claudio Cuello* & James L. Henry**

Département de physiologie, Faculté de médecine, Université de Montréal, C.P. 6208, Succursale A, Montréal, Québec, Canada H3C 3T8, Neuroanatomy/Neuropharmacology Group*, University Departments of Pharmacology & Human Anatomy, South Parks Road, Oxford OX1 3QT and Departments of Physiology and Psychiatry**, McGill University, Montréal, Québec, Canada H3G 1Y6

1 Antidromic stimulation of sensory peripheral branches of the trigeminal system (mental nerve) leads to cutaneous vasodilatation and increases vascular permeability in the rat.

2 Antidromic vasodilatation is observed only at high intensity stimulation (10 V, 15 Hz, 0.2 or 5 ms) supporting the participation of afferent C-fibres in cutaneous dilator responses.

3 Both antidromic vasodilatation and neurogenic plasma extravasation are significantly reduced by muscarinic antagonists suggesting that a cholinergic component may be involved in these trigeminal neurogenic responses.

4 Neurogenic plasma extravasation remains unchanged by hexamethonium while antidromic vasodilatation is reduced. This latter effect may be merely a consequence of the dramatic fall in arterial pressure produced by the ganglion blocker.

5 Antidromic vasodilatation is increased or unaffected by acetylcholinesterase inhibitors. On the other hand, the reduction of the plasma extravasation observed with these drugs could be due to their known ability to decrease the amount of acetylcholine released.

Introduction

There is evidence that substance P (SP) might be involved in primary sensory transmission (Jessell, 1983; Pernow, 1983; Salt & Hill, 1983 for reviews). SP administered iontophoretically to dorsal horn neurones in the spinal cord excites only those that respond to noxious cutaneous stimuli (Henry, 1976). SP is present in the cell bodies of primary sensory neurones of the spinal cord and of the trigeminal system (Hokfelt *et al.*, 1975) and in their central (Hokfelt *et al.*, 1975; Cuello *et al.*, 1978; Del Fiacco & Cuello, 1980) and peripheral branches (Cuello *et al.*, 1978). SP, in amounts able to be detected using immunoassay is released from central (Yaksh *et al.*, 1980) and peripheral (Olgart *et al.*, 1977; Bill *et al.*, 1979; Brodin *et al.*, 1981; Helme & White, 1983) ends of primary sensory neurones *in vivo*. It has been suggested that SP may be released from sensory varicose terminals in the skin following antidromic stimulation of the sciatic (Lembeck & Holzer, 1979; Helme & White, 1983) and the mental nerve (Couture & Cuello, 1983; 1984) at intensities which produce vasodilatation and increased vascular permeability. Substance P antagonists inhibit antidromic vasodilatation (Lembeck *et al.*, 1982; Rosell *et al.*, 1981; Couture & Cuello, 1983; 1984) and neurogenic plasma extravasation (Lembeck *et al.*, 1982; Couture & Cuello, 1983; 1984) in these two areas.

It is well accepted that histamine, probably liberated by mast cells, is also involved in antidromic vasodilatation and neurogenic plasma extravasation (Lembeck, 1983; Couture & Cuello, 1984). In addition 5-hydroxytryptamine seems to participate in neurogenic plasma extravasation but not vasodilatation (Couture & Cuello, 1984). On the other hand, sympathetic noradrenergic neurones do not seem to participate in these responses because sympathetic ganglionectomy and α- and β-adrenoceptor blockers have no effect on antidromic responses (Couture & Cuello, 1984).

Atropine reduces both antidromic vasodilatation and neurogenic plasma extravasation (Couture & Cuello, 1983; 1984) and small levels of choline acetyltransferase, which stimulates biosynthesis of acetylcholine, can be detected in the mental nerve (Couture *et al.*, 1985), suggesting a cholinergic component in these neurogenic responses in the trigeminal territory.

There is, however, no evidence for the presence of trigeminal cholinergic neurones in the mental nerve, and autonomic cholinergic postganglionic fibres running with this nerve are possible candidates.

We have explored further the possible effects of acetylcholine antagonists and cholinesterase inhibitors on antidromic vasodilatation and neurogenic plasma extravasation in the peripheral trigeminal territory. This paper summarizes our findings using anticholinesterases and antimuscarinic drugs on antidromically elicited neurogenic responses. Some of these results have been briefly presented elsewhere (Couture & Henry, 1984a, b).

Methods

Animals

Male Wistar rats (250–350 g) were anaesthetized with urethane (1.4 g kg^{-1}, i.p.) or sodium pentobarbitone (65 mg kg^{-1}, i.p.) with a supplement as necessary. Body temperature was kept at 37°C with a warming lamp. A tracheal cannula was inserted via a tracheotomy to facilitate respiration. All surgery was performed with the aid of a dissecting microscope.

Antidromic vasodilatation

The experimental set-up was similar to that described previously and schematically represented by Couture & Cuello (1984), with some slight modifications. The two mental nerves (the sensory trigeminal branch which supplies the skin of the chin and mucosa of the lower lip) were carefully exposed and cut at their exit from the mental foramina and the distal ends placed on bipolar stainless steel electrodes. They were stimulated electrically with trains of rectangular pulses of 2–10 V and 0.2–5 ms at 15 Hz for two periods of 5 min each using a Grass stimulator (S 88). The periods between trains were determined as described below.

The systemic arterial pressure was recorded via a catheter (PE-50) in the right femoral artery using a Statham pressure transducer (P23 ID) and recorded on a Grass Polygraph (Model 79). One jugular vein and the two anterior facial veins were cannulated using polyethylene tubing (PE-10). The jugular vein was used for i.v. injection of heparin (2000 iu) and of drugs. Blood from both anterior facial veins was collected (using an LKB fraction collector) at 5 min intervals before, during and after antidromic stimulation. The venous outflow was monitored for the assessment of the A.V. by measuring the weight of blood and converting it into volume (mg in μl). Heparinized blood from a donor rat was infused continuously into the jugular vein throughout the experiment at a rate corresponding to the rate of loss via the anterior facial veins (70–110 μl 5 min^{-1}) by using a syringe pump (Sage Instruments Model 341A).

Neurogenic plasma extravasation

The two mental nerves and one jugular vein were prepared as described above for antidromic vasodilatation. Evans Blue dye (Sigma) was injected intravenously (50 mg kg^{-1}) and after 10 min the two mental nerves were stimulated for 5 min as above for antidromic vasodilatation. The rats were killed by exsanguination 5 min after the end of stimulation and the skin of the lower lip which is innervated by the mental nerve was removed. The Evans Blue was extracted from the skin with 4 ml formamide (Sigma) for 24 h at 60°C for colorimetric determination at 620 nm. Neurogenic plasma extravasation was assessed by measuring the excess Evans Blue in the skin as compared with the skin of non-stimulated control animals.

Substances

Physostigmine sulphate, hyoscine and heparin were purchased from Sigma Chemical Co. Other drugs were purchased as indicated: atropine (Mann Research Lab), hexamethonium HCl (National Biochem. Co.), phospholine iodide (Ayerst).

Concentrated solutions (1–10 mg ml^{-1}) of all drugs were prepared in saline (0.9% w/v NaCl solution) and kept at − 20°C. Phospholine iodide was dissolved in diluent for ophthalmic solution as supplied by the manufacturers. Atropine was dissolved in acetic acid and the pH was subsequently adjusted to pH 7.4 with sodium hydroxide (0.5 M) and then saline was added to obtain the 3 mg ml^{-1} solution required.

Statistical analysis

The results were expressed as means ± s.e.mean. Values from control and test groups were evaluated using Student's t test for paired or unpaired data. Probability values (P) smaller than 0.05 were considered to be significant.

Results

Selection of parameters for antidromic stimulation

Low intensity stimulation (2 V, 15 Hz, 0.2 ms) applied bilaterally for 5 min to the distal ends of the cut mental nerves caused only a small vasodilator response and did not affect or only slightly altered arterial blood pressure (Figure 1). At high intensity stimulation (10 V, 15 Hz, 0.2 or 5 ms) a clear cut vasodilatation was observed; the response with a 5 ms pulse width

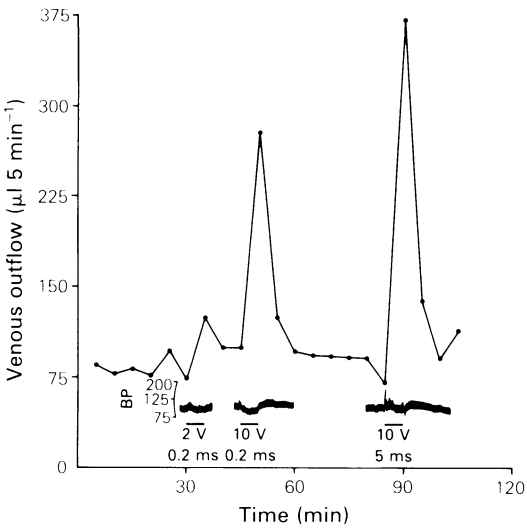

Figure 1 Effect of submaximal and maximal electrical stimulation of the mental nerves according to different parameters (2–10 V, 15 Hz, 0.2–5 ms) for 5 min (−) on the venous outlfow and the arterial blood pressure. Abscissae: time (min), ordinates: venous outflow (μl blood collected at 5 min intervals from both anterior facial veins); and arterial blood pressure (BP) in mmHg.

was greater than that with a 0.2 ms pulse width and was found to be maximal under these experimental conditions (Figure 1).

As our experimental protocol called for more than one sampling run we determined the minimum time period between stimulus trains which would give responses of equal magnitude to successive trains. In preliminary experiments we found that when using a pulse width of 0.2 ms, responses were of roughly equal in magnitude when the stimulation trains were given 20–30 min apart. However, as previously demonstrated (Couture & Cuello, 1984), when using pulses of 5 ms, 1 h or more was required between stimulus trains to obtain responses of equal magnitude and the application of more than two stimulation trains in the same preparation with 5 ms left a distinct brown mark on the nerve. Therefore, in all the experiments described below, we used 10 V, 15 Hz and 0.2 ms. Neurogenic plasma extravasation was unaffected by increasing the pulse width from 0.2 to 5 ms (0.397 ± 0.031 μg Evans Blue mg^{-1} wet tissue at 0.2 ms, $n = 7$, and 0.404 ± 0.019 μg at 5 ms, $n = 10$).

Stimulation at 10 V, 15 Hz and 0.2 ms did affect arterial pressure (Figure 1). The amplitude and the sign of such changes were variable and independent of the basal arterial pressure. These effects were blocked by 80–90% with hexamethonium (10 mg kg^{-1}, i.v., 25 min pretreatment) but remained unchanged by atropine (3 mg kg^{-1}, i.v., 25 min pretreatment), physostigmine (100 μg kg^{-1}, i.v., 20 min pretreatment) and phospholine (10 μg kg^{-1}, i.v., 20 min pretreatment). Hexamethonium (10 mg kg^{-1}, i.v.) decreased arterial pressure (107.1 ± 5.5 mmHg to 64.3 ± 5.1 mmHg, $n = 8$; $P < 0.01$ Student's t test for paired samples). Arterial pressure was decreased by atropine (3 mg kg^{-1}, i.v.) and increased by physostigmine (100 μg kg^{-1}, i.v.) and phospholine (10 μg kg^{-1}, i.v.). The effects of atropine, physostigmine and phospholine were transient, and the pressure returned to its pre-administration level after 5–20 min. Trigeminal antidromic vasodilatation was remarkably insensitive to changes in arterial pressure between 165 and 110 mmHg and from 110 to 70 mmHg. However, the amplitude of the antidromic vasodilatation was significantly reduced at mean arterial pressures lower than 85 mmHg and, therefore, the results below were obtained only from animals with mean arterial pressures of 100 mmHg or greater.

Effects of anaesthetics on neurogenic responses

Comparisons were made between the effects of two anaesthetics, urethane and sodium pentobarbitone on neurogenic plasma extravasation. The results obtained showed that neurogenic plasma extravasation was 34% less in magnitude in animals anaesthetized

Table 1 Effects of various cholinergic drugs on the basal venous outflow (mean ± s.e.mean in μl 5 min^{-1}) measured from both anterior facial veins in the rat

		Before administration	*After administration*
Atropine	($n = 20$)	137.4 ± 11.1	140.6 ± 11.6
Physostigmine	($n = 8$)	129.0 ± 11.5	147.5 ± 12.2*
Phospholine	($n = 19$)	111.8 ± 8.2	131.3 ± 8.2**
Hexamethonium	($n = 8$)	116.9 ± 9.5	115.1 ± 9.3

Statistical significance of differences between venous outflow before and after i.v. administration of drugs, calculated using Student's t test for paired samples; $^*P < 0.005$, $^{**}P < 0.001$. Atropine was given at a dose of 3 mg kg^{-1}, physostigmine and phospholine at 10 μg kg^{-1} and hexamethonium at 10 mg kg^{-1}. n = no. of experiments.

with urethane ($0.255 \pm 0.022\,\mu g\,mg^{-1}$ wet weight tissue, $n = 24$) than in animals anaesthetized with sodium pentobarbitone ($0.385 \pm 0.015\,\mu g\,mg^{-1}$ wet weight tissue, $n = 24$; $P < 0.001$).

As a large variation in the amplitude of the vasodilator responses was observed from one animal to another (200 to 800 µl $5\,min^{-1}$) it was not possible to compare the effects of the two anaesthetics. Nevertheless, clear vasodilator responses were obtained with both anaesthetics.

In the following series of experiments, all the animals were anaesthetized with sodium pentobarbitone.

Effects of acetylcholine antagonists and cholinesterase inhibitors on basal venous outflow

The effects of these compounds on the basal venous outflow are summarized in Table 1. The basal venous outflow was slightly increased by physostigmine (14% increase) and phospholine (17% increase) but remained unaffected by atropine and hexamethonium.

Effects of acetylcholine antagonists and cholinesterase inhibitors on trigeminal antidromic vasodilatation

Venous outflow was reduced by 26% with atropine ($3\,mg\,kg^{-1}$ i.v., 15–20 min pretreatment) and was increased by 34% with phospholine ($10\,\mu g\,kg^{-1}$ i.v., 25 min pretreatment). Hexamethonium ($10\,mg\,kg^{-1}$, i.v., 20 min pretreatment) reduced the antidromic vasodilatation by 36%, but it also reduced mean arterial pressure to below 100 mmHg. Physostigmine ($10\,\mu g\,kg^{-1}$, i.v., 25 min pretreatment) failed to modify venous outflow (Figure 2).

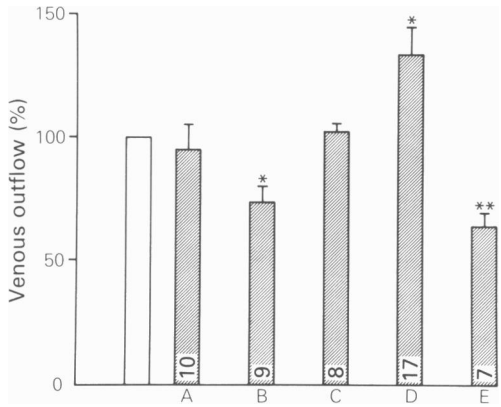

Figure 2 Actions of various compounds on the antidromic vasodilatation induced in the rat lower lip by electrical stimulation of the mental nerves (10 V, 15 Hz, 0.2 ms) for 5 min. Open column, venous outlfow during first stimulation in the absence of compounds, (considered as 100%); hatched columns, venous outflow during second stimulation, (A) control (no drugs), (B) after atropine $3\,mg\,kg^{-1}$ i.v., (C) after physostigmine $10\,\mu g\,kg^{-1}$ i.v., (D) after phospholine $10\,\mu g\,kg^{-1}$ i.v. and (E) after hexamethonium $10\,mg\,kg^{-1}$ i.v.. Columns represent mean and vertical lines s.e.mean of number of experiments indicated (numbers in columns). Statistical significance of differences between venous outflow in absence and in presence of compounds, calculated using Student's *t* test for paired samples $*P < 0.05$, $**P < 0.01$.

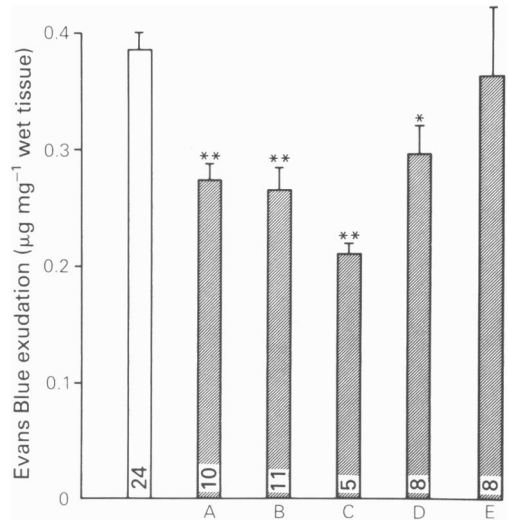

Figure 3 Effect of various compounds on the neurogenic plasma extravasation induced in the rat lower lip by stimulation of the mental nerves (10 V, 15 Hz, 0.2 ms) for 5 min. Open column, control (no drugs); hatched columns, after i.v. (A) atropine $3\,mg\,kg^{-1}$, (B) hyoscine $3\,mg\,kg^{-1}$, (C) physostigmine $10\,\mu g\,kg^{-1}$, (D) phospholine $10\,\mu g\,kg^{-1}$ and (E) hexamethonium $10\,mg\,kg^{-1}$. Columns represent mean and vertical lines s.e.mean of number of experiments indicated (numbers in columns). Statistical significance of differences between Evans Blue exudation in absence and in presence of compounds was calculated using Student's *t* test for unpaired samples; $*P < 0.05$; $**P < 0.001$.

Effects of acetylcholine antagonists and cholinesterase inhibitors on trigeminal plasma extravasation

The effects of these drugs on neurogenic plasma extravasation are shown in Figure 3. Evans Blue exudation was reduced by 29% with atropine ($3\,mg\,kg^{-1}$ i.v., 20 min pretreatment), by 31% with hyoscine ($3\,mg\,kg^{-1}$ i.v., 20 min pretreatment), by 45% with physostigmine ($10\,\mu g\,kg^{-1}$ i.v., 20 min pretreatment) and by 23% with phospholine ($10\,\mu g\,kg^{-1}$ i.v., 20 min pretreatment). This exudation remained unchanged by pretreatment (25 min) with hexamethonium ($10\,mg\,kg^{-1}$ i.v.).

Effects produced by different doses of cholinesterase inhibitors

Neurogenic plasma extravasation was reduced by phospholine and physostigmine, down to doses of 1.0 and 0.1 $\mu g\,kg^{-1}$ i.v. On the other hand, at these low doses there was no effect with physostigmine on the antidromic vasodilatation and some potentiation with phospholine.

After 100 $\mu g\,kg^{-1}$ of physostigmine and 50 $\mu g\,kg^{-1}$ of phospholine the animal showed fibrillary twitching, lacrimation, salivation, defaecation and urination. However, at smaller doses (10 $\mu g\,kg^{-1}$ of each) these effects were not apparent for physostigmine and only slightly apparent for phospholine. Higher doses than the maximum given above produced a high rate of mortality.

Discussion

In the present study bilateral antidromic stimulation of mental nerves, trigeminal branches to the lower lip, caused local cutaneous vaosodilatation and plasma extravasation. The stimulation parameters that produced changes in the nerve response in our study are known to activate afferent C-fibres involved in cutaneous dilator responses (Hensey & Gasser, 1930; Chahl & Ladd, 1976), whereas stimulation with a lower voltage was shown to have a slight effect, and probably activates only A-fibres (Chahl & Ladd, 1976; Akre & Aars, 1977; Yaksh *et al.*, 1980; Kenins, 1981). These findings suggest that activation of high threshold fibres is important for the observed neurogenic responses.

Mental nerve stimulation also caused a change (either a fall or an increase) in systemic arterial pressure. Although this cardiovascular effect was abolished by hexamethonium, the Evans Blue extravasation upon nerve stimulation was not altered. The reduction of the antidromic vasodilatation with hexamethonium pretreatment might be merely a consequence of the dramatic fall in arterial pressure produced by the ganglion blocker. This idea is reinforced by the fact that smaller vasodilator responses were observed in animals with low basal arterial pressure. In addition, the absence of correlation between the intensity and the sign of changes on blood pressure induced by mental nerve stimulation and the amplitude of the vasodilatation rule out any link between the neurogenic responses and the changes induced on arterial blood pressure. Assuming that substance P plays a major role in these responses (Lembeck *et al.*, 1982; Couture & Cuello, 1984), it is important to note that the reduction in the perfusion pressure and skin small artery pressure following infusion of substance P into the brachial artery of a perfused canine forelimb was not related to the systemic blood pressure (Dobbins *et al.*, 1979).

Urethane anaesthesia depressed plasma extravasation, as more Evans Blue extravasation was measured when sodium pentobarbitone was used as an anaesthetic. This depressive effect of urethane was not further investigated. However, it has previously been shown that urethane depresses cutaneous vascular permeability responses to histamine and histamine-releasing agents (Chahl, 1983). These findings are compatible with the involvement of histamine in neurogenic plasma extravasation (Lembeck, 1983; Couture & Cuello, 1984). Recently, Hirata *et al.* (1984) showed that primary afferent thermoreceptors recorded from the trigeminal ganglion of cats anaesthetized with urethane showed a significant reduction in thermal sensitivity when compared with neuronal responses obtained from cats anaesthetized with sodium pentobarbitone. Moreover, substance P-induced facilitation of central neuronal activity appears to be especially sensitive to anaesthetics (Sastry, 1978). While these three studies cannot be directly compared, the results suggest that primary afferent activity can be markedly influenced by anaesthetics.

Antidromic vasodilatation and plasma extravasation were slightly but significantly reduced by muscarinic receptor blockers (atropine and hyoscine). Atropine has also been found to abolish the residual antidromic vasodilatation of the hind leg in capsaicin pretreated rats (Lembeck & Holzer, 1979). Therefore, the atropine-induced reduction of the antidromic responses suggests that a cholinergic mechanism might be involved which could have some physiopathological significance. Atropine suppresses the flush, while prostigmine enhances the flushing reaction in the 'Chinese restaurant syndrome' (Ghadimi *et al.*, 1971). This symptom seems to be caused by a transient increase in an acetylcholine-like substance after ingestion of large quantities of monosodium glutamate.

Agents which prevent acetylcholine breakdown (physostigmine and phospholine) produced an increase or had no effect on antidromic vasodilatation while they depressed plasma extravasation. The potentiation of the antidromic vasodilatation by phospholine cannot be explained by a change in systemic blood pressure and cannot be due to local circulatory changes as both physostigmine and phospholine increased equally (14–17%) the basal venous outflow from anterior facial veins in the rat and physostigmine was without effect on the vasodilatation. Previous studies (Holton & Perry, 1951; Holton, 1953) have shown that physostigmine decreases antidromic vasodilatation in the rabbit ear.

The somewhat contradictory results obtained with the anticholinesterase compounds can be better understood by recalling that they not only prevent breakdown of acetylcholine but also decrease the amount of

acetylcoline released (Szerb & Somogyi, 1973; Sacchi *et al.*, 1978; Dunant *et al.*, 1980). These latter effects could themselves account for the reduction in plasma extravasation seen with these drugs.

Our pharmacological studies indicate that the mechanism of a potential cholinergic component involved in trigeminal antidromic vasodilatation and plasma extravasation appears to be complex and different for the two vascular responses.

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