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SPECIAL REPORT

Involvement of endothelin in the pressor response following injection of NMDA to the periaqueductal gray area of rats

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Microinjection of N-methyl-D-aspartate (NMDA) (0.068 to 6.8 nmol) into the periaqueductal gray area (PAG) of anaesthetized rats caused dose-dependent increases in blood pressure. Preinjection (10 min before) of FR 139317 (an ET_A receptor selective antagonist; 5 nmol) or SB 209670 (an ET_A/ET_B receptor non-selective antagonist; 5 nmol) to the PAG reduced the pressor response to NMDA whereas BQ-788 (an ET_B receptor selective antagonist; 5 nmol) did not affect the NMDA-induced hypertension. Pretreatment with DL-2-amino-5-phosphono valeric acid (2-APV) (an NMDA receptor selective antagonist, 5 nmol) also abolished the pressor response induced by NMDA. Dose-dependent increases in blood pressure induced by injection of angiotensin II (0.1-10 nmol) to the PAG were unaffected by FR 139317 or SB 209670. Thus, our data indicate that endogenous ET-1, via an action on ET_A receptors, contributes to the pressor effects of NMDA within the brain.

Keywords: N-methyl-D-aspartate; PAG area; ET-antagonists; hypertension

Introduction Endothelin-1 (ET-1) has been implicated as a regulator of blood pressure via both central and peripheral actions (see Rubanyi & Polokoff, 1994). Stimulation of the periaqueductal gray (PAG) area with ET-1 activates the glutamatergic system, for blockade of N-methyl-D-aspartate (NMDA) receptors prevents the ET-1-induced pressor responses (D'Amico *et al.*, 1995). Here we show that endothelin receptor antagonists reduce the pressor effect caused by injection of NMDA to the PAG. This is a selective effect, for responses to angiotensin II remain unaffected.

Methods Male Wistar rats (250-300 g) were anaesthetized with urethane ethyl carbamate (1.2 g kg^{-1} i.p.) and catheterized through the femoral artery for measurement of blood pressure. 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 10 μl syringe supported in a stereotaxic micromanipulator. The coordinates of the atlas of Paxinos & Watson (1986) (measured in mm from the bregma: posteriorly, -7.8; laterally, 0.8; vertically, 4.5) were used to position the microsyringe.

Experimental protocols After a 30 min stabilization period NMDA (0.068 to 6.8 nmol) or angiotensin II (0.1 to 10 nmol) was injected into the PAG consecutively (each injection being made only when the blood pressure had returned to its basal value) to construct dose-response curves. After this, antagonist studies were performed using submaximal doses of NMDA (0.68 nmol/rat) or angiotensin II (1 nmol). In these experiments once pressor responses to NMDA or angiotensin II injected at 15 min intervals were established, a single dose of antagonist (5 nmol) was microinjected, followed 10 min later by a further injection of NMDA or angiotensin II. Each intracerebral injection was given in a total volume of 1 μl over a period of 10 s. After the experiments, the positioning of the injection site was checked histologically. The following drugs were used: ET-1 (Peptide Institute, Japan), BQ-788 (N-*cis*-2,6-dimethylpiperidinocarbonyl-L-γ-metLeu-D-1-methoxy-carbonylTrp-D-Nle, SB 209670 (+)-(1S, 2R, 3S)-3-(2-carboxymethoxy-4-methoxyphenyl)-1-(3,4-methylenedioxyphenyl)-5-(prop-1-yloxy)indane-2-carboxylic acid, FR 139317 (R)-2-[(R)-2-[(S)-2-[[1-hexahydro-1H-azepinyl)]carbonyl]amino-4-methyl pentanoyl]-amino-3-(2-pyridyl)propionic acid, NMDA, DL-2-amino-5-phosphono valeric acid (2-APV) and angiotensin II (Sigma Chemical Co, St. Louis, U.S.A.). ET-1 was solubilized in 0.1% of acetic acid in 0.9% w/v saline. All the antagonists, NMDA and angiotensin II were dissolved in 0.9% NaCl. All solutions were 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 $\pm$ standard error (s.e.), with $P<0.05$ being considered significant. Cardiovascular changes were compared by analysis of variance (ANOVA) and Newman Keuls test for multiple comparisons (Tallarida & Murray, 1987).

Results The basal mean blood pressure of the rats was 99 ± 4.4 mmHg ($n=7$). This was increased in a dose-dependent manner by NMDA (0.068-6.8 nmol) microinjected into the PAG area (Figure 1a). Pretreatment of the PAG with FR 139317 or SB 209670 (both 5 nmol) caused, respectively, reductions of 56.2 ± 11.0 and 53.1 ± 6.7% in the NMDA-induced pressor effect ($P<0.01$). In contrast, microinjection of BQ-788 (5 nmol) did not affect the hypertensive response to NMDA (-9.4%, $P>0.05$). Administration of 2-APV (5 nmol) into the PAG abolished the pressor response to NMDA (data not shown). Angiotensin II (0.1-10 nmol) increased the blood pressure in a dose-dependent manner when injected into the PAG (Figure 1b). These responses were unaffected by treatment with FR 139317, SB 209670 or BQ-788 (Figure 2b). In additional experiments, increases in blood pressure induced by ET-1 injected into the PAG were greatly reduced by FR 139317 or SB 209670, but were unaffected by BQ-788 (data not shown). Injected into the PAG, BQ-788, FR 139317 or SB 209670 had no effects on the rat blood pressure.

Discussion Here we show that the endothelin receptor antagonist FR 139317 and SB 209670 reduce the increase in blood pressure induced by the injection of NMDA to the PAG. Conversely, BQ-788 does not cause any reduction in the pressor effects of NMDA, suggesting no involvement of ET_B receptors. Thus, our data suggest that in the PAG en-

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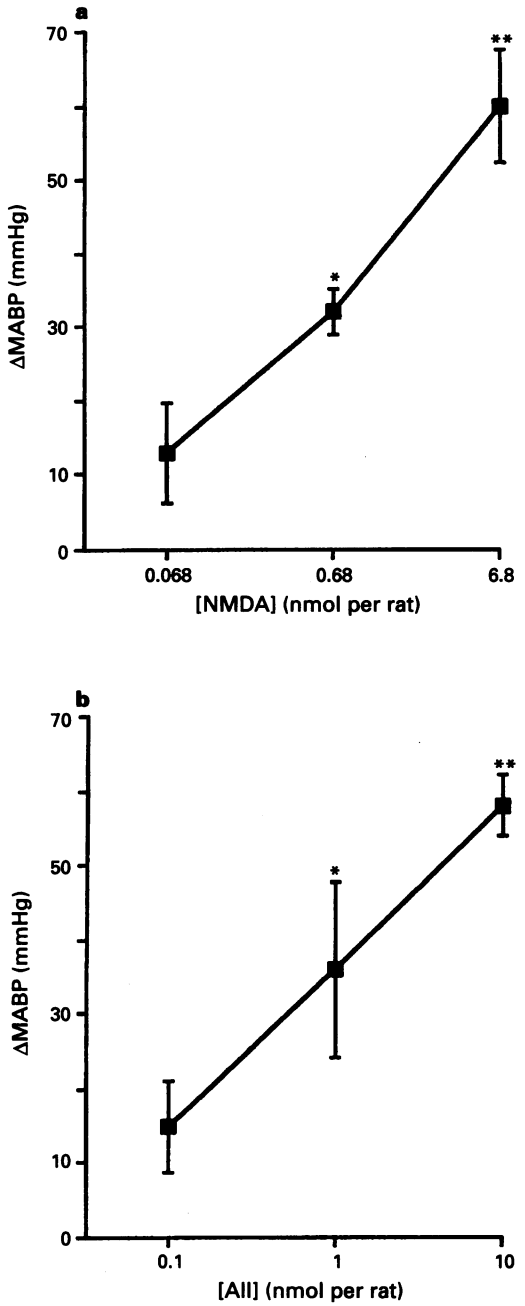

Figure 1 (a) Changes in mean arterial blood pressure (ΔMABP) (mmHg ± s.e.) after microinjection of N-methyl-D-aspartate (NMDA, 0.068–6.8 nmol) or (b) angiotensin II (AII, 0.1–10 nmol) into the dorsolateral periaqueductal gray (PAG) area of rat. Each point represents the mean of 7 observations ± s.e. Significant differences from vehicle-treated animals are shown by asterisks (*$P<0.05$ and **$P<0.01$).

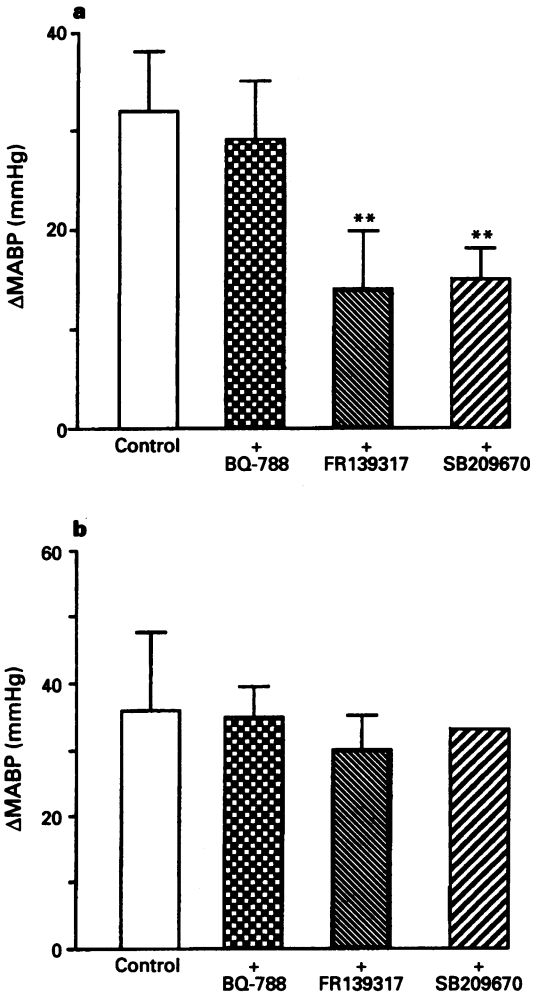

Figure 2 (a) Changes in mean arterial blood pressure (ΔMABP) (mmHg ± s.e.) after microinjection of N-methyl-D-aspartate (NMDA, 0.68 nmol) into the dorsolateral periaqueductal gray (PAG) area of rats treated, 10 min before, with BQ-788 (5 nmol), FR139317 (5 nmol) or SB 209670 (5 nmol). Each point represents the mean of 7 observations ± s.e. Significant differences from groups treated with NMDA are shown as **$P<0.01$. (b) Changes in mean arterial blood pressure (ΔMABP) (mmHg ± s.e.) after microinjection of angiotensin II (1 nmol) in the absence or presence of antagonists. Each point represents the mean of 5 observations ± s.e.

dogenously produced ET-1 modulates the pressor effects of NMDA, probably via an action on ET_A receptors. This is most likely a selective influence on the glutamatergic system, for increases in blood pressure caused by injection of angiotensin II were unaffected by any of the endothelin receptor antagonists. It is also most unlikely that this interaction is due to an action of NMDA at endothelin receptors for the pressor response to NMDA was abolished by 2-APV.

It is not clear where the ET_A receptors that modulate the responses to NMDA are sited. However, it is tempting to speculate that ET-1 may interact with ET_A receptors present on postsynaptic membranes also expressing NMDA receptors. This interaction could well promote cell excitability and so increase responsiveness to both endogenous and exogenous NMDA. Indeed, ET-1 may well be a signalling molecule that, following activation of NMDA receptors, promotes cell hyperstimulation, through changes in the membrane's ionic conductance (Gross *et al.*, 1993). In conclusion, our data suggest that via an action on ET_A receptors endogenous ET-1 modulates the pressor effects of NMDA within the brain.

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References

D'AMICO, M., BERRINO, L., MAIONE, S., FILIPPELLI, A., PIZZIRUSSO, A., VITAGLIANO, S. & ROSSI, F. (1995). Endothelin-1 in rat periaqueductal gray area induces hypertension via glutamatergic receptors. *Hypertension*, **25**, 507–510.

GROSS, P.M., BENINGER, R.J., SHAVER, S.W., WAINMAN, D.S., ESPINOSA, F.J. & WEAVER, D.F. (1993). Metabolic and neuroanatomical correlates of barrel-rolling and oculoclonic convulsions induced by intraventricular endothelin-1: a novel peptidergic signaling mechanism in visuovestibular and oculomotor regulation? *Exp. Brain Res.*, **95**, 397–408.

PAXINOS, G. & WATSON, C. (1986). *The Rat Brain in the Stereotaxic Coordinates*, 2nd edn. San Diego: Academic Press.

RUBANYI, G.M. & POLOKOFF, M.A. (1994). Endothelins: molecular biology, biochemistry, pharmacology, physiology and pathophysiology. *Pharmacol. Rev.*, **46**, 325–415.

TALLARIDA, R.J. & MURRAY, R.B. (1987). *Manual of Pharmacologic Calculations with Computer Programs*. New York: Springer-Verlag

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