British Journal of Pharmacology (1995) 116, 2557-2562

Heterogeneity of neurogenic responses in intra- and extrameningeal arteries of dogs

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1 Neurogenic responses to transmural electrical stimulation were examined in endothelium-denuded extrameningeal (vertebral and carotid) and intrameningeal (spinal, basilar and middle cerebral) arteries isolated from dogs.

2 In the extrameningeal arteries, transmural electrical stimulation produced a phasic contraction. This contraction was abolished by tetrodotoxin, prazosin and guanethidine. However, α,β-methylene ATP and N^G-nitro-L-arginine (L-NOARG) had no significant effect on the contractile responses.

3 In the intrameningeal arteries, the neurogenic responses to electrical stimulation were composed of a transient contraction and relaxation. The transient contraction was selectively inhibited by guanethidine or after desensitization of P_{2X}-purinoceptors with α,β-methylene ATP. L-NOARG abolished the relaxation but not the contraction induced by electrical stimulation. Prazosin had no effect on either neurogenic response.

4 Noradrenaline produced a large contraction in the extrameningeal arteries which was selectively inhibited by prazosin. α,β-Methylene ATP produced neither contraction nor inhibition of the response to noradrenaline in the extrameningeal arteries.

5 In the intrameningeal arteries, α,β-methylene ATP produced a greater contraction than noradrenaline. The response to α,β-methylene ATP was selectively abolished by desensitization of P_{2X}-purinoceptors with α,β-methylene ATP itself. The contractile response to noradrenaline was inhibited by rauwolscine but not by prazosin.

6 ATP produced endothelium-dependent relaxations in the extrameningeal and intrameningeal arteries, which were attenuated by endothelium removal.

7 NADPH diaphorase-positive fibres were dense in the middle cerebral and basilar arteries but rare or absent in the spinal artery. In the extrameningeal arteries diaphorase-positive traces were observed in the vasa vasorum.

8 The present findings indicate that the neurogenic responses of intrameningeal arteries of dogs are composed of NO-ergic and sympathetic purinergic components, while the extrameningeal arteries tested produced only sympathetic adrenergic responses, suggesting that regional heterogeneity may be associated with a sudden transition in innervation and receptor expression at the meninx.

Keywords: Sympathetic responses; adrenergic contraction; purinergic contraction; NO-ergic relaxation; intra- and extrameningeal arteries of dog

Introduction

Cerebral and peripheral blood vessels are densely innervated by sympathetic nerves (Ohgushi, 1968; Kajikawa, 1968; Iwayama *et al.*, 1970; Edvisson & Owman, 1975; Duckles *et al.*, 1977; Alafaci *et al.*, 1986). However, the neuronal function in the cerebral vessels remains controversial, in contrast to that in peripheral vessels (D'Alegy & Feigel, 1972; Marcus & Heistad, 1979; Bevan & Van Riper, 1989). Most peripheral arteries produce sympathetic adrenergic responses, while studies on isolated cerebral arteries have revealed a variety of neurogenic responses and atypical features which cannot be explained simply by adrenergic mechanisms (Lee *et al.*, 1976; Duckles & Bevan, 1976). For example, in the dog basilar artery, transmural electrical stimulation produces both contraction and relaxation which are resistant to α_1- and α_2-adrenoceptor blocking agents (Muramatsu *et al.*, 1981; Muramatsu & Kigoshi, 1987). There are also marked differences in the sensitivity of cerebral and peripheral arteries to various vasoconstrictor agents (Nielsen & Owman, 1971; Muramatsu *et al.*, 1978). In general, noradrenaline and other α-adrenoceptor agonists cause only a modest contraction of cerebral arterial strips amounting to less than 20% of their capacity to contract (Bevan & Van Riper, 1989). 5-Hydroxytryptamine is the most potent contractile agent in the canine basilar artery (Sakakibara *et al.*, 1982; Tsukahara *et al.*, 1986), whereas noradrenaline is the most potent agent in the peripheral arteries. However, it is still unknown whether such a regional transition occurs gradually or suddenly from extrameningeal to intrameningeal artery and whether the regional difference is associated with innervation. In the present study, we used dog carotid and vertebral arteries as extrameningeal, and spinal, basilar and middle cerebral arteries as intrameningeal arteries, and examined the regional heterogeneity of neurogenic responses.

Methods

Mongrel dogs of either sex (7-15 kg) were killed under pentobarbitone anaesthesia. The carotid, vertebral, spinal, basilar and middle cerebral arteries were isolated and cut helically under a dissecting microscope. Unless mentioned elsewhere, the endothelial cells of the blood vessels were removed by rubbing with filter paper in order to avoid the involvement of endothelium-derived relaxing and contractile factors in the

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mechanical response (Furchgott, 1981). The functional loss of endothelial cells was confirmed by the loss of the relaxant response to acetylcholine (1 μM). Each strip was mounted vertically in an organ bath containing 20 ml modified Krebs-Henseleit solution of the following composition (mM): NaCl 112, KCl 5.9, $MgCl_2$ 1.2, $CaCl_2$ 2, $NaHCO_3$ 25, NaH_2PO_4 1.2 and glucose 11.5. The medium was maintained at 37°C, pH 7.4, and equilibrated with a gas mixture consisting of 95% O_2 and 5% CO_2. The tension was recorded isometrically through a force-displacement transducer. The preparations were equilibrated for 90 min before starting the experiments.

Transmural electrical stimulation was applied through a pair of platinum wire electrodes at 10–15 min intervals (Muramatsu *et al.*, 1989). The preparation was placed in parallel between the electrodes, the distance between them being about 2 mm. The stimulus parameters were 0.2 ms in duration and supramaximum voltage of 12 V for 10 s. The stimulus frequency was 20 Hz in all preparations. In this series of experiments, DG-5128 (10 μM) and propranolol (1 μM) were added to the bath medium to block prejunctional α_2-adrenoceptors and postjunctional β-adrenoceptors, respectively (Muramatsu *et al.*, 1983; 1989).

Contractile responses to noradrenaline, α,β-methylene ATP and KCl were compared in all tissues. When noradrenaline was used, desipramine (0.1 μM),deoxycorticosterone (5 μM) and propranolol (1 μM) were added to the bath solution to block neuronal and extraneuronal uptake of noradrenaline and to block β-adrenoceptors, respectively. When the effects of prazosin or rauwolscine on the noradrenaline response were examined, the preparation was treated with the antagonist for 30 min before and during determination of the concentration-response curves. EC_{50} was estimated from the concentration of drug which produced a half maximal contraction. The maximal contractions induced by noradrenaline or α,β-methylene ATP were compared with those of 60 mM KCl in the same strip. The responses to ATP were also examined in endothelium-intact and -denuded preparations. In this case, the intrameningial arteries were contracted by 0.1 μM 5-hydroxytryptamine beforehand, whereas the extrameningial arteries were contracted by 3 μM noradrenaline.

For NADPH diaphorase histochemistry, isolated blood vessels were fixed with 4% paraformaldehyde in 0.1 M sodium phosphate buffer (pH 7.4). The vascular segments were then incubated for 30 min at 37°C in 0.1 M phosphate buffer (pH 7.4) containing 0.3% Triton X-100, 1 mM nitroblue tetrazolium and 1 mM NADPH. Finally, the segments were rinsed in 0.1 M phosphate buffer, dehydrated and mounted with Entellan.

Drugs used were: (−)-noradrenaline bitartrate, desipramine hydrochloride, ATP, α,β-methylene ATP, N^G-nitro-L-arginine (L-NOARG), nitroblue tetrazolium, prazosin, NADPH, 5-hydroxytryptamine creatinine sulphate (Sigma, St Louis, U.S.A.), deoxycorticosterone acetate, (±)-propranolol hydrochloride (Nacalai Tesque, Kyoto, Japan), rauwolscine hydrochloride (RBI, Natick, U.S.A.), tetrodotoxin (Sankyo, Tokyo, Japan), guanethidine sulphate (Tokyo-Kasei, Tokyo, Japan) and DG-5128 (2-(-(4,5-dihydro-1H-imidazol-2-yl)-1-phenylethyl)pyridine dihydrochloride sesquihydrate) (Daiichi Seiyaku, Tokyo, Japan).

Results

Neurogenic responses to transmural electrical stimulation

Transmural electrical stimulation (20 Hz, for 10 s) produced different patterns in the various arteries tested. In the basilar artery, electrical stimulation elicited a transient contraction which was followed by relaxation. Both the responses were abolished by tetrodotoxin (0.5 μM, $n=5$). As shown in Figure 1a, L-NOARG (10 μM, $n=6$) abolished the relaxation response to electrical stimulation with a gradual increase in the resting tension. However, the initial transient contraction was not affected by L-NOARG: the remaining contraction response was inhibited by subsequent treatment with α,β-methylene ATP (10 μM, $n=6$). Guanethidine (3 μM, $n=6$) also abolished the contractile but not the relaxation response. On the other hand, prazosin (0.1 μM) and rauwolscine (30 nM) did not attenuate either response ($n=6$, for each drug).

In the middle cerebral artery, electrical stimulation produced relaxation (Figure 1b), and in 6 of 16 preparations, a small contraction preceded the relaxation. The relaxation was abolished by L-NOARG (10 μM, $n=16$), and thereafter the response reverted to a transient contraction (Figure 1b). This contraction was abolished by tetrodotoxin (0.5 μM, $n=3$), guanethidine (3 μM, $n=6$), or α,β-methylene ATP (10 μM, $n=7$) but not by prazosin (0.1 μM, $n=4$) or rauwolscine (30 nM, $n=6$).

The responses to electrical stimulation of the spinal artery were similar to those of the basilar artery (Figure 1c and 1d). However, the amplitude of relaxation was small as compared with those of basilar and middle cerebral arteries, and no relaxation was observed in 7 out of 19 preparations. Prazosin (0.1 μM, $n=4$) failed to affect either the initial transient contraction or the subsequent relaxation. L-NOARG (10 μM, $n=4$) abolished the relaxation and slightly augmented the contractile response to electrical stimulation. Continuous treatment with α,β-methylene ATP (10 μM, $n=11$) abolished the initial contraction without inhibiting the relaxation.

In the vertebral and carotid arteries, electrical stimulation produced contractions (Figure 2), which were not affected by L-NOARG (10 μM) or α,β-methylene ATP (10 μM) but were abolished by prazosin (0.1 μM), guanethidine (3 μM) or tetrodotoxin (0.5 μM) ($n=6$, each drug and each artery).

Responses to noradrenaline, α,β-methylene ATP and ATP

The vertebral and carotid arteries responded well to noradrenaline, resulting in a large contraction. However, the contractile responses to noradrenaline in the basilar, middle cerebral and spinal arteries were small. Figure 3 shows the relative amplitudes of contractions induced by noradrenaline compared with those induced by KCl (60 mM) in the same strips of each artery. The contractile responses to noradrenaline were significantly inhibited by prazosin (0.1 μM) in the vertebral and carotid arteries but not in the basilar, middle cerebral or spinal arteries. On the other hand, rauwolscine (30 nM) significantly attenuated the response to noradrenaline in the basilar, middle cerebral and spinal arteries but not in the vertebral or carotid arteries (Figure 4).

α,β-Methylene ATP produced large contractions in the basilar, middle cerebral and spinal arteries (Figure 3). The contractile responses to α,β-methylene ATP were transient and were eventually abolished in the continuous presence of a high concentration (10 μM) of α,β-methylene ATP (Figure 1). Under such conditions, additional α,β-methylene ATP failed to produce a contraction (data not shown). In the carotid and vertebral arteries, no responses were elicited by α,β-methylene ATP (1 and 10 μM).

ATP (10 nM ~ 100 μM) produced relaxations in endothelium-intact preparations of all the arteries tested. However, with 10 μM ATP a transient contraction preceded the sustained relaxation in the basilar and middle cerebral arteries. Figure 5 shows the maximum relaxations induced by each concentration of ATP, which were attenuated in endothelium-denuded preparations.

NADPH diaphorase histochemistry

NADPH diaphorase-positive nerves were clearly observed in the middle cerebral and basilar arteries, and the occurrence of positive nerve fibres was rare or absent in the spinal artery in the study. Thick nerve bundles ran mostly along the major axis of the artery and ramifying thin nerve bundles formed a meshwork (Figure 6). The middle cerebral artery had a greater

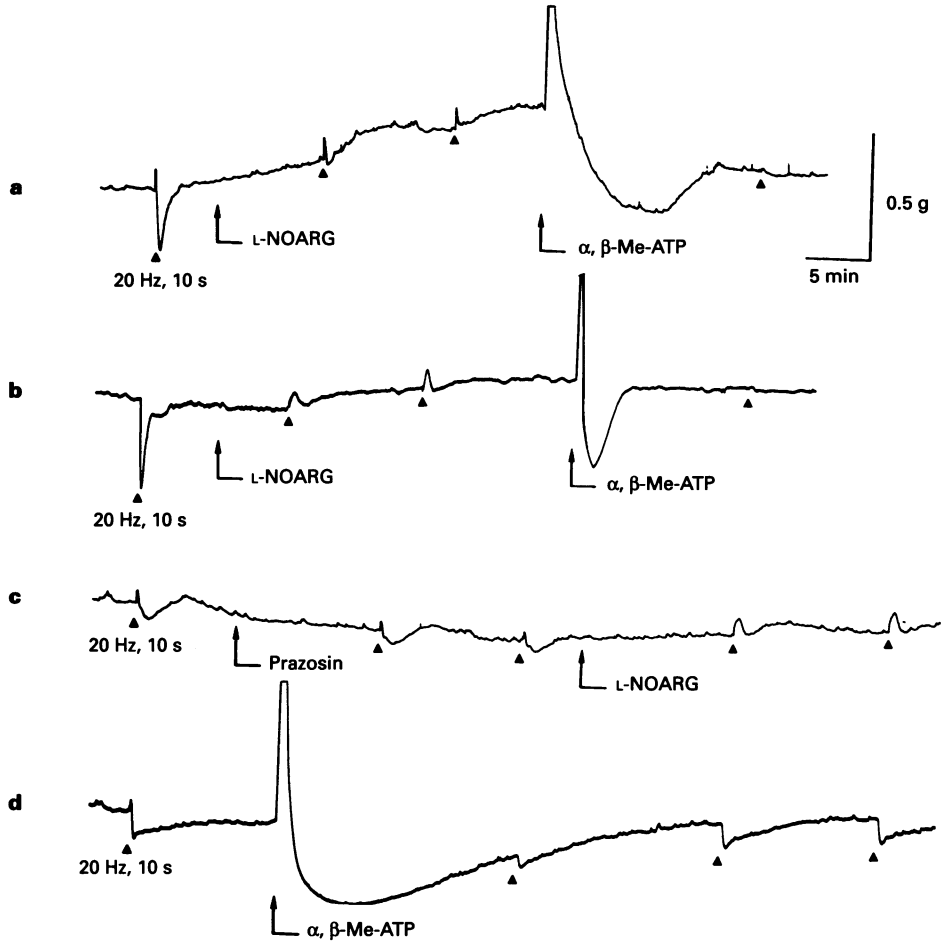

Figure 1 Effects of L-NOARG, α,β-methylene ATP (α,β-Me-ATP) or prazosin on the responses to electrical transmural stimulation in the basilar (a), middle cerebral (b) and spinal (c,d) arteries isolated from dogs. Endothelium-denuded preparations were stimulated electrically at 20 Hz for 10 s. L-NOARG (10 μM) and α,β-methylene ATP (10 μM) or prazosin (0.1 μM) were applied cumulatively. A transient contractile response to α,β-methylene ATP exceeded the recording range.

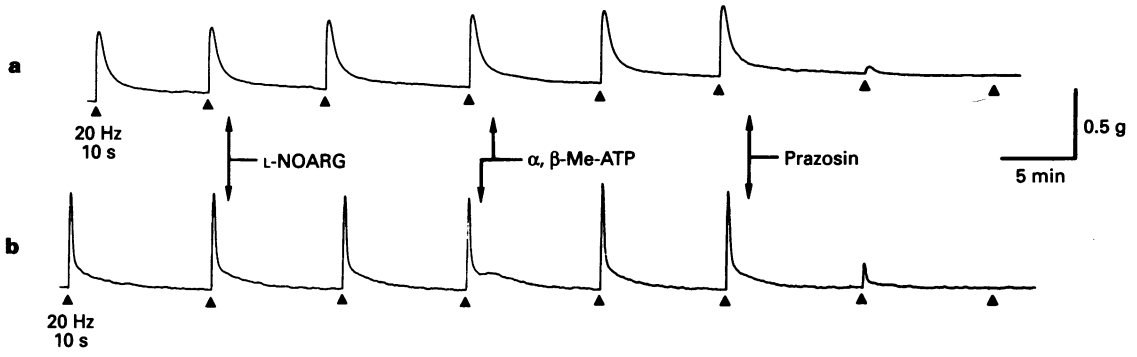

Figure 2 Effects of L-NOARG, α,β-methylene ATP and prazosin on the responses to electrical transmural stimulation in the dog vertebral (a) and carotid (b) arteries. L-NOARG (10 μM), α,β-methylene ATP (α,β-Me-ATP, 10 μM) and prazosin (0.1 μM) were applied cumulatively. The other experimental conditions were the same as those in Figure 1.

number of fibres than the basilar artery. In the vertebral and carotid arteries, diaphorase-positive traces were observed in the vasa vasorum, and very few, if any positive nerves, were detected.

Discussion

The present study clearly shows the existence of regional differences in the neurogenic responses between canine intra- and extrameningeal arteries. Simple contractions similar to those observed in the peripheral blood vessels of dogs (Muramatsu, 1991; Kohno *et al.*, 1994) were produced by transmural electrical stimulation in the vertebral and carotid arteries. In contrast, basilar, middle cerebral and spinal arteries produced a complex pattern composed of a small transient contraction and a large relaxation.

The contractile responses to electrical stimulation in the vertebral and carotid arteries were abolished by tetrodotoxin, guanethidine and prazosin. However, the responses were not affected by L-NOARG or α,β-methylene ATP. These results indicate that the contractile responses induced by electrical

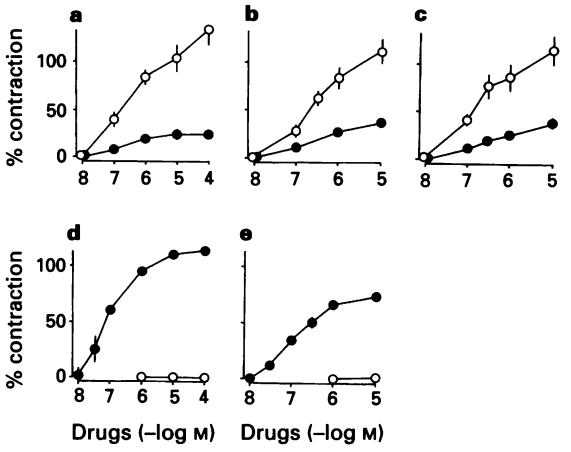

Figure 3 Concentration-response curves for noradrenaline (●) and α,β-methylene ATP (○) in the dog basilar (a), middle cerebral (b), spinal (c), vertebral (d) and carotid arteries (e). The endothelium was removed in all preparations. The maximum contractions induced by 60 mM KCl in the same preparations were taken as 100%. Mean ± s.e.mean of 4–6 experiments.

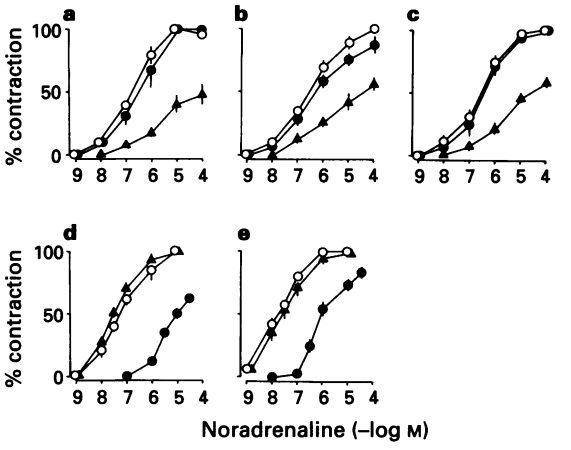

Figure 4 Effects of rauwolscine and prazosin on the contractile responses to noradrenaline in the dog basilar (a), middle cerebral (b), spinal (c), vertebral (d) and carotid (e) arteries. (○) Control; (▲) 30 nM rauwolscine, (●) 30 nM prazosin. The maximum contraction induced by 100 μM noradrenaline in control was taken as 100%. The other experimental conditions were the same as those in Figure 3. Mean ± s.e.mean of 4–6 experiments.

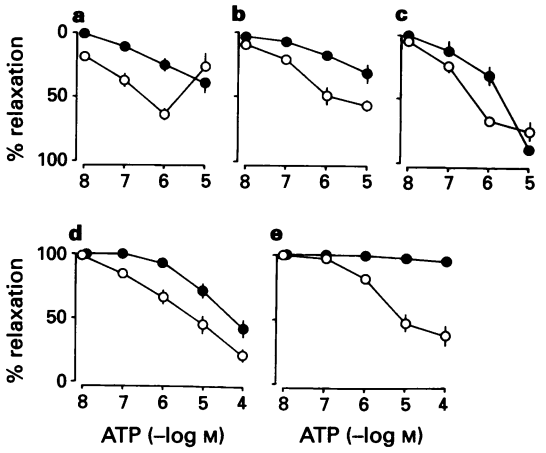

Figure 5 Effects of ATP on the dog basilar (a), middle cerebral (b), spinal (c), vertebral (d) and carotid (e) arteries. The intrameningeal and extrameningial arteries were precontracted by 0.1 μM 5-hydroxytryptamine or 3 μM noradrenaline, respectively. The contractile amplitude induced by 5-hydroxytryptamine or noradrenaline was taken as 100%. (○) Endothelium-intact preparations; (●) endothelium-denuded preparations. Mean ± s.e.mean of 4–6 experiments.

stimulation are sympathetic and adrenergic, and are mediated through α_1-adrenoceptors. Recently, we demonstrated that the α_1-adrenoceptors of dog vertebral and carotid arteries are of the α_{1B} subtype (Kohno *et al.*, 1994).

In contrast, neurogenic responses in the intrameningeal vessels (basilar, middle cerebral and spinal arteries) were biphasic, as mentioned above. The initial transient contraction was abolished by α,β-methylene ATP or guanethidine. α,β-Methylene ATP is a highly selective P_{2X}-purinoceptor agonist and persistent treatment finally desensitizes the P_{2X}-purinoceptor itself in many tissues (Kasakov & Burnstock, 1983; Burnstock & Kennedy, 1985; Muramatsu *et al.*, 1989). Therefore, it is likely that the initial contractions induced by electrical stimulation in spinal, basilar and middle cerebral arteries are sympathetic in origin but are purinergic in nature, as suggested previously (Muramatsu & Kigoshi, 1987). In fact, the sympathetic neurogenic contraction was not inhibited by prazosin or rauwolscine. On the other hand, the relaxations induced by electrical stimulation were resistant to α,β-methylene ATP and guanethidine, and were selectively inhibited by L-NOARG, a NO synthase inhibitor, suggesting that the relaxation is produced by NO or a related substance (Bredt & Hwang, 1990).

Another interesting finding in the present study was the differences in responsiveness to exogenous drugs between intrameningeal and extrameningeal arteries. The vertebral and carotid arteries responded well to noradrenaline, producing large contractions. However, the contractile responses to noradrenaline of the spinal, basilar and middle cerebral arteries were remarkably small compared with the responses to KCl and α,β-methylene ATP. Further, the contractile responses to noradrenaline showed a completely reverse sensitivity to prazosin and rauwolscine between the intra- and extrameningeal arteries. These results strongly suggest that α-adrenoceptors in dog arteries convert from α_1 to α_2-subtype upon meningeal passage.

In contrast to the responses to noradrenaline, α,β-methylene ATP, a P_{2X}-purinoceptor agonist, produces large contractions in the spinal, basilar and middle cerebral arteries but failed to produce any response in the vertebral and carotid arteries. ATP or a related compound is known to coexist with noradrenaline in sympathetic nerve terminals and to produce sympathetic purinergic responses in addition to the adrenergic responses in many blood vessels (Su, 1978; Kügelgen & Starke, 1985; Burnstock, 1986; Muramatsu *et al.*, 1981; 1989). High reactivity of the P_{2X}-purinoceptors, low reactivity of the α_2-adrenoceptors and lack of functional α_1-adrenoceptors in the spinal, basilar and middle cerebral arteries all may account for the predominant appearance of sympathetic purinergic but not adrenergic contraction in the intrameningeal arteries. On the other hand, only sympathetic adrenergic responses are produced in the vertebral and carotid arteries because of the absence of functional P_{2X}-purinoceptors and the high reactivity of the α_1-adrenoceptors present.

Basilar and middle cerebral arteries are innervated by sympathetic nerves derived from superior cervical ganglia, whereas spinal, vertebral and carotid arteries are innervated sympathetically by stellate ganglia in dogs (Ohgushi, 1968; Kajikawa, 1968). Therefore, it is unlikely that the observed regional heterogeneity of sympathetic neurogenic responses is associated with the difference in origin of sympathetic nerves. Rather, expression of receptors such as α-adrenoceptors and P_{2X}-purinoceptors in the vascular smooth muscle cells appears to be controlled in regionally different ways, causing regional heterogeneity of sympathetic responses. Recently, we suggested that different subtypes of α_1-adrenoceptors may be expressed in different tissues (Kohno *et al.*, 1994; Muramatsu *et al.*, 1995).

As mentioned above, NO-ergic relaxation was produced by electrical stimulation only in the intrameningial arteries and no NO-ergic relaxation was observed in the vertebral or carotid

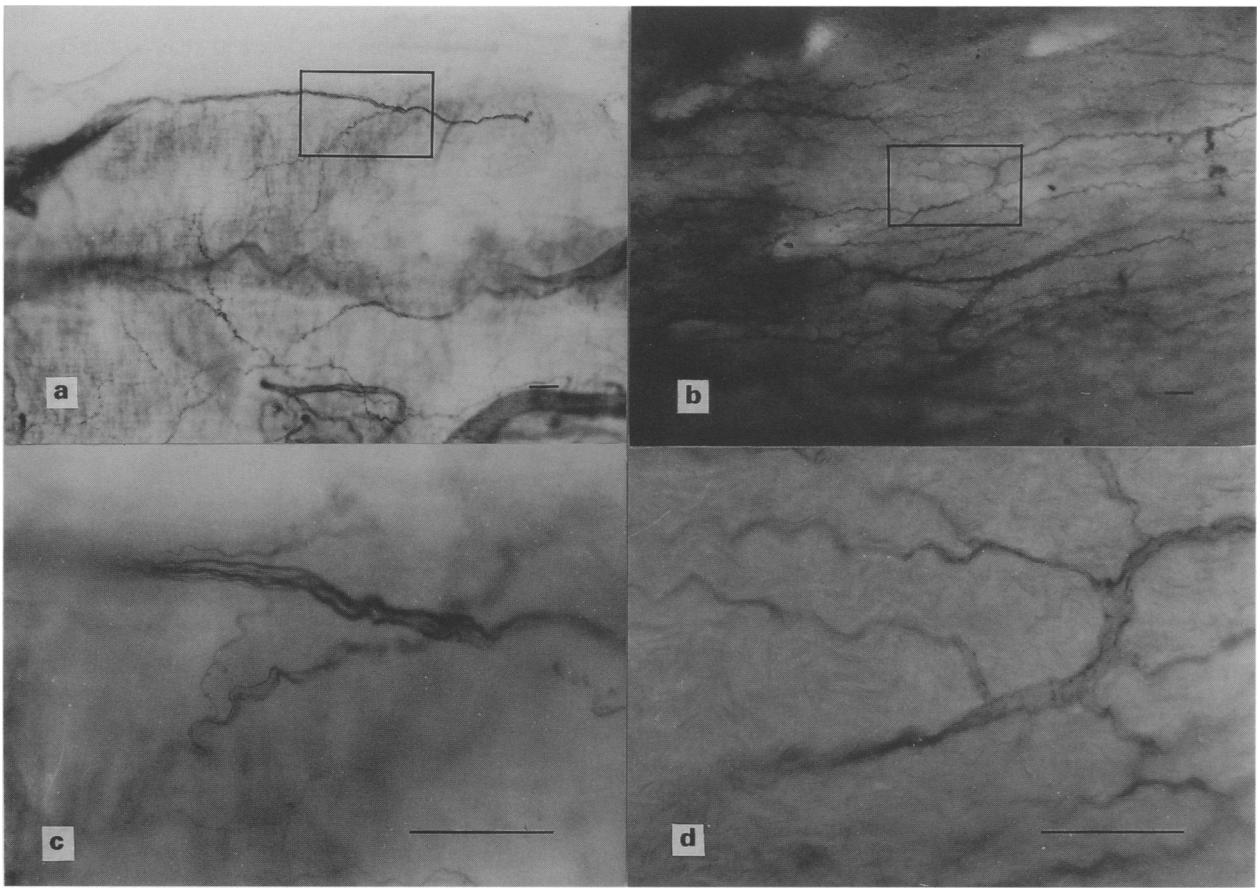

Figure 6 Whole-mount preparations of the middle cerebral and carotid arteries of the dog processed for NADPH diaphorase histochemistry. (a,c) Middle cerebral artery; thick nerve bundles run mostly along the major axis of the artery and ramifying thin nerve bundles form a mesh work. (b,d) Carotid artery: diaphorase-positive traces are shown to the vasa vasorum. Bars = 100 μm.

arteries. However, endothelium-dependent relaxations were elicited by ATP in all the arteries tested. Histological examination clearly showed the high density of NADPH diaphorase-positive fibres in the middle cerebral and basilar arteries, a few positive fibres in the spinal artery, and none in the vertebral and carotid arteries. Therefore, lack of NO-ergic relaxation in the vertebral and carotid arteries appears to be accounted for by the absence of NO-ergic innervation rather than the absence of NO-sensitive relaxation mechanisms in such arteries. Cerebral arteries are known to be densely innervated by NO-ergic nerves (Bredt *et al.*, 1990; Iadecola *et al.*, 1993).

In conclusion, the present study clearly shows the occurrence of a sudden transition in the neurogenic responses of the arteries at the meninx in dogs, which may be in part associated with differences in the innervation and the receptor expression in vascular smooth muscle cells.

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(Received December 20, 1994
Revised July 10, 1995
Accepted July 21, 1995)