mine-adenosine relationship, promoting a more marked manifestation of the dopaminergic effects.

In agreement with recent suggestions pointing to a specific purinergic contribution to the neurological function in the basal ganglia (Jarvis et al 1989), the hypothetical role of adenosine in the modulation of movements seems to be confirmed.

Further studies are needed to investigate whether the drugs affecting the adenosine system may be useful in the treatment of dopaminergic disorders.

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# Response of canine cerebral arteries to endothelin-1

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**Abstract**—The effects of endothelin-1 ($10^{-10}$–$10^{-7}$ M) were isometrically recorded in 4 mm cylindrical segments from the middle cerebral artery of dogs. Cumulative application of endothelin-1 produced marked, sustained contraction of arteries in a concentration-dependent-manner, the maximal response being about 2·6 times higher than that achieved with KCl (50 mM). The contraction by endothelin-1 was unaffected either by endothelium removal or by the cyclo-oxygenase inhibitors indomethacin ($10^{-6}$ M) and meclofenamate ($10^{-6}$ M). In a $Ca^{2+}$-low (25 μM) solution the endothelin-1-induced arterial contraction was decreased. Therefore, the cerebral vasoconstriction induced by endothelin-1 could be caused by activation of specific receptors located on smooth muscle cells which would lead to the influx of extracellular calcium and vascular musculature contraction.

It has been demonstrated that the endothelium plays a main role in the regulation of vascular tone by releasing relaxing and contracting factors (Furchgott & Vanhoutte 1989). Endothelin is an endothelium-derived 21 amino acid peptide which has been isolated and sequenced by Yanagisawa et al (1988b). This peptide is similar among some mammalian species: human and porcine endothelin have the same amino acid sequence (Itoh et al 1988), whereas rat endothelin has 76% homology, and a slightly different pharmacological potency (Yanagisawa et al 1988a). Endothelin-1 produces a potent, long-lasting constrictor effect in several vascular beds in-vivo and in-vitro (Hughes et al 1988; Yanagisawa et al 1988b; Eglen et al 1989; Hinojosa-Laborde et al 1989; Kasuya et al 1989; King et al 1989). However, endothelin-1 appears to be a vasoconstrictor selective for some vascular beds (Clozel & Clozel 1989) and its effects are not limited to vasoconstriction as it can also produce vasodilation in-vivo and in-vitro (de Nucci et al 1988; Folta et al 1989; Lippton et al 1988).

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Few experiments have been reported on the effects of endothelin-1 on cerebral blood vessels and its possible involvement in the regulation of the cerebral circulation has not been determined.

The present experiments were designed to examine the effects of endothelin-1 on canine isolated cerebral arteries and the role of the endothelium, prostanoids and extracellular calcium on these effects.

### Materials and methods

Eight mongrel dogs, 18–27 kg, were anaesthetized with sodium pentobarbitone injected intravenously, and exsanguinated. The brain was then carefully removed and both middle cerebral arteries were dissected out and cut into cylindrical segments 4 mm in length and approximately 950 μm in external diameter. Two stainless-steel pins, 150 μm in diameter, were introduced through the arterial lumen. One pin was fixed to the organ bath wall while the other was connected to a strain gauge. The recording system included a Universal transducing cell (UC3), a Statham microscale accessory (U15) and a Beckman type RS recorder. Each arterial segment was set up in a 6 mL bath containing modified Krebs-Henseleit solution with the following composition (mM): NaCl 115; KCl 4·6; $KH_2PO_4$ 1·2; $CaCl_2$ 2·5; $NaHCO_3$ 25; $MgSO_4$. 7 $H_2O$ 1·2; glucose 11·1 and $Na_2H_2$ EDTA 0·01. The solution was equilibrated with 95% $O_2$- 5% $CO_2$ to give a pH of 7·3 to 7·4 and temperature was held at 37°C. Arterial segments were equilibrated at a passive tension of 1·5 g for 1 h.

Response of vascular segments to endothelin-1 ($10^{-10}$–$10^{-7}$ M) was determined in a cumulative manner. This was carried out in intact, non-treated arteries (control), in arteries without endoth-

elium, in arteries placed in the organ bath containing a $Ca^{2+}$-low (25 $\mu$M) Krebs-Henseleit solution and in arteries treated with the inhibitors of cyclo-oxygenase indomethacin ($10^{-6}$ M) or meclofenamate ($10^{-6}$ M). The endothelium was previously removed by rubbing the luminal surface of the arteries. The presence of endothelium and the adequacy of endothelium removal in rubbed arteries was examined morphologically at the end of the experiments by direct observation after enface silver staining. Only results from vascular segments with more than 60–70% of the endothelium were considered as arteries with endothelium. Arteries without endothelium were considered to be those that had been rubbed and showed less than 5% of their intima covered with endothelium. Arteries with more than 60–70% of the endothelium exhibited relaxation when acetylcholine ($10^{-8}$–$10^{-4}$ M) was added to the bath during the contraction induced by endothelin-1, whereas in the arteries with less than 5% of endothelium, acetylcholine failed to cause relaxation, thus indicating that morphological and functional tests correlated well. The composition of the $Ca^{2+}$-low Krebs-Henseleit solution only differed from the normal solution in the concentration of calcium ions. A 25 $\mu$M $Ca^{2+}$ solution was used because it was effective in reducing the vascular contraction to high KCl (50 mM), a contractile process that primarily depends on extracellular calcium ions. Indomethacin ($10^{-6}$ M) or meclofenamate ($10^{-6}$ M) was added to the organ bath 20–30 min before endothelin-1 was applied to the tissues.

The ability of each vessel preparation to contract was tested in the presence of KCl (50 mM). This was performed in the arteries under control and the different experimental conditions.

The contraction of endothelin-1 causing 50% of the maximal response (EC50) was calculated.

Drugs used were: endothelin-1 (human, porcine; Peninsula Laboratories Europe, Ltd), indomethacin (Sigma) and sodium meclofenamate (Parke Davis).

The data, expressed as means ± s.e.m. were evaluated by Student's *t*-test, considering as significant a probability value of less than 0·05.

## Results

Fig. 1 summarizes the concentration-response curves for endothelin-1 obtained in control arteries, in arteries de-endothelialized, in arteries treated with indomethacin or meclofenamate, and in arteries placed in the calcium-low solution.

Endothelin-1, added cumulatively, produced a concentration-dependent, sustained contraction in control arteries (Fig. 2). The increased tone produced by endothelin-1 took a long time to return to the resting level despite repeated washings of the vessels. The maximal endothelin-1-induced contraction was about 2·6 times higher than the KCl (50 mM)-induced contraction.

The values of EC50 and maximal contraction for endothelin-1 as well as the contraction evoked by KCl in arteries under the different conditions are displayed in Table 1.

## Discussion

The EC50 for endothelin-1 in canine cerebral arteries was $9 \pm 1{\cdot}3 \times 10^{-9}$ M similar to that reported for other cerebral vessels (Vila et al 1990) and blood vessels (Eglen et al 1989; Yanagisawa & Masaki 1989). Endothelial cells have no significant influence on the action of endothelin-1 since the concentration-response curve for this peptide in the intact arteries was virtually identical to that in the de-endothelialized arteries. Also the effects of endothelin-1 were unaffected by the inhibitors of cyclo-oxygenase indomethacin and meclofenamate. Therefore, it can be considered that endothelin-1 does not act indirectly by releasing endothelium-derived factors or prostanoids, but acts directly on the arterial smooth musculature.

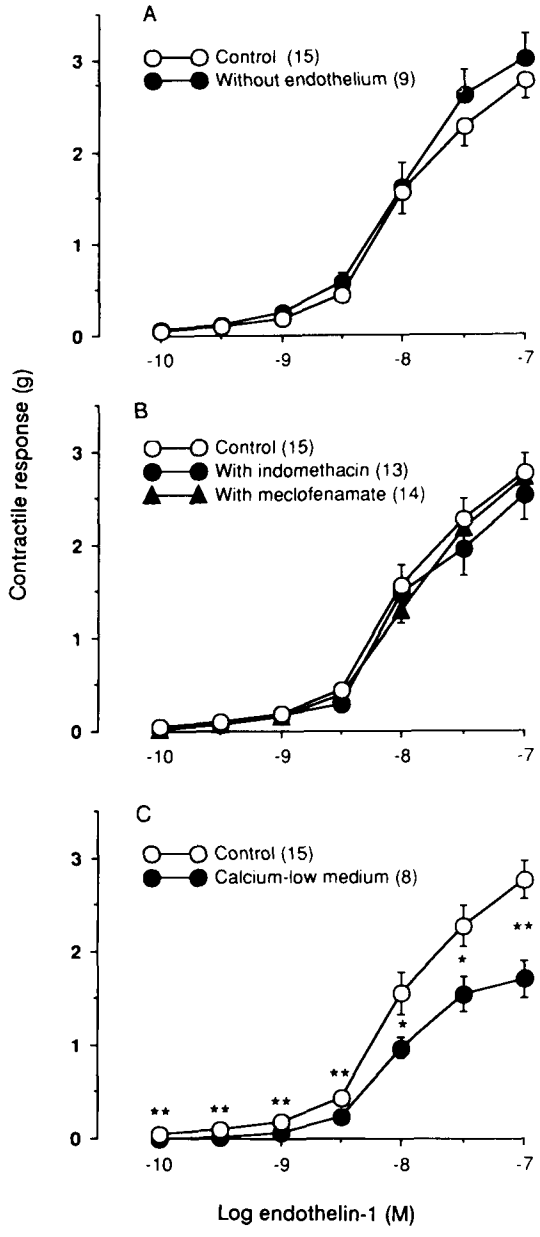


FIG. 1. Concentration-response curves for endothelin-1 obtained in the canine middle cerebral artery under control conditions, without endothelium (A), treated with indomethacin ($10^{-6}$ M) or meclofenamate ($10^{-6}$ M) (B) and placed in a calcium-low medium (C). Values are means ± s.e.m. In parentheses, number of experiments. * $P < 0{\cdot}05$, ** $P < 0{\cdot}01$ compared with control.

A lack of endothelium-dependent modulation of the vascular response to endothelin-1 has been also observed in cerebral arteries from man and rats (Hardebo et al 1989), dogs (Asano et al 1989) and goats (Vila et al 1990) as well as in isolated aortic rings (Marsden et al 1989) and helical strips of the porcine coronary artery (Kasuya et al 1989). Observations in cat cerebral arteries (Edvinsson et al 1989; Jansen et al 1989; Saito et al 1989) however, show that the endothelium attenuates the constrictor effects of endothelin-1 and this is also found in rat isolated perfused mesentery (Warner et al 1989) and rat aorta (Eglen et al 1989). Although the reason for these differences is still to be clarified, differences from species to species may exist.

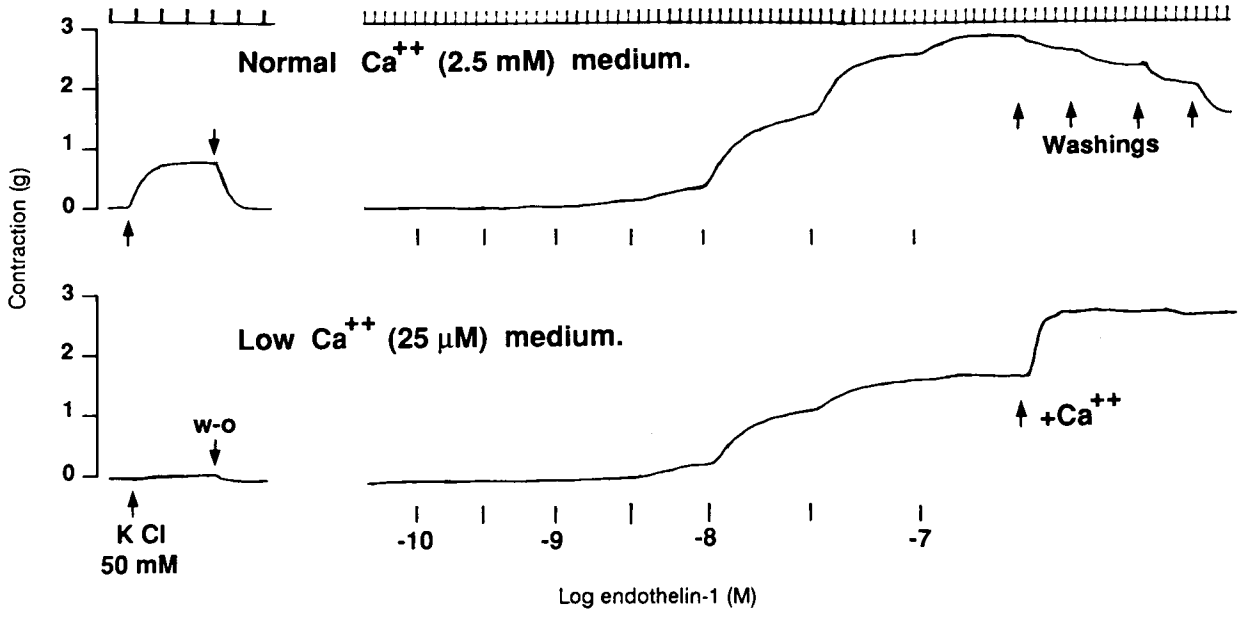


FIG. 2. Representative tracings showing the effects of KCl and endothelin-1 on the canine middle cerebral artery placed in the normal and low $Ca^{2+}$ solutions. At right ($\downarrow + Ca^{2+}$) of the lower recording the effects produced by the addition of calcium to the bath containing the $Ca^{2+}$-low solution and endothelin-1 is shown. Top trace, 1 min time divisions. w − o = washout.

Table 1. EC50 values and maximal contraction for endothelin-1 and contractile response to KCl (50 mM) obtained in the canine middle cerebral artery under control conditions, without endothelium, treated with indomethacin ($10^{-6}$ M) or meclofenamate ($10^{-6}$ M) and placed in a calcium-low medium.

| | Endothelin-1 | | KCl |
|---|---|---|---|
| | EC50 (M) | Maximal contraction (mg) | Contraction (mg) |
| Control (15) | $9{\cdot}0 \pm 1{\cdot}3 \times 10^{-9}$ | $2757 \pm 209$ | $1039 \pm 113$ |
| Without endothelium (9) | $9{\cdot}4 \pm 1{\cdot}2 \times 10^{-9}$ | $2994 \pm 310$ | $931 \pm 128$ |
| With indomethacin (13) | $1{\cdot}1 \pm 0{\cdot}14 \times 10^{-8}$ | $2515 \pm 269$ | $1115 \pm 147$ |
| With meclofenamate (14) | $9{\cdot}3 \pm 1{\cdot}4 \times 10^{-9}$ | $2708 \pm 233$ | $1272 \pm 155$ |
| $Ca^{2+}$-low medium (8) | $8{\cdot}6 \pm 0{\cdot}7 \times 10^{-9}$ | $1675 \pm 183$* | $131 \pm 49$** |

Values are means ± s.e.m. In parentheses, number of experiments. * $P < 0{\cdot}01$, ** $P < 0{\cdot}001$ compared with control.

Experiments in-vivo indicate that prostanoids appear to mediate piglet cerebral vasodilation induced by low concentrations of endothelin-1 and contribute to constriction induced by higher concentrations of endothelin-1 (Armstead et al 1989). However, constrictor effects of endothelin-1 on cat cerebral arteries (Yanagisawa et al 1988b; Saito et al 1989) appear to be resistant to indomethacin as also occurs in other vascular beds of different species (Yanagisawa et al 1988b; Yanagisawa & Masaki 1989). These studies and ours suggest that prostanoids are not primarily involved in the cerebrovascular constriction of endothelin-1.

The contraction of several vascular preparations, including cerebral vessels, produced by endothelin-1 seems to be dependent on the influx of extracellular calcium (Hughes et al 1988; Yanagisawa et al 1988b; Eglen et al 1989; Hardebo et al 1989; Jansen et al 1989; Saito et al 1989). This phenomenon has not been observed, however, in goat cerebral arteries in which the endothelin-1-induced contraction appears to be independent of calcium influx (Vila et al 1990). Our results show that canine cerebral arteries exhibit a lower contraction to each dose of endothelin-1 in a low calcium medium, thus suggesting that the contraction is caused in part by activating the influx of extracellular calcium ions into the smooth muscle cells. The addition of calcium into the calcium-low solution evoked a contraction in the presence of endothelin-1. In addition, the KCl-induced contraction, a process that mostly depends on extracellular calcium ions, is reduced in arteries placed into the calcium-low solution. This observation indicates that the $Ca^{2+}$-low solution used in our study consistently limits the ability of arteries to contract when activated with agents that increase membrane permeability to extracellular $Ca^{2+}$. Therefore, the present study supports previous observations on the importance of extracellular calcium for endothelin-1-induced contraction in cerebral vessels. Experiments performed in cat cerebral arteries (Jansen et al 1989; Saito et al 1989) indicate that the endothelin-1-induced contraction is produced by activating the influx of calcium ions through voltage sensitive calcium channels of smooth muscle cells.

Autoradiographic studies with [$^{125}$I]endothelin-1 indicate that putative endothelin-1 receptors are located in brain blood vessels of various species including man (Hoyer et al 1989). This is consistent with the contractile effects observed in the present

study and suggests a direct action of endothelin-1 on specific receptors. Endothelin-1 could initially stimulate a specific receptor which would lead to the activation of the influx of extracellular calcium and vascular smooth musculature contraction as has been suggested by Marsden et al (1989).

The potent cerebral vasoconstrictor effects of endothelin-1 are in favour of the hypothesis that this peptide could participate in the control of the cerebral circulation under physiological and some pathophysiological conditions. The presence of endothelin-1 in normal human cerebrospinal fluid at concentrations seven times those in plasma (Hoffman et al 1989) and its elevated plasma concentrations in patients with cerebral vasospasm after subarachnoid haemorrhage (Masaoka et al 1989) also support this hypothesis. A functional change of endothelial cells and local generation of spasmogenic substances have been suggested to occur during the development of delayed vasospasm following subarachnoid haemorrhage (Kassel et al 1985; Bevan & Bevan 1988). Since endothelin-1 is produced by the endothelium and evokes a marked, sustained vascular contraction, this endogenous peptide is a potential spasmogenic substance.

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