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RAPID COMMUNICATION

Inhibition of NO Synthase in the Posterior Hypothalamus Increases Blood Pressure in the Rat

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Summary

Decrease of nitric oxide level in the posterior hypothalamus induced by inhibition of NO synthase elevates blood pressure in the rat.

Key words

Nitric oxide – Nitric oxide synthase – Hypothalamus – Hypertension

A new model of hypertension, named according to Dananberg *et al.* (1993) "nitric oxide (NO) deficient hypertension", has been developed by chronic inhibition of nitric oxide synthase (NOS) and/or production of NO from arginine in endothelial cells (Ribeiro *et al.* 1992, Manning *et al.* 1993, Bernátová and Pecháňová 1994). As a consequence of NOS blockade, the pathway: NO activation of guanylate cyclase – increase of cGMP – relaxation of smooth muscles in vessel walls, is compromised and the tone of smooth muscles increases.

The nitric oxide-cGMP relation in central nervous system was already described by Miki *et al.* (1977) and by Deguchi and Yoshimoka (1982) who brought forward experimental data on the involvement of arginine in the above metabolic pathway. Rodrigo *et al.* (1994) and Egberongbe *et al.* (1994) provided a map of NO synthase localization, an enzyme crucial for nitric oxide production, in individual areas of the rat and human brain. NOS was also described in the autonomic nervous system (Toda and Okamura 1992). The question arises how does the level of NO in central nervous system areas, relevant for cardiovascular control, affect the blood pressure. Shapoval *et al.* (1991) demonstrated that alteration of NO level in rostral and/or caudal ventrolateral medulla induced immediate changes in blood pressure. El Karib *et al.* (1993) elicited a blood pressure increase after the infusion of NOS-inhibitor into the brain-ventricle system. Horn *et al.* (1994) described a small blood pressure decrease after infusion of sodium nitroprusside into the paraventricular nucleus.

Hypothalamus has been generally accepted as the area involved in cardiovascular control (Folkow and Neil 1971). Moreover, Rodrigo *et al.* (1994) found an abundant amount of NOS in this area. The aim of our study was to block the NOS activity in the posterior hypothalamus and to follow the blood pressure changes.

Experiments were performed on male Wistar rats (300 g b.w.). Pentobarbital (50 mg/kg, i.p.) was used for anaesthesia. The tail artery was prepared, cannulated and connected to a Stattham pressure transducer for blood pressure recording. The animals were placed in a stereotaxic apparatus and a small hole, about 1 mm in diameter, was made in the skull. Stereotaxic coordinates were used according to Paxinos and Watson (1982): 3.8 mm posterior of bregma, 0.4 mm lateral (left). A micropipette (about 150 μm in diameter) was inserted in the trephine opening, 8 mm vertically into the posterior hypothalamus area. Nitro-L-arginine methyl ester (L-NAME, Sigma) in a dose 0.3 mg/100 g b.w. (i.e. a total of 0.9 mg/per animal), diluted in 3 μl of ACSF was applied into the posterior hypothalamus *via* the micropipette. Blood pressure was monitored continuously before, during and at least 30

min after application. In three of the seven experimental animals the same dose was repeated after 30 minutes.

In seven control animals, prepared in the same way as the experimental ones, 3 μl ACSF, without L-NAME, was applied into the posterior hypothalamus.

The data expressed as means ± S.E.M. are compared using Student's t-test for unpaired observations. Probability values for $p<0.05$ were considered as significant.

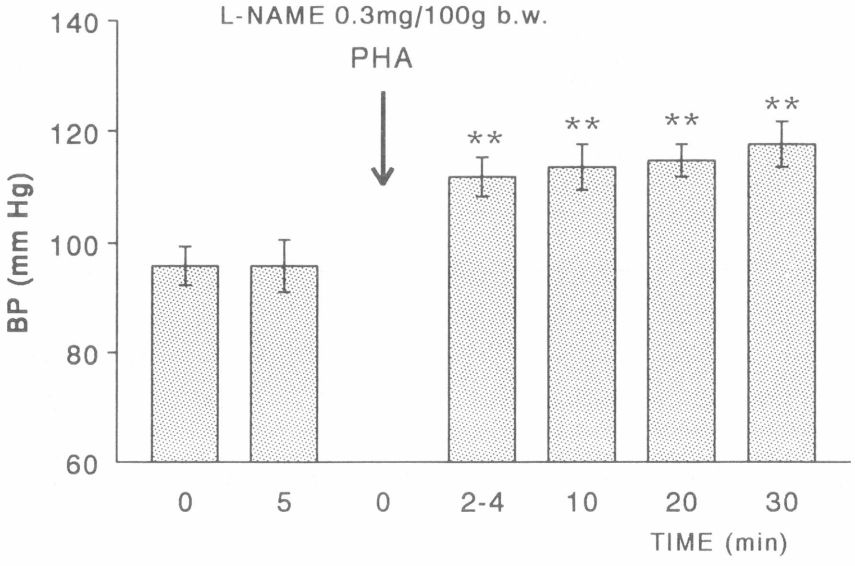

Fig. 1
Mean blood pressure before and after inhibition of NO synthase in the posterior hypothalamus. L-NAME was applied in a dose 0.3 mg/100 g b.w. diluted in 3 μl ACSF. ** $p<0.01$

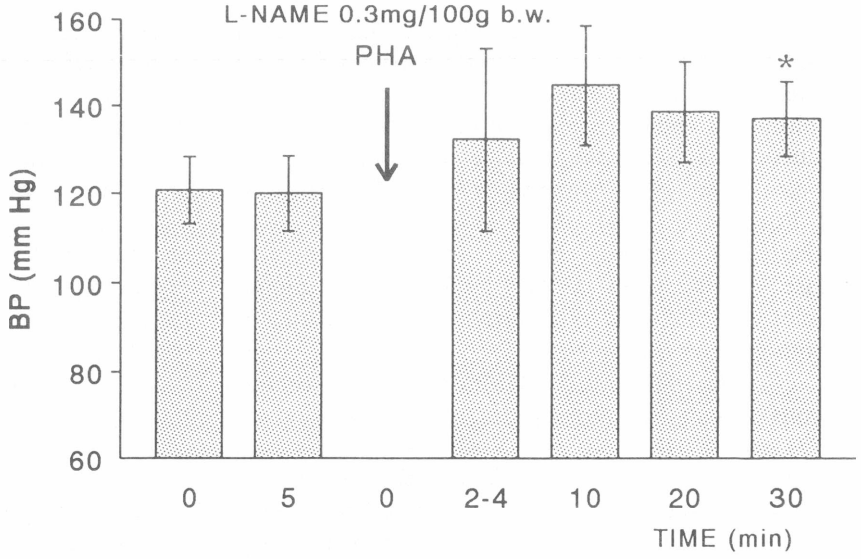

Fig. 2
Mean blood pressure before and after an additional dose L-NAME (0.3 μg/100g b.w.) had been administered into the posterior hypothalamus. The additional dose was applied 30 min after the first application during a sustained increase of blood pressure. * $p<0.05$

Fig. 1 represents blood pressure values from the experimental animals. In all seven animals, after administering NO synthase inhibitor L-NAME into the posterior hypothalamus, blood pressure increased significantly by at least 20 %, and the increase was sustained for 30 min or longer.

In 3 animals the additional dose was applied during the sustained blood pressure increase, i.e. 30 min after the first application. This induced an additional significant blood pressure increase as is demonstrated in Fig. 2.

Fig. 3 represents control experiments. 3 μl of ACSF applied into the posterior hypothalamus did not affect the blood pressure significantly.

The experiments have shown that NO levels in the posterior hypothalamus, affected by inhibition of NO synthase, are relevant for blood pressure regulation. A decrease of NO level induced blood pressure increase.

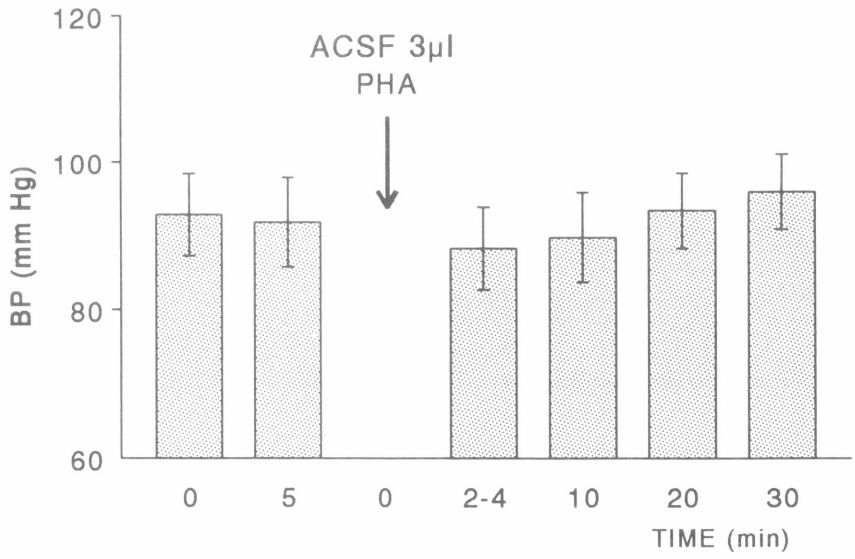

Fig. 3
Mean blood pressure of control rats before and after 3 μl ACSF (without L-NAME) had been applied into the posterior hypothalamus.

When comparing the experiments of Shapoval *et al.* (1991) on the rostral ventrolateral medulla with the present experiments, a substantial difference should be noted, namely the time course of blood pressure changes. Alterations of NO levels in the rostral ventrolateral medulla caused changes in blood pressure which occurred within seconds and returned to the steady state value in about 60 s. On the contrary, the alteration of NO levels in the posterior hypothalamus increased blood pressure in 2–4 min, and its elevation lasted for 30 min or longer. This suggests the involvement neurohumoral mechanisms, triggered in posterior hypothalamus. The analysis of the factors as well as the haemodynamic pattern of this type of hypertension should by addressed in future experiments.

In conclusion, the decrease in NO levels in the posterior hypothalamus after inhibition of NO synthase induces an increase in blood pressure.

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