ELSEVIER

European Journal of Pharmacology 328 (1997) 65–67

ejp

Short communication

Regulation of blood pressure by L-arginine-nitric oxide pathway within the superior colliculus of rats

Michele D'Amico [a,b,*], Francesco Rossi [a], Timothy D. Warner [b]

[a] *Institute of Pharmacology and Toxicology, 2nd University of Naples, Via Costantinopoli 16, I-80138 Naples, Italy*
[b] *The William Harvey Research Institute, Charterhouse Square, London, UK*

Received 11 February 1997; revised 7 April 1997; accepted 11 April 1997

Abstract

Injection into the superior colliculus of anaesthetised rats of the nitric oxide (NO) synthase inhibitor N^{ω}-nitro-L-arginine methyl ester (L-NAME; 1 μmol), but not its inactive enantiomer N^{ω}-nitro-D-arginine methyl ester (D-NAME; 1 μmol), significantly ($P < 0.01$) increased the mean arterial blood pressure. Injection to the superior colliculus of L-arginine (L-Arg; 1 μmol), the substrate for NO synthase, significantly ($P < 0.01$) lessened the pressor effect of L-NAME, while D-arginine (D-Arg; 1 μmol) did not affect it. L-Arg (7.5 μmol), but not D-Arg (7.5 μmol) administered at the peak of the pressor response to L-NAME (1 μmol) also partially reversed this pressor response ($P < 0.05$). These data would suggest that endogenously produced NO acts within the superior colliculus to modulate the arterial blood pressure.

Keywords: N^{ω}-Nitro-L-arginine methyl ester (L-NAME); L-Arginine; Superior colliculus; Blood pressure

1. Introduction

Immunohistochemical studies have demonstrated that the enzyme nitric oxide (NO) synthase which leads to production of NO is located discretely throughout the midbrain of rats, and notably in the superior colliculus (Bredt et al., 1990; Föstermann et al., 1990). NO formed in the brain participates to the central regulation of arterial blood pressure by reducing vascular tone (Togashi et al., 1992). 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 or inhibiting the synthesis of NO within the superior colliculus. Therefore, we have investigated the effects of microinjection into the superficial layer of the superior colliculus of anaesthetised rats of N^{ω}-nitro-L-arginine methyl ester (L-NAME), an L-arginine (L-Arg) analogue which is an inhibitor of NO synthase, and L-arginine, the substrate for NO synthase. Both agents were used in single dose(s) which are efficacious within the brain (D'Amico et al., 1994).

2. Materials and methods

Male Wistar rats (230–260 g) were anaesthetised with urethane ethyl carbamate (1.2 g/kg 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 1 μl syringe. 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

In a first series of experiments, after a 30 min stabilisation period D-NAME (1 μmol) was injected into the superior colliculus, as a control. 10–15 min later L-NAME (1 μmol) was microinjected followed by D-Arg (7.5 μmol) at the sustained portion to L-NAME. A further 10–15 min later L-Arg (7.5 μmol) was microinjected.

* Corresponding author at address a. Tel.: (39-81) 566-5877/566-5878; Fax: (39-81) 566-5877.

In a second series of experiments, after a 30 min stabilisation period D-NAME (1 μmol) was again microinjected into the superior colliculus as a control, followed 10–15 min later by microinjection of either L-arginine or D-arginine (1 μmol). L-NAME (1 μmol) was injected 10–15 min after the injection of L-Arg. Each intracerebral injection was given in a total volume of 200 nl over a period of 5 s. After 5 experiments the positioning of the injection site was checked histologically. The following drugs were used: D-arginine, L-arginine, D-NAME (N^{ω}-nitro-D-arginine methyl ester) and L-NAME (N^{ω}-nitro-L-arginine methyl ester) purchased from Sigma Chemical Co. (Poole, Dorset, UK). All drugs were dissolved in saline. 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 (S.E.), with $P < 0.05$ being taken as significant. Cardiovascular changes were compared by analysis of variance (ANOVA) and Newman-Keuls test for multiple comparisons (Tallarida and Murray, 1987).

3. Results

The basal mean arterial pressure of the rats was 97 ± 4 mmHg ($n = 6$). This was increased by injection of L-NAME (1 μmol; 33 ± 2%, $P < 0.01$; Fig. 2A) but not by D-NAME (1 μmol) into the superior colliculus (Fig. 1A). L-Arg (7.5 μmol), microinjected into the superior colliculus during the sustained phase of the pressor response to L-NAME (1 μmol) significantly (51 ± 7%, $P < 0.05$; Fig. 2A) lowered the blood pressure (Fig. 1B). In contrast, D-Arg (7.5 μmol) did not affect the pressor response to L-NAME (Fig. 1A and Fig. 2A). Similarly, treatment of the superior colliculus with L-Arg (1 μmol), but not with D-Arg (1 μmol), before L-NAME significantly ($P < 0.01$) lessened the increase in blood pressure induced by L-NAME (Fig. 1C and Fig. 2B). Intravenous injection of the same dose of L-NAME did not alter blood pressure (data not shown). Finally, the injection of L-NAME into the superior colliculus caused a reduction (−15 ± 2%) of the basal values (484 ± 17 bpm, $P > 0.05$) of heart rate.

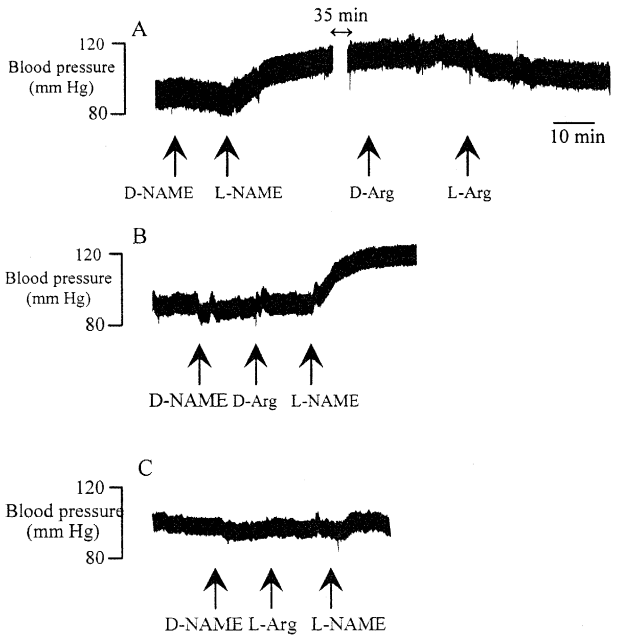

Fig. 1. Original traces of changes in arterial blood pressure induced by microinjection of (A) N^{ω}-nitro-D-arginine methyl ester (D-NAME; 1 μmol), N^{ω}-nitro-L-arginine methyl ester (L-NAME; 1 μmol), D-arginine (D-Arg; 7.5 μmol) or L-arginine (L-Arg; 7.5 μmol), (B) D-NAME (1 μmol), D-Arg (1 μmol) or L-NAME (1 μmol), (C) D-NAME, L-Arg or L-NAME (doses as in B) to the superficial layer of the superior colliculus of anaesthetised rats.

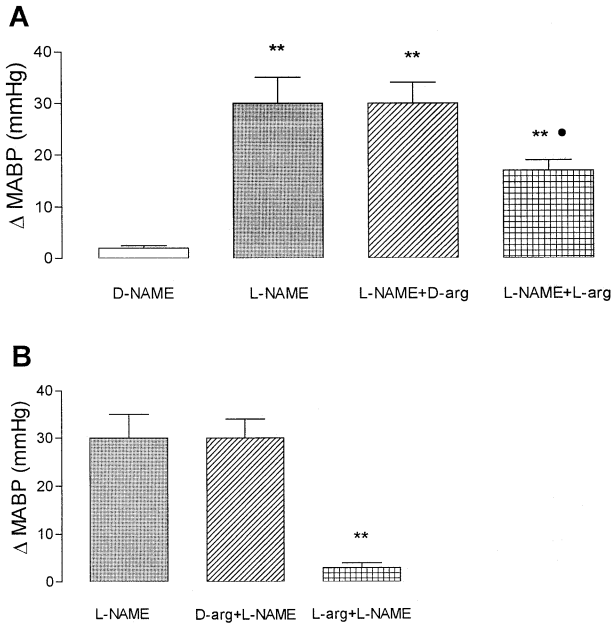

Fig. 2. (A) Changes in mean arterial blood pressure (ΔMABP, mmHg) after subsequent microinjections of N^{ω}-nitro-D-arginine methyl ester (D-NAME; 1 μmol), N^{ω}-nitro-L-arginine methyl ester (L-NAME; 1 μmol), L-NAME + D-arginine (D-Arg; 7.5 μmol) or L-NAME + L-arginine (L-Arg; 7.5 μmol) into the superficial layer of the superior colliculus of the rat. Each column represents the mean of four observations ± S.E. Significant differences to vehicle-treated animals are shown by asterisks ($^{**}P < 0.01$) while differences to L-NAME are shown as $^{*}P < 0.05$. (B) ΔMABP (mmHg) after microinjection of L-NAME (1 μmol) into the superior colliculus with or without preinjection of either D-Arg (1 μmol) or L-Arg (1 μmol). Each column represents the mean of four observations ± S.E. Significant differences to L-NAME are shown as $^{**}P < 0.01$.

4. Discussion

Our results show that the L-arginine-NO pathway plays a role in the regulation of blood pressure at the level of the superior colliculus, for injection of L-NAME increases blood pressure, an effect which is lessened, or reversed, by L-arginine. This cannot be due to a systemic effect, for

intravenous injection of the same dose of L-NAME did not alter blood pressure. The response we observed is in accordance with our previous studies done in the periaqueductal gray area (D'Amico et al., 1994). However, currently it is not clear through which pathways the effects of NO on blood pressure are exerted. One would speculate that in agreement with previous reports, NO acts within the superior colliculus to modulate sympathetic outflow (Sakuma et al., 1992; Togashi et al., 1992). Interestingly, pretreatment of the superior colliculus with L-Arg, a precursor of NO, largely prevented the pressor effect of L-NAME, suggesting that in the superior colliculus the activity of NO synthase is not saturated by endogenous levels of L-arginine.

Therefore, we would suggest that our functional data, together with earlier histochemical and hybridisation studies (Bredt et al., 1990; Föstermann et al., 1990), show that endogenously produced NO acts within the superior colliculus to modulate the arterial blood pressure.

Acknowledgements

This study was supported by CNR, Italy and the British Heart Foundation. T.D.W. holds a British Heart Foundation lectureship (BS/95003).

References

Bredt, D.S., Hwang, P.M., Snyder, S.H., 1990. Localization of nitric oxide synthase indicating a neural role for nitric oxide. Nature 347, 768–770.

D'Amico, M., Berrino, L., Filippelli, A., Maione, S., Rossi, F., 1994. Relationship between L-arginine-NO pathway and endothelin-1 effect in the periaqueductal gray area of the rat. J. Cardiovasc. Pharmacol. 24, 974–978.

Föstermann, U., Soroky, L.D., Pullock, J.S., Schimidt, H.H.H.W., Heller, M., Murad, F., 1990. Regional distribution of EDRF/NO-synthesizing enzyme(s) in rat brain. Biochem. Biophys. Res. Commun. 168, 727–732.

Keay, K.A., Redgrave, P., Dean, P., 1988. Cardiovascular and respiratory changes elicited by stimulation of rat superior colliculus. Brain Res. Bull. 20, 13–26.

Paxinos, G., Watson, C., 1986. The Rat Brain in the Stereotaxic Coordinates, 2nd ed. Academic Press, San Diego, CA.

Sakuma, I., Togashi, H., Yoshioka, M., Saito, H., Yanagida, M., Tamura, M., Kobayashi, T., Yasuda, H., Gross, S.S., Levi, R., 1992. N^G-Methyl-L-arginine, an inhibitor of L-arginine-derived nitric oxide synthesis stimulates renal sympathetic nerve activity in vivo. Circ. Res. 70, 607–611.

Tallarida, R.J., Murray, R.B., 1987. Manual of Pharmacologic Calculations with Computer Programs. Springer-Verlag, New York, NY.

Togashi, H., Sakuma, I., Yoshioka, M., Kobayashi, T., Yasuda, H., Kitabatake, A., Saito, H., Gross, S.S., Levi, R., 1992. A central nervous system action of nitric oxide in blood pressure regulation. J. Pharmacol. Exp. Ther. 262, 343–347.