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Effects of capsaicin on vascular smooth muscle

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Summary. 1) Acute administration of capsaicin in vitro produced either vascular smooth muscle contraction (cat middle cerebral artery) or smooth muscle relaxation (guinea pig carotid artery and thoracic aorta).

2) Prior in vivo treatment with capsaicin abolished the relaxation response of guinea pig vessels to acute capsaicin. Instead a contractile response was seen after chronic capsaicin treatment, suggesting that the relaxation response produced by capsaicin is due to release of a vasodilator substance.

3) Substance P caused relaxation in both cat cerebral arteries and the guinea pig thoracic aorta, an effect which was abolished or reduced by endothelial damage. However, responses to acute capsaicin were not altered by endothelial damage, suggesting that substance P does not mediate the relaxation response to acute capsaicin administration.

4) Exposure to capsaicin in vitro did not affect the neurogenic vasodilator response of cat cerebral arteries and did not alter substance P levels.

5) Therefore, it was concluded that the acute effect of capsaicin is composed of two components. A contractile response is most likely due to direct effects on vascular smooth muscle, while a relaxation response is attributed to release of an as yet unidentified bioactive substance distinct from substance P.

Key words: Substance P – Capsaicin – Cat cerebral arteries – Guinea pig thoracic aorta – Guinea pig carotid artery – Neurogenic vasodilation

Introduction

Capsaicin has been well established as a powerful tool for exploration of the effects of primary afferent neurons. For example, capsaicin has been important in establishing the role of substance P as a mediator of antidromic vasodilation seen in the skin, eye and other tissues (Lewis 1937; Jancsó et al. 1967; Lembeck and Holzer 1979; Lembeck 1983). Chronic exposure to capsaicin has been shown to deplete substance P specifically from primary sensory afferents (Jessell et al. 1978; Gamse et al. 1980; Duckles and Levitt 1984). However, acute exposure to capsaicin has also been used as a way to explore the function of sensory nerves. For example, acute exposure to capsaicin of the guinea pig ileum produces a contractile effect which is reduced in preparations desensitized to substance P or treated with atropine (Bartho et al. 1982; Chahl 1982). These studies have been interpreted as meaning that substance P containing nerves can produce contraction of the ileum through both a direct effect and stimulation of acetylcholine release. Vasodilator responses due to direct capsaicin administration have been demonstrated in the cat nasal mucosa (Lundblad et al. 1983), and these also have been attributed to substance P release.

Substance P containing primary afferent neurons have been shown to innervate blood vessels in several different species (Furness et al. 1982; Duckles and Buck 1982; Edvinsson et al. 1981). However, the role of these afferent nerves is incompletely understood. It has been difficult to study the effects of these nerves on vascular smooth muscle, in part because of the presence of other types of vasodilator fibers (Edvinsson et al. 1980; Gibbins et al. 1984; Duckles 1985). Therefore, in order to explore the function of these perivascular afferent neurons and determine whether they produce local effects on vascular smooth muscle cells, we chose to investigate the effects of acute capsaicin administration in vitro on two large blood vessels from the guinea pig as well as cat cerebral arteries.

Methods

Measurement of contractile force in vitro

Hartley guinea pigs (200–300 g) of either sex were killed by decapitation. Adult cats of either sex were given ketamine hydrochloride (100 mg i. m.) followed several minutes later by an overdose of sodium pentobarbital (600 mg i. v.)

The guinea pig carotid artery and thoracic aorta and the cat brain were quickly removed and placed in Krebs' solution at room temperature which was composed as follows (mM): Na^+, 147.6; K^+, 6.4; Ca^{2+}, 1.6; Mg^{2+}, 1.2; Cl^-, 130; HCO_3^-, 26; SO_4^{2-}, 1.2; $H_2PO_4^-$, 1.2; glucose, 11; and disodium ethylenediamine tetraacetate, 0.027. Care was taken during dissection so that vessels were not stretched.

With the aid of a dissecting microscope the cat middle cerebral arteries were removed and cut into segments 4 mm in length. The guinea pig thoracic aorta and carotid artery were trimmed to segments 2 and 4 mm in length, respectively. To mount vessels for measurement of isometric force, two pieces of platinum wire (0.13 mm diameter) were passed through the vessel lumen. One wire was connected to a Gould-Statham UC2 universal transducing cell with microscale accessory for recording changes in force with a potentiometric recorder. The other wire was attached to a

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moveable plastic support for adjustment of the resting force. The entire apparatus was then placed in a tissue bath containing 50 ml of prewarmed (37° C), oxygenated (95% O_2, 5% CO_2) Krebs' solution.

After equilibration for 60 min at 37° C, the bath solution was replaced. Vessels were then stretched to optimum resting force as previously determined: 0.5 g for cat cerebral arteries and 1.0 g for guinea pig thoracic aorta and carotid artery.

Two parallel platinum electrodes (1 cm long) were placed on either side of the vessel segment approximately 5 mm apart for delivery of transmural nerve stimulation (TNS). Electrical stimulation (15 V, 0.3 ms) was delivered from a Grass S-44 stimulator and coupling device to provide a low source impedance (Duckles and Silverman 1980). Endothelium was stripped by pulling a thread back and forth through the vessel lumen.

Capsaicin treatment in vivo

Guinea pigs were treated with capsaicin administered subcutaneously in a vehicle of 10% polyoxyethlenesorbitan monooleate – 10% ethanol – 80% saline (v/v/v). Control animals received vehicle alone. Animals received 100 mg/kg of capsaicin followed by a second injection of 250 mg/kg 24 h later. After the first injection, guinea pigs were placed for a short time in a chamber gassed with 95% O_2 – 5% CO_2 and a mist of 0.75% isoproterenol to prevent fatalities from capsaicin-induced bronchospasm. The animals were killed by decapitation 4 to 10 days after the second injection.

Substance P radioimmunoassay

Tissues were gently blotted with filter paper, weighed and homogenized in 1.5 ml 2 N acetic acid. After centrifugation at 2000 × *g* for 15 min (4° C), the supernatant was frozen and lyophilized. Lyophilized samples were stored at – 15° C for up to 1 month. On the day of the assay, tissue samples were reconstituted with 0.6 ml buffer [0.05 M Na_2HPO_4, 0.9% NaCl, (pH adjusted to 7.4 by addition of HCl) containing 0.1% RIA grade bovine serum albumin and 0.1% NaN_3]. After refrigeration for 2 h (with intermittent vortexing) samples were centrifuged at 2000 × *g* for 10 min. For radioimmunoassay, 0.2 ml substance P standards (3–150 pg) or tissue extracts were incubated for 24 h at 4° C with substance P antibody (Accurate Chemical, Westbury, NY, USA) and 15,000 DPM/tube [^{125}I] substance P(8-L-tyrosine) (New England Nuclear, Boston, MA, USA; 500 μCi/μg) all in buffer indicated above. At the end of this time 0.6 ml buffer and a dextran coated charcoal tablet (West Chem, San Diego, CA, USA) were added to each tube. After vortexing and incubating for 15 min at 4° C, supernatants were decanted and both sets of tubes were counted in a gamma counter. The limit of sensitivity of the assay was 5 pg/tube. Standard curves were fitted and unknowns calculated using a computer program (Statistical Programs Package, R. Barlow, Cambridge, England, 1983).

Drugs used

All drugs used were obtained from Sigma Chemical Co., St Louis, MO, USA: capsaicin, d,l-isoproterenol, l-norepinephrine bitartrate, serotonin creatinine sulfate, acetylcholine chloride and substance P. Drugs were diluted in distilled water and injected in a volume of 0.05 ml in a 50 ml tissue bath with the following exceptions. Substance P was diluted in 0.01 N acetic acid, and norepinephrine was diluted in 0.001 N HCl. For in vitro administration capsaicin was dissolved in ethanol and then diluted 100-fold in saline before diluted 1000-fold into the tissue bath.

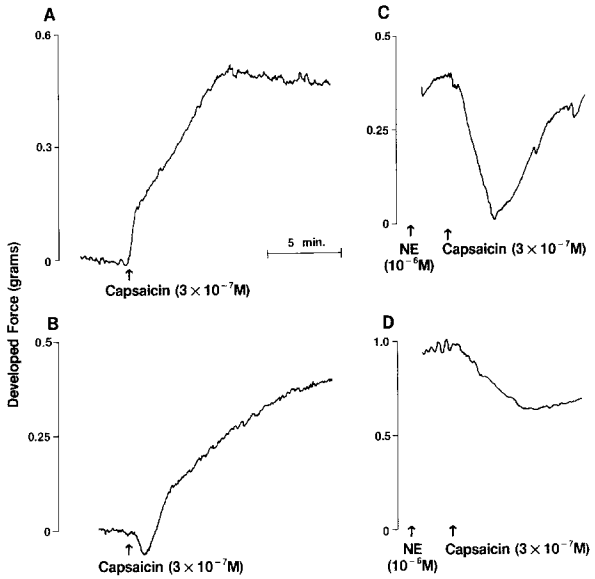

Fig. 1. Effect of capsaicin (3×10^{-7} M) on developed force of the cat middle cerebral artery, **A** and **B**, the guinea pig carotid artery, **C** and the guinea pig thoracic aorta, **D**. The carotid artery and aorta were pre-contracted with norepinephrine (NE), 10^{-6} M. Recorder tracings of developed force are shown, and a time scale is included

Table 1. Response to capsaicin (3×10^{-7} M) in vitro

	Relaxation		
	g	Percent of induced tone[a]	*N*
Guinea pig carotid artery	0.21 ± 0.06	56 ± 9	8
Guinea pig thoracic aorta	0.20 ± 0.03	26 ± 3	9
Cat middle cerebral artery	0.05 ± 0.01	–	6
	Contraction		
	g		*N*
Cat middle cerebral artery	0.68 ± 0.08		24

[a] Tone was induced in the carotid artery and thoracic aorta by prior administration of norepinephrine, 10^{-6} M. No tone was induced in the cat middle cerebral artery

Statistics

Student's *t*-test for unpaired samples was used throughout.

Results

Addition of capsaicin (3×10^{-7} M) to blood vessels in vitro produced markedly varied effects on developed force (Fig. 1). In the cat middle cerebral artery capsaicin produced a small transient relaxation in 6 out of 24 vessels studied (Table 1). Capsaicin also produced a large maintained

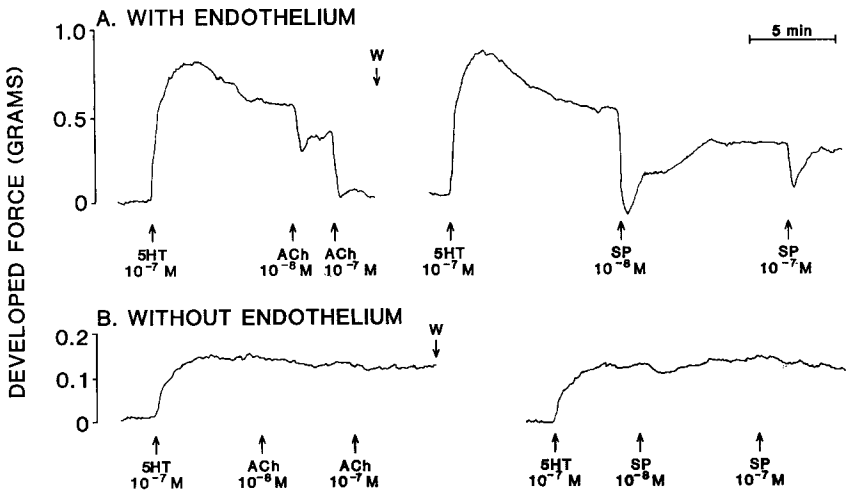

Fig. 2A, B
Effect of removal of the endothelium on relaxation responses of cat middle cerebral artery segments in vitro. Responses with intact endothelium are shown in **A** and without endothelium in **B**. Developed force is shown in g, and the time scale is indicated. *5HT* Serotonin; *ACh* acetycholine; *SP* substance P

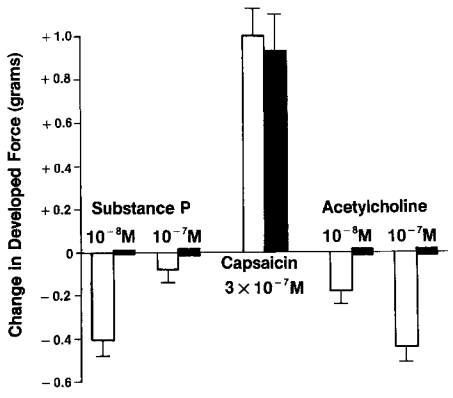

Fig. 3. Effect of endothelium removal on responses of the cat middle cerebral artery to substance P, capsaicin and acetylcholine. Change in developed force in g is plotted for control segments (*open bars*, $n = 9$) and those with endothelium removed (*filled bars*, $n = 5$). Vessels were pre-contracted with serotonin (10^{-7} M) prior to testing relaxation responses to acetylcholine and substance P (□ = control; ■ = endothelium stripped)

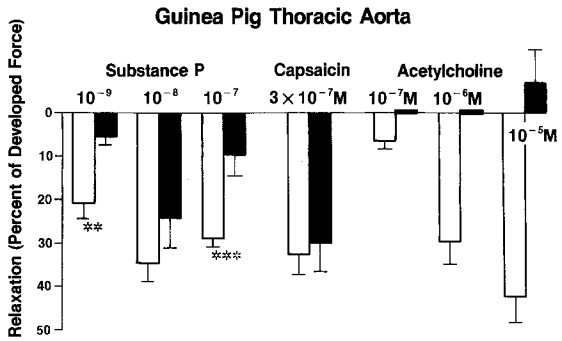

Fig. 4. Effect of endothelium removal on relaxation responses of the guinea pig thoracic aorta to substance P, capsaicin and acetylcholine. Relaxation is plotted as percent of developed force for control segments (*open bars*, $n = 5$) and those with endothelium stripped (*shaded bars*, $n = 3$). Vessels were pre-contracted with norepinephrine, 10^{-6} M. Contractile responses to norepinephrine were not different when the two groups were compared, averaging 0.67 ± 0.06 for the control (□) group and 0.65 ± 0.15 for those with endothelium stripped (■); **, $p < 0.025$; ***, $p < 0.01$

contraction in all segments of the cat middle cerebral artery studied ($n = 24$). In contrast, in the guinea pig carotid artery and thoracic aorta, in the absence of smooth muscle tone, capsaicin produced no contractile effect. In the presence of active smooth muscle tone produced by norepinephrine, capsaicin caused relaxation of both the thoracic aorta and the carotid artery (Fig. 1, Table 1). This brief in vitro exposure to capsaicin (3×10^{-7} M, 15 min) did not alter levels of substance P in the cat middle cerebral artery, as determined by radioimmunoassay. After capsaicin treatment in vivo, substance P levels were 32.3 ± 7.0 pmoles/g wet weight compared to levels of 26.9 ± 4.7 pmoles/g in control tissues ($n = 3$). Incubation of vessel segments in Krebs' solution at 37° C for the time of each experiment (5–6 h) apparently did not alter substance P content, as control values are close to those obtained previously in vessels quickly processed after dissection (Duckles and Buck 1982).

Substance P produced transient relaxations of all three vessels studied when smooth muscle tone was present (Figs. 2–5). Besides their transient nature, relaxation responses to higher concentrations of substance P were often smaller than relaxations produced by lower concentrations, indicating the rapid development of tachyphylaxis, as others have previously observed in some vascular smooth muscle preparations (Regoli et al. 1984).

When the vascular endothelium was removed by rubbing the intimal surface, relaxation reponses to acetylcholine were abolished (Figs. 2–4), also confirming previous reports (Furchgott and Zawadski 1980). In the cat middle cerebral artery, responses to substance P were abolished by endothelial damage (Figs. 2, 3), as has been previously shown for the rabbit aorta and dog carotid artery (Regoli et al. 1984). However in the guinea pig thoracic aorta, relaxation responses to substance P were substantially reduced, but not completely abolished (Fig. 4). We were unable to obtain consistent relaxation responses to acetylcholine in the guinea pig carotid artery, most probably due to damage produced by wires placed in the lumen of this small diameter vessel.

In order to determine what role the endothelium might play in response to in vitro capsaicin, we used endothelium damaged preparations, as defined by loss of responsiveness to acetylcholine. As shown in Fig. 3, contractile responses to capsaicin of the cat middle cerebral artery were not altered

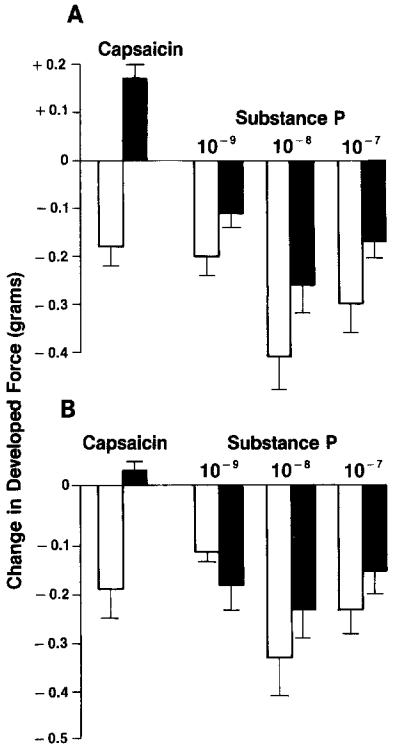

Fig. 5. Effect of prior in vivo capsaicin treatment on contractile responses of the guinea pig thoracic aorta (**A**) and carotid artery (**B**) to in vitro capsaicin (3×10^{-7} M) and substance P. Vessels from control animals are indicated by open bars, vessels from capsaicin-treated (■) animals by filled bars ($n = 4$ or 5). Values are means ± SE. Change in developed force (grams) is plotted. Vessels were pre-contracted with norepinephrine, 10^{-6} M. Contractile responses to norepinephrine were not significantly different when compared between control and capsaicin-treated animals (Table 3) (□ = control)

after endothelial damage. Furthermore, in the guinea pig thoracic aorta, relaxation responses to capsaicin were unchanged in vessels with endothelial damage (Fig. 4).

Treatment with capsaicin in vivo is known to deplete substance P from sensory nerves innervating guinea pig blood vessels (Duckles and Buck 1982; Furness et al. 1982; Papka et al. 1984). Therefore, we studied the effects of in vitro capsaicin administration on blood vessels from animals administered capsaicin in vivo. As can be seen in Fig. 5, responses of both the thoracic aorta and carotid artery to in vitro capsaicin were substantially altered when animals had been administered capsaicin in vivo. Relaxation responses to in vitro capsaicin were abolished in vessels from capsaicin-treated animals. In the thoracic aorta, the response to capsaicin was contraction in vessels from capsaicin-treated animals compared to a relaxation in vessels from control animals. Responses to substance P were not changed when animals were pre-treated with capsaicin (Fig. 5A, B). Despite these changes in response to capsaicin, contractile responses to norepinephrine were not different when vessels from capsaicin-treated and control animals were compared (Table 2).

In the cat cerebral artery in the presence of smooth muscle tone, transmural nerve stimulation produces relaxation (Lee at al. 1978). Therefore, we explored the effect of exposure to capsaicin in vitro on this neurogenic vasodilator

Table 2. Contractile response to norepinephrine of guinea pig arteries with and without in vivo capsaicin treatment

	(g developed force)	
	Control	Capsaicin-treated
Thoracic aorta	0.36 ± 0.05	0.34 ± 0.02
Carotid artery	0.20 ± 0.03	0.19 ± 0.02
N	9	10

Table 3. Effect of exposure to capsaicin (3×10^{-7} M, 15 min) on neurogenic vasodilation of cat cerebral arteries in vitro

	Response to transmural nerve stimulation	
	Control	After capsaicin exposure
2 Hz[a] (mg)	58 ± 10	54 ± 8
(% control)		129 ± 28
4 Hz[a] (mg)	80 ± 16	89 ± 15
(% control)		125 ± 19

[a] Stimulation for 25 s at each fequency
Values are means ± SE, $n = 9$

response. As shown in Fig. 6 and Table 3, exposure to capsaicin, 3×10^{-7} M for 15 min, did not alter the magnitude of vasodilator responses to transmural nerve stimulation at either 2 or 4 Hz.

Discussion

Acute administration of capsaicin to isolated blood vessels produces two distinct effects. A contractile response was most prominent in cat cerebral arteries and was seen in the thoracic aorta and carotid artery from animals treated in vivo with capsaicin. In contrast, a relaxation response to acute capsaicin administration was most prominent in the thoracic aorta and carotid artery and was seen to a slight extent in some cerebral arteries. The experiments detailed in this manuscript were designed to better understand the nature of these responses.

In the guinea pig, chronic in vivo capsaicin treatment abolished the vascular relaxation response to acute capsaicin in vitro, leaving a contractile response in its place. This suggests that capsaicin releases a transmitter, perhaps substance P, to produce vascular smooth muscle relaxation, while the contractile effect of capsaicin is most likely due to a direct smooth muscle effect, as has been shown for the rat vasculature in vivo (Donnerer and Lembeck 1982), as well as the cat nasal mucosa (Lundblad et al. 1983). We have not entirely ruled out the possibility that the relaxation produced by capsaicin is due to a direct effect on the blood vessel wall, although this seems unlikely as capsaicin seems to cause contraction by a direct vascular smooth muscle action.

Our results, however, suggest that the dilator substance released by capsaicin is not likely to be substance P. Although substance P does cause vascular smooth muscle relaxation, as has been shown for other blood vessels, this

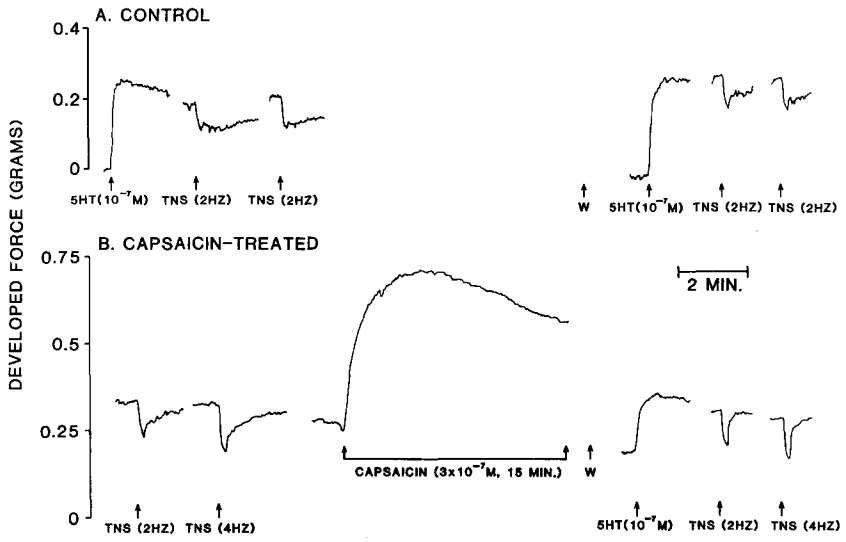

Fig. 6A, B
Contractile responses of cat cerebral arteries to transmural nerve stimulation (TNS) for 25 s trains before and after exposure to capsaicin in vitro (3×10^{-7} M, 15 min). Part **A** shows responses of a control tissue while part **B** shows responses of another segment before and after exposure to capsaicin in vitro. Developed force in g is indicated, as is the time scale. Serotonin (*5HT*) is given to produce developed tone

effect is dependent on an intact endothelium (Regoli et al. 1984). In contrast, damage to the endothelium, which also abolished dilator responses to acetylcholine (Furchgott and Zawadski 1980), did not alter responses to acute capsaicin administration.

Thus these results suggest that capsaicin produces two effects on vascular smooth muscle: 1) a contraction which is most probably due to a direct action on vascular smooth muscle, 2) a dilation mediated by an unknown transmitter released by capsaicin.

In addition, the possible role of substance P containing nerves in mediation of the neurogenic vasodilator response seen in cat cerebral arteries was investigated. It has been shown that chronic capsaicin treatment depletes substance P from cerebral arteries (Duckles and Buck 1982; Duckles and Levitt 1984). Furthermore, these nerves have been shown to originate in the trigeminal ganglion, confirming their sensory nature (Liu-Chen et al. 1983; Mayberg, et al. 1981). Although capsaicin has been shown to cause release of substance P from bovine cerebral arteries in vitro (Moskowitz et al. 1983), the present studies suggest that it is unlikely that substance P released from sensory nerves contributes to neurogenic vasodilation. First of all, acute capsaicin administration to cerebral blood vessels causes a contraction rather than a relaxation. Secondly, endothelial removal abolished cerebrovascular dilator responses to substance P (Figs. 2, 3) but has been previously shown to have no effect on the neurogenic vasodilator response (Lee 1980; Lee 1982), an observation which has been confirmed in this laboratory (Duckles, unpublished observation). Thus, vasoactive intestinal peptide remains the most likely candidate to mediate the neurogenic vasodilator response of cat cerebral arteries (Duckles and Said 1982; Edvinsson et al. 1980; Duckles 1983).

Previous studies of the effects of capsaicin on vascular smooth muscle demonstrate both direct contractile effects and vasodilator effects produced by activation of sensory nerves (Donnerer and Lembeck 1982, 1983). In the rat, intravenously injected capsaicin produces a complex response composed of three components. The vasoconstrictor component of this response, which is augmented in rats treated neonatally with capsaicin, has been attributed to a direct effect of capsaicin on vascular smooth muscle. Isolated canine arteries also contract when capsaicin is added to the bath, an effect which appears to be non-adrenergic and is dependent on extracellular calcium (Toda et al. 1972).

Capsaicin causes contraction of a number of non-vascular smooth muscles, including the guinea pig ileum (Bartho et al. 1982; Chahl 1982), esophagus, urinary bladder, trachea and gall bladder (Lundberg et al. 1984a). In those cases studied, this contractile response is thought to be mediated by release of substance P. However, similar to our findings in guinea pig blood vessels, in the guinea pig atrium, capsaicin produces a response which is abolished by chronic capsaicin pre-treatment but is not mimicked by substance P (Lundberg et al. 1984b). Although these authors could not rule out a direct action of capsaicin on cardiac myocytes, the effect of chronic capsaicin treatment suggested that acute capsaicin exposure causes release of bioactive substances other than substance P.

These findings in the guinea pig atrium are most analogous to the relaxation response to capsaicin we saw in the guinea pig thoracic aorta and carotid artery. However, in our case, we think it even more unlikely that the capsaicin-induced relaxation is a direct effect on the vascular smooth muscle, since we found that capsaicin's direct effect is vasoconstriction. Therefore, we conclude that capsaicin releases an as-yet-unidentified vasodilator substance in the guinea pig. It is possible that this substance is related to the substance released by capsaicin from the guinea pig atrium (Lundberg et al. 1984b).

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