Br. J. Pharmac. (1987), **92**, 901–908

Purinergic and non-purinergic innervation in the cerebral arteries of the dog

[1] Ikunobu Muramatsu & Shigeru Kigoshi

Department of Pharmacology, Fukui Medical School, Matsuoka, Fukui 910-11, Japan

1 Possible involvement of sympathetic purinergic transmission in the neurogenic response of dog cerebral and basilar arteries was examined with the use of α,β-methylene ATP and adrenoceptor, cholinoceptor blocking agents.

2 In the isolated basilar arteries, electrical transmural stimulation produced a transient contraction which was frequently followed by a relaxation. This transient contraction was abolished after desensitization of P_2-purinoceptors with α,β-methylene ATP or by treatment with guanethidine. The relaxant response induced by electrical stimulation was also attenuated but was not abolished by such treatments. Prazosin, propranolol and atropine had no significant effect on the responses to electrical stimulation. Yohimbine augmented both the contractile and relaxant responses.

3 In most preparations of the dog middle cerebral arteries, electrical transmural stimulation produced only a relaxation. This relaxation was little affected after treatment with α,β-methylene ATP or guanethidine, and was not inhibited by the other adrenoceptor and cholinoceptor blocking agents.

4 Tetrodotoxin abolished the responses induced by electrical transmural stimulation in both the basilar and middle cerebral arteries.

5 Exogenous ATP (10^{-6} and 10^{-5} M) produced a transient contraction followed by a relaxation of the basilar arteries and a relaxation of the middle cerebral arteries. Desensitization of P_2-purinoceptors abolished the contractile response to ATP without affecting the amplitude of relaxation.

6 In the basilar and middle cerebral arteries preincubated with [^{3}H]-noradrenaline, electrical transmural stimulation evoked an increase in ^{3}H-efflux and this response was markedly inhibited by guanethidine or tetrodotoxin but was not affected by α,β-methylene ATP. Yohimbine increased the evoked ^{3}H-efflux.

7 These findings indicate that cerebral arteries of the dog are innervated by sympathetic purinergic nerves and non-sympathetic nerves which liberate unknown vasodilator substance(s), and that the former nerves are more dominant in the neurogenic response to electrical stimulation of the dog basilar artery than in the middle cerebral artery.

Introduction

Cerebral blood vessels of several species receive a rich supply of adrenergic, cholingeric, 5-hydroxytryptaminergic and peptidergic fibres (Ohgushi, 1968; Iwayama *et al.*, 1970; Owman *et al.*, 1974; Burnstock, 1977; 1986; Edvinson *et al.*, 1980; 1981; Griffith *et al.*, 1982). However, despite intense investigation, the function of these fibres remains controversal (Evdinsson & Mackenzie, 1977; Heistad & Marcus, 1978; Purves, 1978; Marcus & Heistad, 1979). Studies on isolated cerebral arteries revealed a variety of neurogenic responses and atypical features not explained simply by adrenergic and cholinergic mechanisms. For example, electrical transmural stimulation of isolated cerebral arteries produces a contraction in the rabbit and sheep (Lee *et al.*, 1976; Duckles, 1979), a relaxation in the cat, monkey and human (Lee *et al.*, 1978; Toda, 1981) and both contraction and relaxation in the dog (Muramatsu *et al.*, 1978; 1981). The contractile response is abolished by the adrenergic neurone blocking agents guanethidine and bretylium but is not inhibited by α- or β-adrenoceptor blocking agents (Muramatsu *et al.*, 1977; Lee *et al.*, 1978; Toda, 1981). Rather, α-adrenoceptor blocking agents such as phentolamine enhance the neurogenic response (Muramatsu *et al.*,

[1] Author for correspondence.

1977; 1981). The relaxation induced by electrical stimulation is resistant to adrenoceptor and cholinoceptor blocking agents. These lines of evidence suggest that the neurogenic response of the cerebral artery may be caused by non-adrenergic, non-cholinergic neurotransmitter substances.

We demonstrated that the neurogenic response of the dog isolated basilar artery to electrical transmural stimulation was mimicked by exogenously applied ATP but not by noradrenaline (Muramatsu *et al.*, 1981). Since ^{3}H-purine compounds were released by electrical stimulation of the basilar artery preincubated with [^{3}H]-adenosine and this release was inhibited by bretylium or tetrodotoxin, the possible involvement of adenine nucleotides in the sympathetic response of the dog basilar artery was investigated (Muramatsu *et al.*, 1981). We present here further evidence for sympathetic purinergic transmission in the dog cerebral arteries; that is, the neurogenic response is inhibited by P_2-purinoceptor desensitization with α,β-methylene ATP (Kasakov & Burnstock, 1983; Muramatsu, 1987) as well as by treatment with guanethidine.

Methods

Dogs of either sex, weighing 8 to 15 kg, were anaesthetized with thiopentone sodium (20 mg kg^{-1}, i.v.), exsanguinated from the common carotid arteries, and the basilar and middle cerebral arteries isolated. The arteries were then cut helically into strips approximately 1 to 1.5 mm in length, under a dissecting microscope. These strips were mounted vertically in an organ bath containing 20 ml 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 bath medium was maintained at 37°C, pH 7.4, and was equilibrated with a gas mixture consisting of 95% O_2 and 5% CO_2. Initially a tension of 1.0 g was applied and the preparations were allowed to equilibrate for 90 min in the bathing medium before experiments were started. During the equilibration period the preparations initially relaxed and then a spontaneous tonic tension ranging from 0.7 to 1.5 g developed. This tension was maintained throughout the experiment.

Desensitization of the P_2-purinoceptor was achieved by the addition of α,β-methylene ATP (5×10^{-6} M), which produced a transient contraction followed by a relaxation. This relaxation gradually diminished, thus the tone returned to the original level in many preparations (Figures 1a and 2a). The preparations which showed poor recovery of the tone under persistent treatment with α,β-methylene ATP were not used for analysis. Desensitization of the P_2-purinoceptor was confirmed by the lack of any further response to the addition of the same concentration of α,β-methylene ATP (total concentration in the bath; 10^{-5} M).

Electrical transmural stimulation was applied through a pair of platinum-wire electrodes at 10–15 min intervals. Stimulus parameters were 0.2 ms duration, frequencies of 10 and 30 Hz and supramaximum voltage (12 V) for 10 s. Drugs were added directly to the bath.

The release of [^{3}H]-noradrenaline was determined according to the method of Muramatsu *et al.* (1981). The basilar and middle cerebral arterial preparations were preincubated with [^{3}H]-noradrenaline (2×10^{-7} M) in Krebs-Henseleit solution containing 5.7×10^{-4} M ascorbic acid for 90 min at 37°C. These strips were then suspended between a pair of parallel platinum wire electrodes under 1 g of tension. The Krebs-Henseleit solution containing 5.7×10^{-7} ascorbic acid was bubbled with 95% O_2, 5% CO_2 at 37°C and was superfused using a peristaltic pump at a flow rate of 1 ml min^{-1}. Before the start of the experiments, each strip was equilibrated for 120 min, after which electrical transmural stimulation was applied through a pair of electrodes. Stimulus parameters were of a frequency of 10 Hz, duration of 0.1 ms and supramaximal voltage (7 V) for 10 s. Superfusate solution was collected every 1 min and the radioactivity was determined by counting in a Packard Tri-Carb liquid scintillation spectrometer. The spontaneous ^{3}H-efflux decreased exponentially to reach a plateau during the equilibrated period for 120 min. Thus, the increase in ^{3}H-efflux induced by electrical stimulation was calculated to be the net ^{3}H-efflux by stimulation. The drugs to be tested were superfused 15 min before the initiation of electrical stimulation.

The following drugs were used: lithium salt of α,β-methylene ATP (Sigma, MO, U.S.A.), (±)-propranolol hydrochloride, atropine sulphate, yohimbine hydrochloride (Nakarai, Kyoto, Japan), guanethidine sulphate (Tokyo-Kase, Tokyo, Japan), tetrodotoxin (Sankyo, Tokyo, Japan), prazosin hydrochloride (Taito-Pfizer, Tokyo, Japan), adenosine triphosphate (ATP; Kohjin, Tokyo, Japan) and (−)-[ring-2,5,6-^{3}H]-noradrenaline (43.7 Ci mmol^{-1}, NEN Research Products, Boston, MA, U.S.A.).

Experimental values are given as mean ± s.e.mean. The result were analysed by use of Student's *t* test (unpaired comparison) and a probability of less than 0.05 was considered significant.

Results

Response to electrical transmural stimulation

Previously, we demonstrated that electrical transmural stimulation of isolated basilar arteries of the dog produced a complex response; that is, an initial

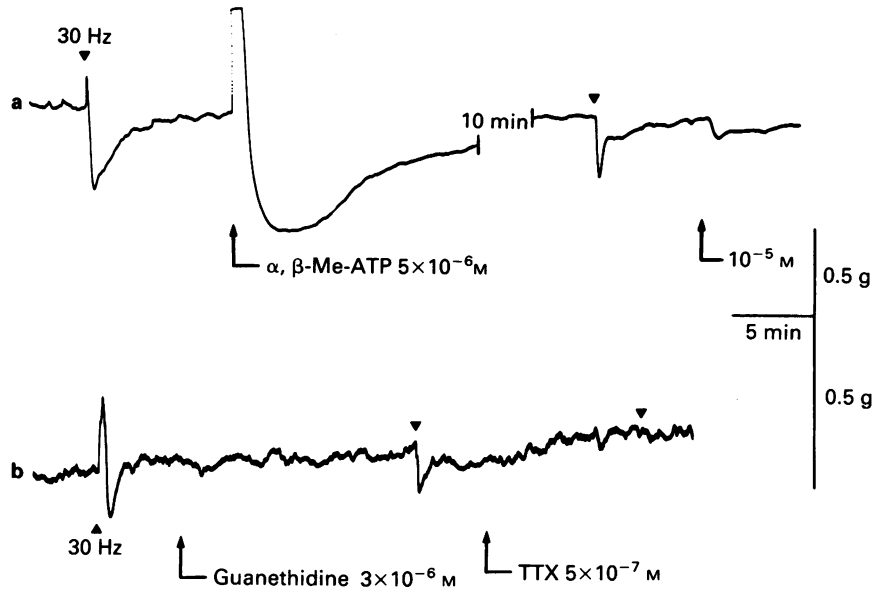

Figure 1 Effects of (a) α,β-methylene ATP (α,β-Me-ATP), (b) guanethidine and tetrodotoxin (TTX) on the responses to electrical transmural stimulation (30 Hz, at maximum voltage for 10 s) in the dog basilar artery. In (a) α,β-methylene ATP at a concentration of 5×10^{-6} M was applied twice; thus the final concentration of the drug was 10^{-5} M. A transient contractile response to α,β-methylene ATP exceeded the recording range.

contraction followed by a relaxation and/or a late contraction (Muramatsu *et al.*, 1981). In this series of experiments also, the basilar arteries elicited a transient contraction ($n = 39$) in response to electrical transmural stimulation (10 or 30 Hz, for 10 s) and this contraction was followed by a relaxation in 31 out of 39 preparations (Figure 1). These responses were abolished by tetrodotoxin (5×10^{-7} M, $n = 7$) but were unaffected by prazosin (10^{-7} M, $n = 5$), propranolol (10^{-6} M, $n = 4$) and atropine (10^{-6} M, $n = 56$). Yohimbine (3×10^{-8} M) enhanced the contractile (34 ± 6% increase) and relaxant (29 ± 8% increase) responses ($n = 6$).

The addition of α,β-methylene ATP (5×10^{-6} M) elicited a large contraction of the dog basilar artery. This contraction was followed by a relaxation which gradually diminished to the original level, even though the drug was present in the bath. Under such conditions, electrical stimulation failed to produce the initial contraction, and the subsequent relaxation was attenuated (33 ± 7% decrease), as compared with the response before treatment with the drug (Figure 1a). Further addition of the same concentration of α,β-methylene ATP (total 10^{-5} M in the bath) elicited neither contractile nor relaxant responses, thereby indicating that the preparation was desensitized to the drug. Guanethidine (3×10^{-6} M) also abolished the contractile response to electrical stimulation and inhibited the relaxation (23 ± 5% decrease) ($n = 5$) (Figure 1b).

On the other hand, upon electrical transmural stimulation, most of the preparations of the middle cerebral artery (25 out of 29) produced relaxation only. This relaxation was unaffected by desensitization to α,β-methylene ATP (5×10^{-6} M, $n = 5$) or by guanethidine (3×10^{-6} M, $n = 5$) (Figure 2). Tetrodotoxin (5×10^{-7} M) abolished the relaxation, whereas prazosin (10^{-7} M), propranolol (10^{-6} M), yohimbine (3×10^{-8} M) and atropine (10^{-6} M) had no significant effect ($n = 3$, for each drug). In the other 4 preparations of the middle cerebral artery, a small, transient contraction was evoked before development of the relaxation in response to electrical stimulation; the initial contraction was abolished and the subsequent relaxation was slightly attenuated (less than 10% decrease) by α,β-methylene ATP or guanethidine, as described in the case of the basilar arteries (data not shown).

The residual relaxant responses of the basilar and middle cerebral arteries to electrical transmural stimulation after treatment with α,β-methylene ATP or guanethidine were not inhibited by further treatment with guanethidine or α,β-methylene ATP, respectively (Figure 2b).

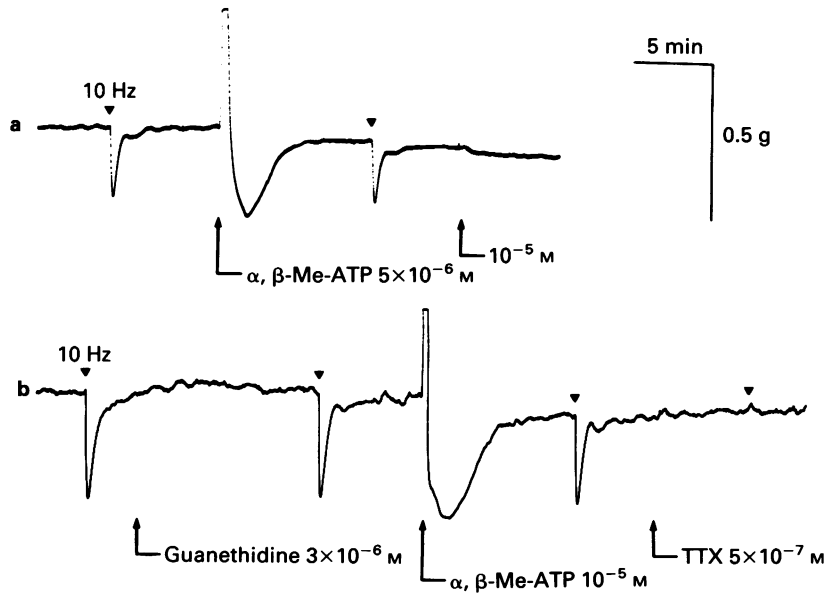

Figure 2 Effects of α,β-methylene ATP (α,β-Me-ATP), guanethidine and tetrodotoxin (TTX) on the responses to electrical transmural stimulation in the dog middle cerebral artery. Experimental conditions were the same as those in Figure 1.

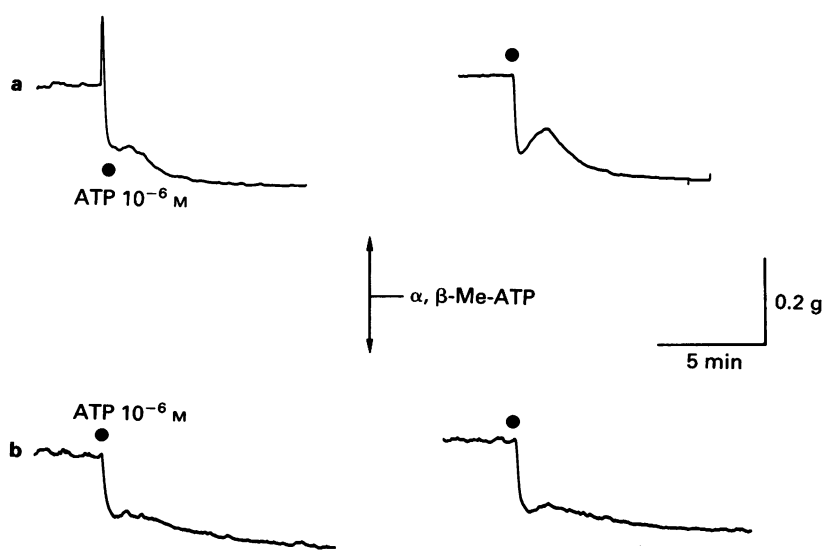

Figure 3 Effects of α,β-methylene ATP (α,β-Me-ATP, 5×10^{-6} M) on the responses to ATP of the basilar (a) and middle cerebral (b) arteries isolated from the dog. The righthand traces were recorded when the tension of the preparations had returned to the original level under treatment with α,β-methylene ATP.

Response to ATP

In the basilar arteries ATP (10^{-6} and 10^{-5} M) produced a transient contraction, followed by a sustained relaxation (Figure 3a). On the other hand, the middle cerebral arteries responded to ATP with only a relaxation (Figure 3b). Desensitization to α,β-methylene ATP abolished the contractile response to ATP but did not reduce the amplitude of relaxation. The pattern of the relaxation induced by ATP became more clearly biphasic under treatment with α,β-methylene ATP. Guanethidine (3×10^{-6} M) and tetrodotoxin (5×10^{-7} M) had no effect on the responses to ATP in the basilar and middle cerebral arteries ($n = 4$ for each drug and each preparation).

^{3}H-efflux induced by electrical transmural stimulation

Electrical transmural stimulation (10 Hz for 10 s) resulted in a remarkable increase in ^{3}H-efflux from the

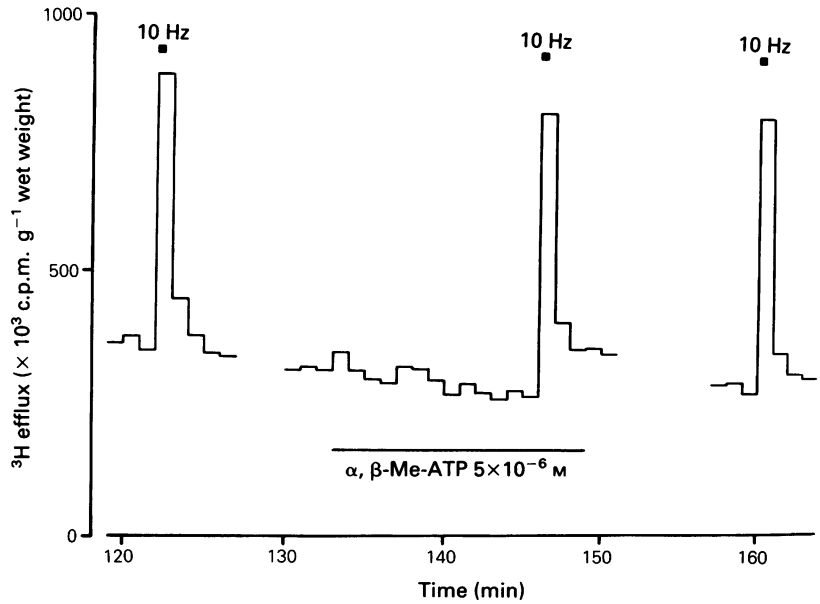

Figure 4 Effects of α,β-methylene ATP (α,β-Me-ATP) on the basal and evoked ^{3}H-efflux from a basilar artery preincubated with [^{3}H]-noradrenaline. Electrical stimulation: 10 Hz at maximum voltage for 10 s. Abscissa scale: superfusion time.

Table 1 Effects of various drugs on ^{3}H-efflux induced by electrical transmural stimulation in the dog basilar and middle cerebral arteries

Treatment	*^{3}H-efflux* (% of the first response)[a] *Basilar artery*	*Middle cerebral artery*
None	102 ± 3 (5)	106 ± 8 (4)
α,β-Methylene ATP (5×10^{-6} M)	98 ± 2 (4)	99 ± 2 (4)
Prazosin (10^{-7} M)	121 ± 4[b] (3)	127 ± 7 (4)
Yohimbine (3×10^{-8} M)	200 ± 14[b] (4)	179 ± 16[b] (4)
Guanethidine (3×10^{-6} M)	12 ± 1[b] (4)	23 ± 10[b] (3)
Tetrodotoxin (5×10^{-7} M)	1 ± 1[b] (3)	1 ± 1[b] (3)

[a] The preparations were stimulated twice electrically (10 Hz for 10 s), and the ^{3}H-efflux evoked by the second stimulation was expressed as a percentage of the first stimulation. Each drug was treated for 15 min before and during the second stimulation.

[b] Significantly different from the value without treatment (time control) (paried t test, $P < 0.01$).

Numbers in parentheses represent the number of experiments.

basilar and middle cerebral arteries preincubated with [^{3}H]-noradrenaline. This ^{3}H-efflux was markedly attenuated by tetrodotoxin (5×10^{-7} M) and by guanethidine (3×10^{-6} M) (Table 1). α,β-Methylene ATP (5×10^{-6} M) caused neither an increase in basal ^{3}H-efflux nor a reduction of evoked ^{3}H-efflux (Figure 4). Yohimbine (3×10^{-8} M), but not prazosin (10^{-7} M), markedly increased the ^{3}H-efflux evoked by electrical transmural stimulation. These results are summarized in Table 1.

Discussion

In most of the preparations of the dog basilar artery, electrical transmural stimulation evoked biphasic responses; an initial contraction and subsequent relaxation. On the other hand, only a relaxation was predominantly observed in the middle cerebral arteries. Since tetrodotoxin abolished the responses to electrical stimulation, the above results clearly indicate the presence of a regional variation of neurogenic responses in dog cerebral arteries.

The contraction and part of the relaxation of the basilar artery induced by electrical stimulation were suppressed by guanethidine. This confirms our previous observations, in which the neurogenic response of the dog basilar artery was inhibited by bretylium or superior cervical ganglionectomy (Muramatsu *et al.*, 1981). Furthermore the present study demonstrates that after desensitization to α,β-methylene ATP, an inhibitory effect similar to that seen with gunathidine is observed; that is the contractile response to electrical stimulation is abolished and the relaxant response suppressed.

Although there are a few exceptional cases (Byrne & Large, 1986; Amobi & Smith, 1987), α,β-methylene ATP is a highly selective P_2-purinoceptor agonist (Burnstock & Kennedy, 1985) and persistent treatment finally desensitizes the P_2-purinoceptor itself in many preparations (Kasakov & Burnstock, 1983; Katsuragi & Furukawa, 1985; Muramatsu, 1987). In the dog basilar artery also, after the contractile and relaxant responses produced by α,β-methylene ATP returned to baseline (and in the continued presence of α,β-methylene ATP) a further addition caused no further response (Figures 1a and 2a). Such treatment with α,β-methylene ATP also abolished the contractile response to exogenous ATP but did not significantly affect the evoked ^{3}H-efflux from the arteries preincubated with [^{3}H]-noradrenaline. These results suggest that, even though α,β-methylene ATP mimics the inhibitory effect of guanethidine, the site of action is postsynaptic (in contrast to the presynaptic site of action of guanethidine), and is probably due to desensitization of the P_2-purinoceptor. Since combined treatment with α,β-methylene ATP and guanethidine did not further attenuate the residual response present after treatment with each drug alone, it is probable that the inhibition induced by each drug reflects abolition of the sympathetic purinergic component. Such sympathetic purinergic transmission has recently been demonstrated in various peripheral blood vessels (Sneddon & Burnstock, 1984; Kügelgen & Starke, 1985; Muramatsu, 1986; 1987; Burnstock & Warland, 1987a).

As in the basilar artery, relaxation of the middle cerebral arteries was resistant to the adrenoceptor and cholinoceptor blocking agents tested. This is reminiscent of the non-adrenergic, non-cholinergic relaxation noted in cerebral arteries of many species (Lee *et al.*, 1978; Muramatsu *et al.*, 1978; Toda, 1981). The present study showed that guanethidine and α,β-methylene ATP has no effect on the relaxant response, thereby suggesting that the relaxation is non-sympathetic and non-purinergic in nature. This component is probably the same one as the residual relaxant response observed in the basilar artery after treatment with guanethidine and/or α,β-methylene ATP. Since the cerebral artery is innvervated by various peptidergic fibres (Edvinsson *et al.*, 1980; 1981; Burnstock, 1986), it is likely that the residual non-sympathetic, non-purinergic relaxation of the dog cerebral artery may be caused through such peptidergic nerve(s).

Dog basilar and middle cerebral arteries are densely innervated by sympathetic adrenergic nerves (Ohgushi, 1968; Muramatsu *et al.*, 1977). In the present study electrical transmural stimulation of the basilar artery and middle cerebral artery evoked an increase in ^{3}H-efflux from preparations which had been incubated with [^{3}H]-noradrenaline and the efflux was markedly inhibited by guanethidine or tetrodotoxin. Thus, it is reasonable to assume that noradrenaline released from sympathetic nerve terminals is involved in the neurogenic response. However, prazosin and propranolol did not inhibit the neurogenic response, and yohimbine enhanced both the mechnical response and ^{3}H-efflux induced by electrical stimulation. The postsynaptic α-adrenoceptor of the dog cerebral artery is the α_2, not the α_1, subtype and the contractile potency of noradrenaline through the α_2-adrenoceptors is much lower than that of ATP or other contractile substances (Muramatsu *et al.*, 1981; Sakakibara *et al.*, 1982; Tsukahara *et al.*, 1986). These lines of evidence support our view that, although noradrenaline is released from the sympathetic nerve terminals, it acts as a neuromodulator (via presynaptic α_2-adrenoceptors) rather than as a neurotransmitter in the dog cerebral arteries (Muramatsu *et al.*, 1981). The evidence that α,β-methylene ATP inhibits the sympathetic component of the neurogenic response without affecting the ^{3}H-efflux, strongly suggests that the sympathetic response may be caused by ATP or related substances which are released concomitantly

with noradrenaline. ^{3}H-purine release from sympathetic nerve terminals has been demonstrated in basilar arteries preincubated with [^{3}H]-adenosine (Muramatsu *et al.*, 1981).

ATP produced contractile and relaxant responses in the basilar artery, whereas only a relaxation was observed in the middle cerebral artery. These results indicate that there is a regional difference in the ATP response of dog cerebral arteries (Muramatsu *et al.*, 1980). Recently, P_2-purinoceptors have been subdivided into P_{2x} and P_{2y}, which are selectively inhibited by α,β-methylene ATP and reactive blue 2, respectively (Burnstock & Kennedy, 1985; Burnstock & Warland, 1987b; Hopwood & Burnstock, 1987). The contraction induced by ATP of the dog basilar artery was abolished by desensitization to α,β-methylene ATP, thereby suggesting that the contractile response, at least, is mediated through the P_{2x}-purinoceptor. On the other hand, the relaxant response to ATP was not attenuated by α,β-methylene ATP although the pattern of the response was modified. This lack of inhibition may mean that the relaxation induced by exogenous ATP is predominantly mediated through different types of purinoceptors such as P_{2y} or P_1 (Burnstock & Warland, 1987b). Previously we observed that ATP-induced relaxation of the dog cerebral artery was partially but not completely inhibited by a P_1-purinoceptor antagonist, theophylline (Muramatsu *et al.*, 1980). Although reactive blue 2 has recently been claimed to block selectively P_{2y}-purinoceptors (Burnstock & Warland, 1987b); Hopwood & Burnstock, 1987), we have not yet used this drug successfully in the dog cerebral artery because of a strong relaxation induced by the drug alone (I. Muramatsu, unpublished observation).

In conclusion, the present study clearly shows that the dog basilar and middle cerebral arteries are innervated by both sympathetic and non-sympathetic nerves and that excitation of the sympathetic nerves causes a purinergic response, predominantly in the basilar artery.

The authors thank M. Ohara for reading the manuscript and Naomi Yamada for secretarial assistance. This work was supported in part by a grant from the Japan Ministry of Education, Science and Culture, and by a grant from the Smoking Research Foundation of Japan.

References

AMOBI, N. & SMITH, C.H. (1987). Effects of α,β-methylene ATP on biphasi : responses of rat vas deferens. *Eur. J. Pharmacol.*, **133** 75–82.

BURNSTOCK, G. ( )77). Ultrastructure of the vascular neuroeffector apparatus, with special regard to brain vessels. In *Neurogenic Control of the Brain Circulation*, ed. Owman, C. & Edvinsson, L. pp. 3–14, Oxford: Pergamon Press.

BURNSTOCK, G. (1986). The changing face of autonomic neurotransmission. *Acta Physiol. Scand.*, **126**, 67–91.

BURNSTOCK, G. & KENNEDY, C. (1985). Is there a basis for distinguishing two types of P_2-purinoceptor? *Gen. Pharmacol.*, **16**, 433–440.

BURNSTOCK, G. & WARLAND, J.J.I. (1987a). A pharmacological study of the rabbit saphenous artery *in vitro*: a vessel with large purinergic contractile response to sympathetic nerve stimulation. *Br. J. Pharmacol.*, **90**, 111–120.

BURNSTOCK, G. & WARLAND, J.J.I. (1987b). P_2-purinoceptors of two subtypes in the rabbit mesenteric artery: reactive blue 2 selectively inhibits responses mediated via the P_{2y}- but not the P_{2x}-purinoceptor. *Br. J. Pharmacol.*, **90**, 383–391.

BYRNE, N.G. & LARGE, W.A. (1986). The effect of α,β-methylene ATP on the depolarization evoked by noradrenaline (γ-adrenoceptor response) and ATP in the immature rat basilar artery. *Br. J. Pharmacol.*, **88**, 6–8.

DUCKLES, S.P. (1979). Neurogenic dilator and constrictor responses of pial arteries *in vitro*. *Circ. Res.*, **44**, 482–490.

EDVINSSON, L., FAHRENKRVG, J., HANKO, J., OWMAN, Ch., SUNDLER, F. & UDDMAN, R. (1980). VIP nerve supply of intracranial arteries of mammals. *Cell & Tissue Res.*, **208**, 135–142.

EDVINSSON, L. & MACKENZIE, E.T. (1977). Amine mechanisms in the cerebral circulation. *Pharmacol. Rev.*, **28**, 275–348.

EDVINSSON, L., McCULLOCH, J. & UDDMAN, R. (1981). Substance P: immunohistochemical localization and effect upon cat pial arteries *in vitro* and *in situ*. *J. Physiol.*, **318**, 251–258.

GRIFFITH, S.G., LINCOLN, J. & BURNSTOCK, G. (1982). Serotonin as a neurotransmitter in cerebral arteries. *Brain Res.*, **247**, 388–392.

HEISTAD, D.D. & MARCUS, M.L. (1978). Evidence that neural mechanisms do not have important effects on cerebral blood flow. *Circ. Res.*, **42**, 295–302.

HOPWOOD, A.M. & BURNSTOCK, G. (1987). ATP mediates coronary vasoconstriction via P_{2x}-purinoceptors and coronary vasodilatation via P_{2y}-purinoceptors in the isolated perfused rat heart. *Eur. J. Pharmacol.*, **136**, 49–54.

IWAYAMA, T., FURNESS, J.B. & BURNSTOCK, G. (1970). Dual adrenergic and cholinergic innervation of the cerebral arteries of the rat. *Circ. Res.*, **26**, 635–646.

KASAKOV, L. & BURNSTOCK, G. (1983). The use of the slowly degradable analog, α,β-methylene ATP, to produce desensitisation of the P_2-purinoceptor: effect on non-adrenergic, non-cholinergic responses of the guinea-pig urinary bladder. *Eur. J. Pharmacol.*, **86**, 291–294.

KATSURAGI, T. & FURUKAWA, T. (1985). Novel, selective purinoceptor antagonists: investigation of ATP as a neurotransmitter. *Trends Pharmacol. Sci.*, **6**, 337–339.

KENNEDY, C., SAVILLE, V.L. & BURNSTOCK, G. (1986). The contribution of noradrenaline and ATP to the responses

of the rabbit central ear artery to sympathetic nerve stimulation depend on the parameters of stimulation. *Eur. J. Pharmacol.*, **122**, 291–300.

KÜGELGEN, I.V. & STARKE, K. (1985). Noradrenaline and adenosine triphosphate as co-transmitters of neurogenic vasoconstriction in rabbit mesenteric artery. *J. Physiol.*, **367**, 435–455.

LEE, T.J-F., HUME, W.R., SU, C. & BEVAN, J.A. (1978). Neurogenic vasodilation of cat cerebral arteries. *Circ. Res.*, **42**, 535–542.

LEE, T.J-F., SU, C. & BEVAN, J.A. (1976). Neurogenic sympathetic vasoconstriction of the rabbit basilar artery. *Circ. Res.*, **39**, 120–126.

MARCUS, M.L. & HEISTAD, D.D. (1979). Effects of sympathetic nerves on cerebral blood flow in awake dogs. *Am. J. Physiol.*, **236**, H549–H553.

MURAMATSU, I. (1986). Evidence for sympathetic, purinergic transmission in the mesenteric artery of the dog. *Br. J. Pharmacol.*, **87**, 478–480.

MURAMATSU, I. (1987). The effect of reserpine on sympathetic, purinergic neurotransmission in the isolated mesenteric artery of the dog: a pharmacological study. *Br. J. Pharmacol.*, **91**, 467–474.

MURAMATSU, I., FUJIWARA, M., MIURA, A. & SAKAKIBARA, Y. (1981). Possible involvement of adenine nucleotides in sympathetic neuroeffector mechanisms of the dog basilar artery. *J. Pharmacol. Exp. Ther.*, **216**, 401–409.

MURAMATSU, I., FUJIWARA, M., MIURA, A. & SHIBATA, S. (1980). Reactivity of isolated canine cerebral arteries of adenine nucleotides and adenosine. *Pharmacology*, **21**, 198–205.

MURAMATSU, I., FUJIWARA, M., OSUMI, Y. & SHIBATA, S. (1978). Vasoconstrictor and dilator actions of nicotine and electrical transmural stimulation of isolated dog cerebral arteries. *Blood Vessels*, **15**, 110–118.

MURAMATSU, I., IKUSHIMA, S. & FUJIWARA, M. (1977). Enhancement by phentolamine of sympathetic contraction of dog basilar artery and α-receptor mediated feedback. In *Neurogenic Control of the Brain Circulation*, ed. C. Owman, Edvinsson, L. pp. 197–206, Oxford: Pergamon Press.

OHGUSHI, N. (1968). Adrenergic fibers to the brain and spinal cord vessels in the dog. *Arch. Jap. Chir.*, **37**, 294–303.

OWMAN, C., EDVINSSON, L. & NIELSEN, K.C. (1974). Autonomic neuroreceptor mechanisms in brain vessels. *Blood Vessels*, **11**, 2–31.

PURVES, M.J. (1978). Do vasomotor nerves significantly regulate cerebral blood flow? *Circ. Res.*, **43**, 485–493.

SAKAKIBARA, Y., FUJIWARA, M. & MURAMATSU, I. (1982). Pharmacological characterization of the alpha adrenoceptors of the dog basilar artery. *Naunyn-Schmiedebergs Arch. Pharmacol.*, **319**, 1–7.

SNEDDON, P. & BURNSTOCK, G. (1984). ATP as a co-transmitter in rat tail artery. *Eur. J. Pharmacol.*, **106**, 149–152.

TODA, N. (1981). Non-adrenergic, non-cholinergic innervation in monkey and human cerebral arteries. *Br. J. Pharmacol.*, **72**, 281–283.

TSUKAHARA, T., TANIGUCHI, T., USUI, H., MIWA, S., SHIMOHAMA, S., FUJIWARA, M. & HANDA, H. (1986). Sympathetic denervation and alpha adrenoceptors in dog cerebral arteries. *Naunyn-Schmiedebergs Arch Pharmacol.*, **334**, 436–443.

(Received May 28, 1987.
Revised July 28, 1987.
Accepted August 6, 1987.)