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Short communication

Cardiovascular responses induced by injection of endothelin-1 into the superior colliculus of rats

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Abstract

Microinjection of endothelin-1 (1–10–100 pmol) into the superficial layer of the superior colliculus of anaesthetised rats caused dose-dependent decreases in blood pressure. This is unlikely to be a non-specific effect, for angiotensin II (1 nmol) caused increases in blood pressure. Injection of endothelin-1 (10 pmol) into the superior colliculus also caused falls in renal blood flow as measured by an ultrasonic flow probe. Decreases in blood pressure and falls in renal blood flow induced by injection of endothelin-1 into the superior colliculus were unaffected by bilateral vagotomy suggesting that attenuations in the activity of the sympathetic nervous system were involved in these events.

Keywords: Endothelin-1; Superior colliculus; Blood pressure; Renal blood flow

1. Introduction

Autoradiographic studies have demonstrated that endothelin-1 binds discretely throughout the midbrain of rats, and notably in the superior colliculus (Kohzuki et al., 1991). This is interesting, for the superior colliculus, in addition to integrating visual inputs, takes part in the central control of cardiovascular function via descending neuronal pathways originating in its superficial, intermediate or deep layer (Keay et al., 1988). However, there has been no study of the cardiovascular consequences of stimulating endothelin's receptors within the superior colliculus. Therefore, we have investigated the effects of microinjecting endothelin-1 into the superficial layer of the superior colliculus of anaesthetised rats. In particular, we show that endothelin-1 microinjected into the superficial layer of the superior colliculus induces pressor responses and reductions in renal blood flow.

2. Materials and methods

Male Wistar rats (250–300 g; 6 for each group) were anaesthetised with urethane ethyl carbamate (1.2 g/kg i.p.) and catheterized through the femoral artery for measurement of blood pressure. The left kidney was exposed via a mid-line laparatomy and the renal artery was isolated. An ultrasonic flow probe was placed around the left renal artery for measurement of total renal blood flow. In some animals the vagus nerves were also isolated to facilitate later cutting. The animals, spontaneously breathing, were then placed in a stereotaxic head frame and the dorsal surface of the brain exposed by a craniotomy to permit intracerebral microinjections using a Hamilton 1 μl syringe supported in a stereotaxic micromanipulator. The coordinates of the atlas of Paxinos and Watson (1986) (measured in mm from the bregma: posteriorly, −8.0; laterally, 0.5; vertically, 3.2) were used to position the microsyringe.

2.1. Experimental protocols

After a 30 min stabilisation period consecutive injections of endothelin-1 (1–10–100 pmol) were made into the

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superior colliculus (each injection being made only when the blood pressure had returned to its basal value) to construct dose-response curves. Angiotensin II was injected at a single dose of (1 nmol). Renal blood flow studies were performed using submaximal doses of endothelin-1 (10 pmol/rat). In these experiments once pressor responses to endothelin-1 injected at 15 min intervals were established, a bilateral vagotomy was performed followed 15 min later by a further injection of endothelin-1. Each intracerebral injection was given in a total volume of 100 nl over of a period of 5 s. After some experiments the positioning of the injection site was checked histologically. Endothelin-1 (Peptide Institute, Osaka, Japan) was solubilized in 0.1% acetic acid in 0.9% (w/v) saline and the solution adjusted to pH 7.2. Control injections were carried out with the same amount of solvent in which the drugs were dissolved. These did not produce any changes in blood pressure. All results are expressed as mean ± standard error, with $P < 0.05$ being considered significant. Cardiovascular changes were compared by analysis of variance (ANOVA) and Newman-Keuls test for multiple comparisons (Tallarida and Murray, 1987). Renal vascular resistance was calculated as renal blood flow/mean arterial blood pressure.

3. Results

The basal mean blood pressure of the rats was 102 ± 3.5 mmHg ($n = 6$). This was decreased in a dose-dependent manner by endothelin-1 (1–10–100 pmol) microinjected into the superficial layer of the superior colliculus (Fig. 1). Injection of endothelin-1 (10 pmol) into the superior colliculus also caused left renal blood flow to fall (control, 7.7 ± 0.4 ml/min) by 27 ± 2% ($P < 0.01$) ($n = 4$), with the peak decrease in renal blood flow coinciding with the peak decrease in mean arterial blood pressure ($n = 4$) (Fig. 2). The renal vascular resistance following injection of endothelin-1 (10 pmol) into the superior colliculus was 0.062 ± 0.01 mmHg · min/ml (control, 0.075 ± 0.01 mmHg · min/ml). Both the pressor and the renal responses were unaffected by bilateral vagotomy (Fig. 2). Angiotensin II (1 nmol) injected into the superior colliculus of bilaterally vagotomized rats caused an increase in basal mean blood pressure of 41 ± 5 mmHg ($n = 4$).

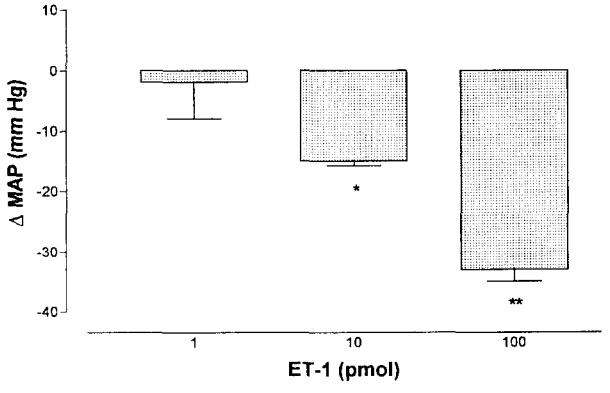

Fig. 1. Changes in mean arterial bood pressure (ΔMABP) (mmHg ± S.E.) after microinjection of endothelin-1 (ET-1; 1–100 pmol) into the superficial layer of the superior colliculus (SC) of the rat. Each column represents the mean of 6 observations ± S.E. Significant differences to vehicle-treated animals are shown by asterisks (* $P < 0.05$ and ** $P < 0.01$).

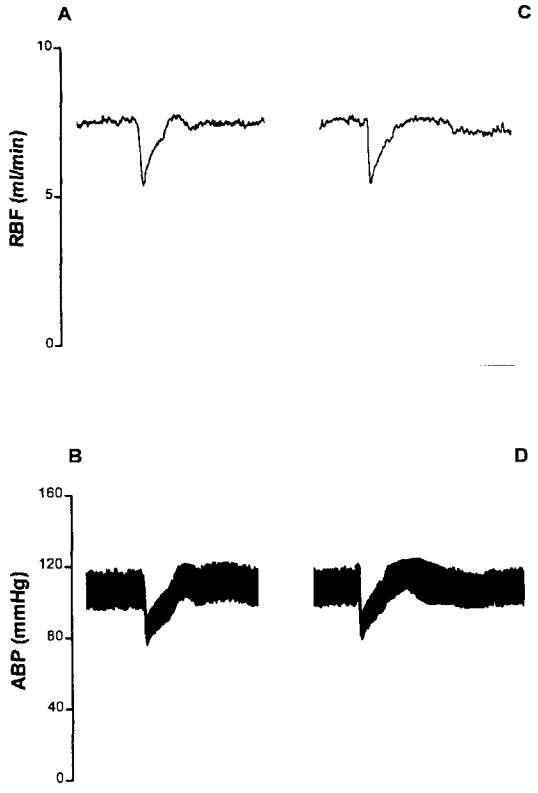

Fig. 2. Representative traces of changes in arterial blood pressure (ABP) and renal blood flow (RBF) induced by microinjection of endothelin-1 (ET-1; 10 pmol) into the superficial layer of the superior colliculus (SC) of rats before (A and B) or after bilateral vagotomy (C and D). Scale bar = 2 min.

4. Discussion

Here we show that endothelin-1 microinjected into the superficial layer of the superior colliculus reduces the blood pressure of anaesthetised rats. This is unlikely to be a non-selective effect, for angiotensin II caused increases in blood pressure. Endothelin-1 injected into the superior colliculus also reduced renal blood flow, which is in contrast to the expected increases in renal blood flow that follow injection of endothelin-1 into areas such as the

nucleus tractus solitarius and the cerebral ventricles (Hashim and Tadepalli, 1992). Interestingly, the decrease in renal blood flow had the same time of onset as the decrease in mean arterial pressure, and reached its lowest value at the same time as the maximum decrease in mean arterial pressure. Thus, there was no net change in renal vascular resistance.

Falls in renal blood flow associated with falls in blood pressure are thought to be vagally mediated (see Ludbrook, 1990). Interestingly, our data show that a bilateral vagotomy did not affect reductions in renal blood flow induced by endothelin-1, suggesting no involvement of the vagus. It is tempting to suggest, therefore, that exogenously applied endothelin-1 may within the superior colliculus inhibit the activity of descending sympathetic pathways. This suggestion is supported by evidence that (a) the superior colliculus has axonal projections to the intermediolateral cell column (Yasui et al., 1994), which is the final site at which the central nervous system interacts with sympathetic preganglionic neurons (see Coote, 1988); (b) central inhibition of sympathetic activity results in an immediate fall in renal blood flow and blood pressure (Hsieh et al., 1988). However, an answer to the question of whether endogenously produced endothelin-1 can cause a reduction in sympathetic drive by an action within the superior colliculus would require further investigation.

In conclusion, our data indicate that endothelin-1 microinjected into the superficial layer of the superior colliculus causes decreases in blood pressure and renal blood flow which are not mediated by changes in vagal activity.

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