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Cerebrovascular responses to capsaicin *in vitro* and *in situ*

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1 The cerebrovascular effects of capsaicin have been examined *in vitro*, in feline isolated cerebral arteries (circular segments, 2–3 mm long, 300–400 μm extended diameter) and, *in situ*, in pial arterioles (diameter 40–200 μm) on the cortical surface of chloralose-anaesthetized cats.

2 In isolated middle cerebral arteries, low concentrations of capsaicin (10^{-14}–10^{-10} M) effected a concentration-dependent relaxation of vessels precontracted with prostaglandin $F_{2\alpha}$. This relaxant response was markedly attenuated by repeated administration of capsaicin but was minimally affected by the presence of atropine, propranolol, cimetidine or spantide in the tissue bath.

3 In isolated middle cerebral arteries, higher concentrations of capsaicin effected a marked concentration-dependent contraction. This contraction was not modified by 10^{-6} M phentolamine or 10^{-6} M ketanserin. A markedly reduced contraction by capsaicin was found upon the removal of calcium ions from the buffer solution. Also the calcium entry blocker nimodipine reversed the capsaicin-induced contraction.

4 Subarachnoid perivascular microapplication of capsaicin around individual pial arterioles *in situ* elicited a biphasic response (an immediate vasoconstriction followed by a sustained vasodilatation). The maximum vasoconstriction was a 60 ± 6% reduction in diameter from base line and the maximum vasodilatation a 38 ± 7% increase in diameter. Vasodilatation occurred at lower concentrations of capsaicin (EC_{50}, approximately 5×10^{-8} M) than those required for vasoconstriction (EC_{50} 3×10^{-7} M).

5 Trigeminal ganglionectomy 10–16 days before the microapplication abolished the *in situ* vasodilator effects of capsaicin (10^{-6} M) applied perivascularly, but was without effect on the vasoconstrictor actions of this agent.

6 Repeated administration of capsaicin (10^{-6} M) around the same arteriole resulted in a progressive attenuation of the vasodilator phase of the response, with no modification of the vasoconstrictor phase.

7 The present study suggests that capsaicin-induced cerebral vasodilatation is due to the release of vasoactive agents from cerebrovascular trigeminal nerve fibres, whereas the vasoconstrictor effect of capsaicin is due to a direct effect on the cerebral vasculature which is mediated via the transmembrane passage of extracellular calcium.

Introduction

Cerebral blood vessels are innervated by a population of small diameter unmyelinated nerve fibres which originate in the trigeminal ganglion (Mayberg *et al.*, 1984). The trigemino-cerebrovascular nerve fibres and perikarya in the trigeminal ganglion contain, often co-localized in the same cellular elements, substance P, calcitonin gene-related peptide (CGRP), cholecystokinin, dynorphin B, neurokinins A and B, as well as putative precursors such as preprotachykinin A (Edvinsson, 1985; Uddman *et al.*, 1985; Hanko *et al.*, 1985; Liu-Chen *et al.*, 1985; Saito & Goto, 1986; Moskowitz *et al.*, 1986; McCulloch *et al.*, 1986; Edvinsson *et al.*, 1987a,b,c). The trigemino-cerebrovascular system has long been considered the primary sensory afferent system involved in the transmission to the CNS of nociceptive information of a vascular origin. However, many of the peptides which are present in trigemino-cerebrovascular fibres are vasoactive in the cerebral circulation (Edvinsson *et al.*, 1987a,b; Edvinsson & Jansen, 1987) and recent evidence suggests that this system is of major vasomotor significance in restoring normal cerebrovascular calibre after excessive vasoconstriction (McCulloch *et al.*, 1986).

Capsaicin, 8-methyl-N-vanillyl-6-nonenamide is a pungent constituent of some red peppers and its uses, both chronically, as a neurotoxin, and acutely to release peptides, in the elucidation of the function of primary sensory afferent neurones, is well established (Nagy, 1982). As in peripheral blood vessels (Nagy, 1982; Furness *et al.*, 1982; Wharton *et al.*, 1986), chronic administration of capsaicin results in a specific depletion of perivascular substance P- and CGRP-immunoreactivity from cerebral vessels (Duckles & Buck, 1982; Duckles & Levitt, 1984; Saito & Goto, 1986; Saito *et al.*, 1988). In peripheral blood vessels, the acute administration of capsaicin produces a sustained vasodilatation and increased vascular permeability, which are putatively mediated via the release of neuropeptides from sensory nerve endings (Lembeck, 1983). Acute capsaicin administration is now being extended to cerebrovascular research to explore the influence of the trigeminal system upon blood-brain barrier permeability (Reid & McCulloch, 1987). Capsaicin has, however, a diverse range of effects on smooth muscle. In some arterial preparations *in vitro*, capsaicin provokes contraction whereas in other vessels, capsaicin elicits relaxation (Toda *et al.*, 1972; Duckles, 1986). In guinea-pig ureter, the effects are dose-dependent, with low concentrations causing an inhibition of ureter motility and high concentrations stimulating motility (Hua *et al.*, 1985; 1986). In the present study, we have characterized in detail the cerebrovascular effects of capsaicin by use of sensitive *in vitro* and *in situ* methods and the insight gained adds to our understanding of the functional significance of the trigemino-cerebrovascular system.

Methods

Responses of middle cerebral artery segments in vitro

Ten adult cats (weighing 2–4 kg) were exsanguinated under pentobarbitone anaesthesia (Nembutal 30 mg kg^{-1} i.p.). The brains were removed, the middle cerebral arteries dissected free and placed in cold Krebs-Ringer solution aerated with

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95% O_2 plus 5% CO_2. The composition of the buffer solution was (mM): NaCl 119, KCl 4.6, $CaCl_2$ 1.5, $MgCl_2$ 1.2, $NaHCO_3$ 15, NaH_2PO_4 1.2 and glucose 11, pH 7.4. Circular vessel segments, 2–3 mm long, were mounted between two L-shaped metal prongs in 2.5 ml mantled tissue baths at 37.5°C for recording of vasomotor activity (Högestätt *et al.*, 1983). Isometric tension was measured with Grass FT03C force displacement transducers and recorded on a Grass Polygraph. The segments were given a passive load of 4 mN, allowed to attain a steady level of tension and to stabilize for 90 min before testing. In an initial set of tests each vessel segment was first exposed to a buffer solution containing 60 mM potassium (achieved by an equimolar substitution of NaCl for KCl in the above buffer solution). This resulted in strong contractions, which in the present series of experiments amounted to 14 ± 3 mN ($n = 19$). In experiments performed in Ca^{2+}-free buffer the calcium was replaced with 0.1 mM EGTA (ethylene glycol-bis (β-aminoethyl ether)-N,N,N,N,-tetraacetic acid). After an incubation time of 0.5–1 h, during which the Ca^{2+}-free buffer in the tissue bath had been exchanged every 5 min, capsaicin was given in cumulative (10^{-9}–10^{-6} M) concentrations until maximum contraction was achieved. In subsequent tests we were only able to observe a contractile effect of capsaicin (see Saito *et al.*, 1988). These contractile responses to capsaicin were reproducible. When relaxation of the vessels was being examined, by cumulative application of capsaicin in the concentration range 10^{-14}–10^{-10} M, the vascular segments were first constricted by the addition of 3×10^{-6} M prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$)(EC_{80}), which resulted in a contraction that was stable for at least 30 min. However, in vessels precontracted by potassium chloride (60 mM) no vasodilator response was observed.

In some experiments the effect of capsaicin was examined in the presence of different specific receptor antagonists, each given approximately 20 min before and being present during the tests.

Exposure to capsaicin was performed only once. Experiments with antagonists were performed in parallel tests, six vessel segments were run in separate tissue baths in order to avoid tachyphylaxis or depletion of stored neurotransmitter, with one or two segments serving as control.

The integrity of the endothelium in the present set-up was assessed in separate experiments where vasodilator responses to acetylcholine (10^{-9}–10^{-4} M) in $PGF_{2\alpha}$-precontracted vessel segments were found to be unaltered (60 ± 7% of the $PGF_{2\alpha}$-induced contraction).

Vasomotor responses of pial arterioles in situ

Eight cats (weighing 3–4 kg) were anaesthetized with a mixture of alphaxolone (6.75 mg kg^{-1}) and alphadolone acetate (2.25 mg kg^{-1}) i.v., intubated and connected to an intermittent positive-pressure ventilation system delivering room air in an open circuit. The right femoral artery and vein were cannulated. Anaesthesia was maintained during the subsequent course of the experiments with α-chloralose (60 mg kg^{-1} i.v.). The animals were maintained normocapnic (arterial carbon dioxide tension, P_aCO_2, close to 32 mmHg throughout the course of the experiments). The end-tidal concentration of carbon dioxide was monitored continuously by means of an infra-red analyzer, and samples of arterial blood were taken frequently during the experiments for the estimation of arterial oxygen tension, P_aCO_2 and pH. In each cat, the mean arterial blood pressure was always greater than 80 mmHg. Rectal temperature was maintained at 38°C with a heating blanket. The animals were placed in a stereotaxic frame, and a craniotomy, measuring 2.5 cm × 1.5 cm, was made over the left parietal cortex. The dura was removed carefully and any bleeding from the cut dural edges was sealed by bipolar diathermy. The exposed cortex was bathed by prewarmed liquid paraffin maintained at 38°C. Vascular calibre was measured by an image-splitting technique (Baez, 1966). Individual pial vessels on the convexity of the brain were viewed in focus through a microscope, and the image was passed through a Vickers image-splitting eye-piece to a closed circuit television camera and displayed on a television monitor. Vascular diameter was measured from the degree of shear applied to the image splitter that had been calibrated against wire and thread of known diameter. Capsaicin was dissolved in artificial cerebrospinal fluid immediately before use. The composition of the artificial cerebrospinal fluid was (mM): NaCl 133, KCl 3, $CaCl_2$ 2.5, $NaHCO_3$ 12 and glucose 3.3. It was adjusted to pH 7.2 by aeration with 5% CO_2 and 95% O_2. Glass micropipettes were filled with artificial cerebrospinal fluid under mineral oil. By use of a micromanipulator, the micropipettes were inserted through the arachnoid into the perivascular space close to a cerebral arteriole. For further details see Harper & MacKenzie (1977). Approximately 5 μl of artificial cerebrospinal fluid was injected into the perivascular space over 15 s, and any resulting alterations in vascular calibre were monitored until the vessel diameter was restored, often for periods of up to 5 min following the injection. The maximum changes in calibre in response to the perivascular microinjection of the drug (expressed as % changes from the diameter of the vessel before drug administration) were compared with those following the administration of artificial cerebrospinal fluid alone. Microinjection was usually made only on one occasion at each site.

Surgical division of the trigeminal nerve

In five cats anaesthetized with pentobarbitone, the trigeminal nerve was unilaterally divided under sterile operating conditions by a neurosurgeon (T.A.K.) using a previously described technique (Liu-Chen *et al.*, 1983). The temporal muscle was resected to expose the calvarium and a subtemporal craniectomy was made with a dental drill. Each division of the left trigeminal nerve was surgically sectioned immediately distal to the trigeminal ganglion. Care was taken not to enter the subdural space and to prevent bleeding from the bone and cavernous sinus. In five sham-operated animals the nerve was exposed, but not divided. Post-operative recovery was generally excellent, with all animals eating normally within 48 h of the procedure. The vasomotor responses of pial arterioles *in situ* were investigated 10–16 days after surgery (see above).

Statistical analysis

Data are presented as mean values ± s.e.mean. Differences between the mean values were assessed by Student's t test and the Bonferroni correction factor was employed to maintain an α-level of 0.05 or smaller.

Drugs

Capsaicin (Sigma, U.S.A.), prostaglandin $F_{2\alpha}$ (Amoglandin, Astra, Sweden or Sigma, U.S.A.), atropine (Atropin, ACO, Sweden), propranolol (Inderal, ICI, U.K.), cimetidine (Sigma, U.S.A.), spantide (Ferring AB, Sweden), phentolamine (Regitin, Ciba-Geigy, Switzerland), ketanserin (Janssen, Belgium) and nimodipine (Bayer AG, F.R.G.) were used.

For the investigations *in vitro*, capsaicin was dissolved in ethanol (70%) and then further diluted in saline. Spantide was dissolved and diluted in saline containing 0.1 mM ascorbic acid. All other drugs were dissolved and/or diluted in saline. The concentrations given below are the final concentrations (M) in the tissue bath.

Results

Vasomotor responses of middle cerebral artery segments in vitro

Under the condition of resting tone, the administration of capsaicin markedly increased the tension developed by the

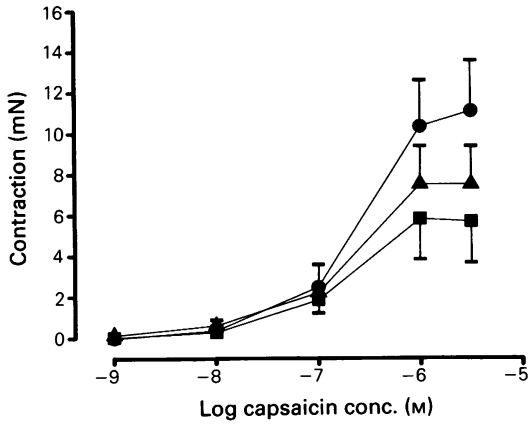

Figure 1 Contractions induced by capsaicin alone (●) or in presence of the α-adrenoceptor antagonist phentolamine (10^{-6} M) (▲), or the 5-hydroxytryptamine-antagonist ketanserin (10^{-6} M) (■). Mean values are shown with vertical lines indicating s.e.mean; number of vessels, 6–8.

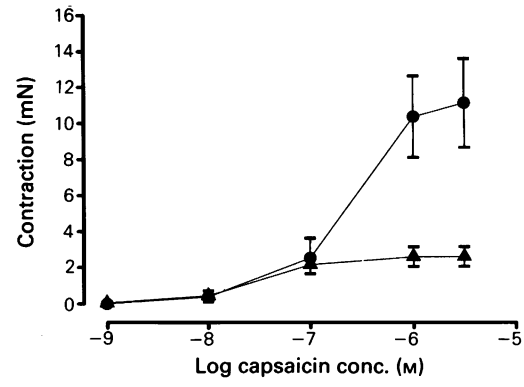

Figure 2 Contraction induced by capsaicin in Ca^{2+}-free media (▲) compared to control (●). Mean values are shown with vertical lines indicating s.e.mean; number of vessels, 5.

pial arteries. The threshold concentration of capsaicin for the induction of contraction was between 10^{-9} and 3×10^{-8} M (Figure 1) and the maximum contraction, at a concentration of 3×10^{-6} M was 11.1 ± 2.5 mN or 63.4 ± 12.1% of the contraction produced by potassium (60 mM). The contractile response to capsaicin was reproducible upon a second exposure (not shown). However, as the experimental design of this study was to avoid a second capsaicin exposure to a particular vessel segment, the antagonist studies were performed in studies in which six parallel tissue baths with one or two ring segments as control were utilized.

The contractions induced by capsaicin were minimally attenuated by the presence of phentolamine (10^{-6} M) or ketanserin (10^{-6} M) in the tissue bath (Student's t test; $P > 0.05$) (Figure 1). The contractions induced by capsaicin were significantly ($P < 0.05$) reduced in calcium-free medium (Figure 2). Nimodipine produced a concentration-related reversal of the contraction to capsaicin (at concentrations between 10^{-11}–10^{-6} M) with the maximum relaxation being 99.1 ± 0.6% and the IC_{50} value $7.6 \pm 3.6 \times 10^{-10}$ M.

The administration of prostaglandin $F_{2\alpha}$ (3×10^{-6} M) elicited a strong and stable contractile response (6.1 ± 1.1 mN, $n = 13$). The cumulative administration of capsaicin to precontracted cerebral arteries elicited a concentration-dependent relaxation in the majority of animals examined (7 of the 10 cats) (Figure 3). In arterial segments from the three remaining cats, capsaicin failed to relax the precontracted arteries despite the testing of multiple segments from each animal. The concentration of capsaicin producing half-maximum relaxation in the seven responding cats was $3.7 \pm 1.3 \times 10^{-14}$ M and the maximum relaxation was 24.5 ± 7.1% of the $PGF_{2\alpha}$-induced contraction. The cerebrovascular relaxation induced by low concentrations of capsaicin was unaffected by the presence of propranolol (10^{-7} M), cimetidine (10^{-6} M), atropine (10^{-4} M) or spantide (3×10^{-6} M) (Figure 4). Repeated exposure of cerebral arterial segments to capsaicin successively reduced the relaxant response to the agent (Figure 3). Furthermore, a slight contraction was seen with 10^{-10} M capsaicin.

Vasomotor responses of pial arterioles in situ

The perivascular microapplication of capsaicin around individual pial arterioles elicited time-dependent and concentration-dependent constrictions and dilatations. Capsaicin, particularly at higher concentrations, effected an immediate transient (duration less than one minute) reduction in arteriolar calibre, followed, two to three minutes after the microinjection, by a sustained increase in arteriolar calibre which was sustained for more than 15 min after the microinjection. A statistically significant dilatation of pial arterioles was observed with capsaicin (10^{-7}–10^{-5} M) whereas significant constriction was noted only with capsaicin (10^{-6} and 10^{-5} M) (Table 1). The concentration of capsaicin eliciting the half-maximal relaxation of pial arterioles was approximately 5×10^{-8} M whereas that for constriction was approximately 3×10^{-7} M (Figure 5, Table 1). The administration of the appropriate vehicle (CSF containing 0.8% Tween 80 and 0.2% ethanol) was without significant effect upon arteriolar calibre (mean alteration in calibre, -4.1 ± 1.5%, $n = 8$).

Repeated microapplication of capsaicin (10^{-6} M), every 15 min at the same site indicated that the initial vasoconstrictor response was unaltered by prior exposure to capsaicin, but that the delayed vasodilator component was markedly

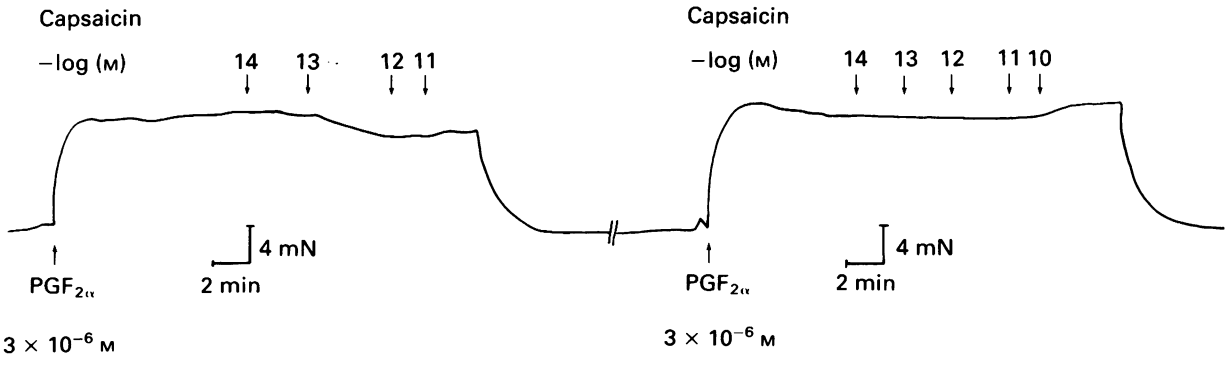

Figure 3 Typical response obtained when capsaicin was given to arteries precontracted by prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$). Repeating the experiment reduced the relaxant response to capsaicin. Calibration bars are inserted. The concentrations of capsaicin, −log (M), are given above the traces.

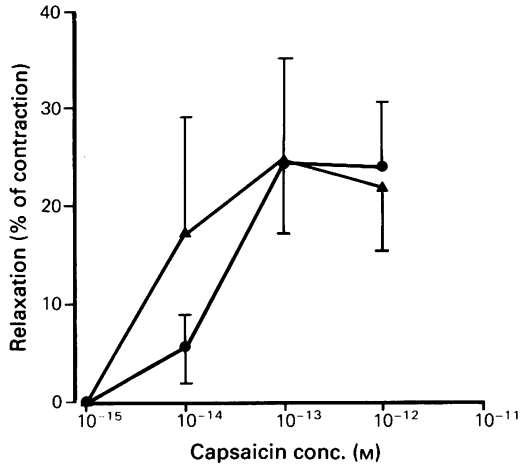

Figure 4 Capsaicin-induced relaxation of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$)-contracted vessels in the presence of the substance P antagonist [D-Pro^2, D-Trp^{7,9}]-SP (3×10^{-6} M) (▲) compared to control (●). Mean values are shown and vertical lines indicate s.e.mean; number of vessels, 4–6.

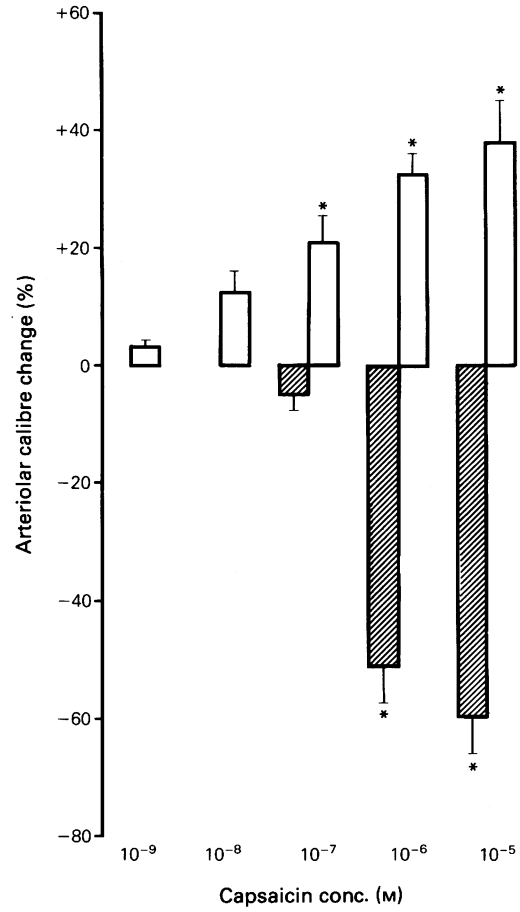

Figure 5 Vasomotor responses of feline pial arterioles *in situ* to perivascular microapplication of capsaicin eliciting concentration-dependent reduction followed by a sustained increase in arteriolar calibre. The maximal arteriolar calibre changes induced by the different capsaicin concentrations are shown; vertical bars indicate s.e.mean. For details on number of tests and statistics, see Table 1; *, $P < 0.05$. Shaded columns represent initial shortlasting constriction upon capsaicin administration; open columns, the more sustained increase in vessel calibre.

reduced after a single prior microapplication of capsaicin at the same site (Figure 6).

A separate series of experiments was performed in cats in which the ipsilateral trigeminal ganglion had been lesioned surgically 10–16 days before the microapplication study, and in cats subjected to a sham-operation. The perivascular microapplication of capsaicin (10^{-6} M) elicited an initial vasoconstriction of a similar magnitude in sham-operated and trigeminal-lesioned cats (Figure 7). However, the duration of the initial vasoconstriction in the sham-operated animals (time for half restoration of preinjection calibre 27 ± 4 s, $n = 12$) was significantly less ($P < 0.01$) than in cats with trigeminal lesions (time for half restoration of preinjection calibre 78 ± 13 s, $n = 11$). Moreover, no delayed vasodilatation was observed after perivascular microapplication of capsaicin (10^{-6} M) in cats in which the trigeminal nerve had been surgically lesioned before the micro-application study (Figure 7).

Discussion

In this detailed characterization of the cerebrovascular effects of capsaisin, the dual vasomotor effects of capsaicin upon cerebral arteries *in vitro* and arterioles *in situ* have been defined. A vasodilator effect was observed at lower concentrations of capsaicin and required the integrity of the trigeminal system. A vasoconstrictor effect of capsaicin was found at higher concentrations and this was not dependent upon the trigeminal system.

The acute administration of capsaicin is known to cause the release of neuropeptides such as substance P, neurokinin A and CGRP from perivascular nerve fibres (Duckles & Buck, 1982; Saria *et al.*, 1983; Franco-Cereceda & Lundberg, 1985; Hua *et al.*, 1986). Although the release from cerebral vessels *in vitro* of substance P and CGRP has been demonstrated with capsaicin administration (Moskowitz *et al.*, 1983; Saito & Goto, 1986), it seems likely in view of the evidence from other tissues that the release of a range of other neuropeptides (neurokinin A, neurokinin B, dynorphin, *inter alia*) may contribute to the cerebral vasomotor effects of capsaicin. Although capsaicin administration has been demonstrated to relax the thoracic aorta and carotid artery of the guinea-pig, only contractions of the cerebral vasculature have been shown with capsaicin (Toda *et al.*, 1972; Duckles, 1986; Saito *et al.*, 1988). In the present study, vasodilatation of cerebral blood vessels could be demonstrated both *in vitro* and *in situ* after

Table 1 Cerebral vasomotor effects to the microapplication of capsaicin *in situ*

Capsaicin conc. (M)	*Initial vasoconstriction*	*No. of arterioles responding/ No. of arterioles tested*	*Delayed vasodilatation*	*No of arterioles responding/ No. of arterioles tested*
10^{-9}	None	0/5	103.0 ± 0.9	0/5
10^{-8}	None	0/9	112.7 ± 3.6	4/9
10^{-7}	95.1 ± 2.5	1/6	121.3 ± 4.3*	6/6
10^{-6}	49.1 ± 6.1*	9/9	132.8 ± 4.1*	9/9
10^{-5}	39.9 ± 5.9*	9/9	138.0 ± 7.2*	9/9

Data on vessel calibre are expressed as a percentage of calibre before microinjection of an agent (i.e. 100%). Values given represent means ± s.e.mean. Statistical comparison between microapplication of cerebrospinal fluid and different concentrations of capsaicin were analysed; *$P < 0.05$ (derived from t statistic with use of Bonferroni's inequality).

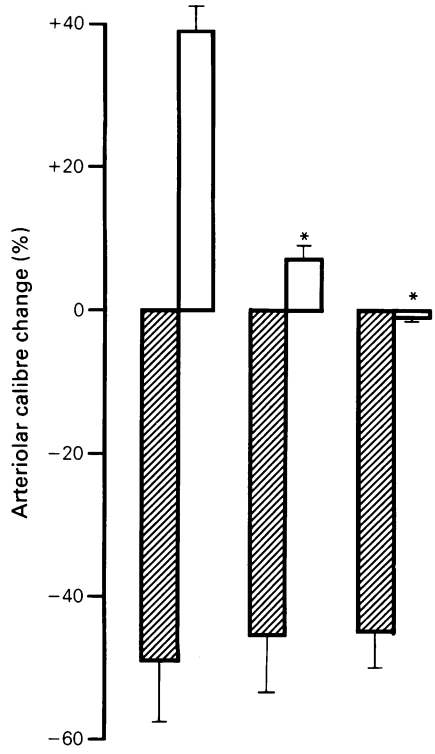

Figure 6 Vasomotor responses of feline pial arterioles *in situ* to repeated perivascular microapplication of capsaicin (10^{-6} M) every 15 min. The initial vasoconstrictor response (shaded columns) was unaltered while the delayed vasodilator response (open columns) was markedly reduced (Student's *t* test with Bonferroni correction; $*P < 0.05$). Arteriolar calibre changes are given and vertical bars show s.e.mean. Number of experiments, 8–9.

capsaicin administration. Notably the vasodilator response was seen at concentrations lower than those required to produce vasoconstriction. The loss of the vasodilator response to capsaicin after trigeminal lesions *in situ* or after repeated

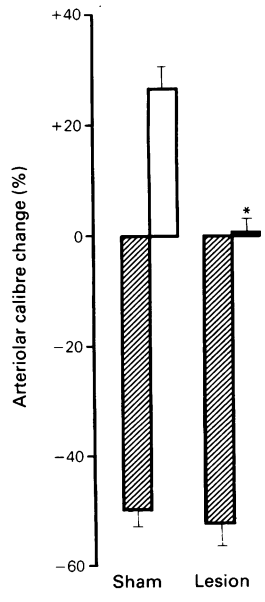

Figure 7 Vasomotor responses of feline pial arterioles *in situ*. The magnitude of the vasoconstrictor response (shaded columns) capsaicin (10^{-6} M) was unchanged while the delayed vasodilatation (open column) was abolished in cats in which the trigeminal nerve had been surgically lesioned before the microapplication study. Arteriolar calibre changes are given and vertical bars show s.e.mean. Number of experiments, 11–12. Student's *t* test; $*P < 0.01$.

exposure to the agent *in vitro* or *in situ* is consistent with the view, well established in peripheral tissue, that relaxation induced by capsaicin is produced indirectly via the release of vasoactive agents from the trigeminal nerve endings. The failure of previous studies *in vitro* to observe relaxation of cerebral vessels after capsaicin (Toda *et al.*, 1972; Duckles, 1986; Saito *et al.*, 1988) may be a consequence of the widespread use of depolarization with potassium, at the outset of the study, to assess the maximal contractile capacity of the vessel. This was avoided in the present study because high concentrations of potassium may effect the release of stored perivascular neurotransmitters such as substance P (Edvinsson *et al.*, 1983; Moskowitz *et al.*, 1983). In some cases we were unable to demonstrate the relaxation of middle cerebral arteries induced by capsaicin after exposure of the vessel to potassium (see Methods). Moreover, in vessels from a minority of cats (30%), no relaxation was observed upon capsaicin administration. This may reflect either the release of neuropeptides during the killing of the animal or interanimal variability in endogenous levels of neuropeptides. There is support for both views (see Uddman *et al.*, 1985; McCulloch *et al.*, 1986).

The trigemino-cerebrovascular system putatively contains substance P, neurokinin A, calcitonin gene-related peptide, gastrin-releasing peptide, dynorphin B and cholecystokinin (Edvinsson *et al.*, 1981; 1987a,c; Uddman *et al.*, 1983; 1985; Hanko *et al.*, 1985; Liu-Chen *et al.*, 1985; Moskowitz *et al.*, 1986). Substance P, neurokinin A and particularly CGRP are cerebral vasodilators, both *in vitro* and *in situ* (Edvinsson *et al.*, 1981; 1987a,c; McCulloch *et al.*, 1986), whereas gastrin-releasing peptides, dynorphin B and cholecystokinin have no direct effects on the cerebral vasculature (Uddman *et al.*, 1983; McCulloch & Kelly, 1984; Moskowitz *et al.*, 1986). The cerebrovascular effects of substance P and neurokinin A are markedly attenuated by the substance P analogue, spantide, which does not influence the effects of CGRP (Edvinsson & Jansen, 1987). The capsaicin-induced relaxation of cerebral vessels was not modified by atropine, cimetidine, propranolol or spantide. The weak relaxing effects of substance P on cat middle cerebral artery (Edvinsson *et al.*, 1981) compared to the stronger CGRP relaxation (Edvinsson *et al.*, 1985) suggest that it is the release of CGRP or an equally potent agent rather than substance P or neurokinin A which is responsible for the relaxation of the cerebral vessels elicited by capsaicin in the cat. Our recent observations (Jansen, Alafaci, Brodin, Edvinsson & Uddman, unpublished observations) that the relaxant response to capsaicin is not dependent on an intact endothelium, is further indicative of the involvement of a strong vasodilator such as CGRP in the response. However, one cannot exclude the possibility that, it is the release of some presently unknown material from the trigeminal nerve fibres that is crucial for the effect of capsaicin.

Vasoconstrictor responses to capsaicin have been seen in many smooth muscle preparations, although there is considerable variability between different tissues in their sensitivity to capsaicin (Toda *et al.*, 1972; Nagy, 1982; Wahlestedt *et al.*, 1984; Duckles, 1986). Capsaicin produces contractions of a similar magnitude to noradrenaline in canine mesenteric and renal arteries whereas in rabbit mesenteric or canine pulmonary arteries, capsaicin elicits only small contractions (less than 5% of that to noradrenaline). In contrast, the present study of cerebral vessels revealed that capsaicin is considerably more potent as a vasoconstrictor than noradrenaline (Wahl *et al.*, 1972; Edvinsson & Owman, 1974; Edvinsson *et al.*, 1982). In most tissues, the contractile effects of capsaicin are reproducible and, as in the present study, not dependent upon an intact sensory afferent innervation (persists after trigeminalectomy), although in some muscle preparations (e.g. pupillary sphincter), the contractile effect of capsaicin requires an intact trigeminal system (Wahlestedt *et al.*, 1984). Previous studies have shown that substance P and neurokinin A act as vasoconstrictors in this preparation while CGRP was ineffective but potentiated the substance P-induced contractions

(Wahlestedt *et al.*, 1986). The contractile effect of capsaicin in all tissues including the cerebral vasculature is dependent upon the mobilization of extracellular calcium, as shown here by reduced capsaicin contraction in calcium-free medium with EGTA and reversal of contraction by the calcium entry blocker nimodipine. Furthermore, phentolamine and ketanserin were without blocking effect. Our study complements that of Saito *et al.* (1988), who noted that capsaicin directly constricted cat cerebral arteries *in vitro* via a mechanism that was independent of endothelium and nerve components.

The present data have provided a detailed description of the vasomotor effects of capsaicin in the feline cerebral circulation. They describe not only the sensitivity and selectivity of capsaicin in this vascular bed but also the difficulties which are encountered (biphasic responses) when capsaicin is used *in vivo*.

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