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Modulation by glycine on vascular effects of NMDA: *in vivo* experimental research

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Summary. The present study has been carried out to determine if glycine, an allosteric modulator of NMDA receptor, is involved in the vascular effect induced by the activation of the CNS NMDA receptors. *Icv* NMDA (from 0.01 to 1 μg/rat in the 3rd ventricle) caused a significant increase in arterial blood pressure in conscious freely moving rats. Moreover, the hypertension was associated with behavioural modifications (jumping, rearing, teething and running). Glycine pretreatment (1 and 10 μg/rat *icv*), significantly increased the NMDA hypertension. Glycine alone did not cause any arterial blood pressure modification while it induced a slight sedation. HA-966 (an antagonist of the glycine site on NMDA receptor) administration (1–10 μg/rat *icv* 5 min before glycine) significantly antagonized the glycine effects on NMDA hypertension.

Alone HA-966 neither modified arterial blood pressure nor antagonized NMDA hypertension. In conclusion, our investigations confirm NMDA receptor involvement in cardiovascular function and they demonstrate that *in vivo* glycine positively modulates NMDA receptors.

Keywords: Amino acids – N-Methyl-D-aspartate (NMDA) – Glycine – HA-966 – Rat – Strychnine

Introduction

Our previous investigations have shown the excitatory amino acid (EAA) effects on the cardiovascular system and both physiological and pathological action have been seen. NMDA and KA receptors play a primary role in the cardiovascular effects exerted by EAA (Lampa et al., 1988b; Maione et al., 1992); in conscious rats intracerebroventricular administration of EAA produced hypertension and tachycardia associated with behavioural modifications (Berrino et al., 1990); in anaesthetized rats glutamate, NMDA and kainate when administered intracerebroventricularly produce hypertension and bradycardia (Lampa

et al., 1988a). It has been shown in neuropharmacological studies that glycine greatly potentiates NMDA effects acting on the glycine modulator site of NMDA receptor (Ascher and Nowak, 1987; Henderson et al., 1990; Kaplita and Ferkany, 1990). Recent evidence showed that glycine also inhibited excitatory responses to kainate, quisqualate and NMDA in the rat thalamus *in vivo* (Salt, 1989). The aim of the present investigation has been to determine if the allosteric modulation of glycine on NMDA receptor is also involved in the cardiovascular effects induced by the activation of NMDA receptor *in vivo*.

Material and methods

Subjects

Research has been carried out on conscious freely moving male Sprague Dawley rats (220–240 g).

Surgical preparation and treatment

Arterial blood pressure was measured continuously by a Statham pressure transducer, introduced by catheter into the femoral artery and connected to a Hellige polygraph (mean basal values: 112 ± 8 mm Hg). The direct application of drugs intracerebroventricularly (*icv*) was done by a guide cannula implanted into the 3rd ventricle under ketamine anaesthesia (100 mg/Kg *ip*) 2 days before experimentation, using a stereotaxic apparatus and applying the coordinates of the atlas of Paxinos and Watson (1986). Microinjections were made with a Hamilton micro-syringe, in a volume of 1 μl. Control injections were carried out with the same volume of the solvent (0.2 M phosphate buffer pH 6.5 adjusted to 7.2 with 12 mM NaOH) used to dissolve the drugs. *Icv* (3rd ventricle) NMDA from 0.01 to 1 μg/rat was delivered in 5 sec. Glycine (1 and 10 μg/rat) was delivered in 5 sec (in the 3rd ventricle) 7 min before NMDA. HA-966 (1 and 10 μg/rat in the 3rd ventricle 5 min before glycine) was delivered in 5 sec. Afterwards, the stereotaxic coordinates of the cannula were checked histologically.

Brain tissue was taken out fresh and immersed in buffered formaline for 2 min; the injection site was verified using 2 consecutive sections, one stained with cresyl violet to identify the areas and the other unstained to determine the diffusion and position of the dye.

Drugs

The following drugs were used: NMDA (N-methyl-D-aspartate), glycine and diethylether (Sigma Chemical Co. St. Louis MO, USA), ketamine hydrochloride (Parke-Davis S.p.A., Lainate – Milan, Italy); HA-966 (RBI, Natick, USA); NMDA, glycine and HA-966 [R(+)-3-amino-1-hydroxy-2-pyrrolidinone] were solubilized in 0.2 M phosphate buffer (pH 6.5) and the pH of the solution was adjusted to 7.2 with 12 mM NaOH.

Statistics

All results are expressed as mean ± standard error (S.E.) with $p < 0.05$ considered as the level of significance; cardiovascular changes were compared by a paired or unpaired Student's *t*-test (Burn et al., 1952; Snedecor and Cochran, 1978)

Results

Icv NMDA (from 0.01 to 1 μg/rat 3rd ventricle) determined a significant and dose-dependent increase in arterial blood pressure (Fig. 1 and 2) in freely moving

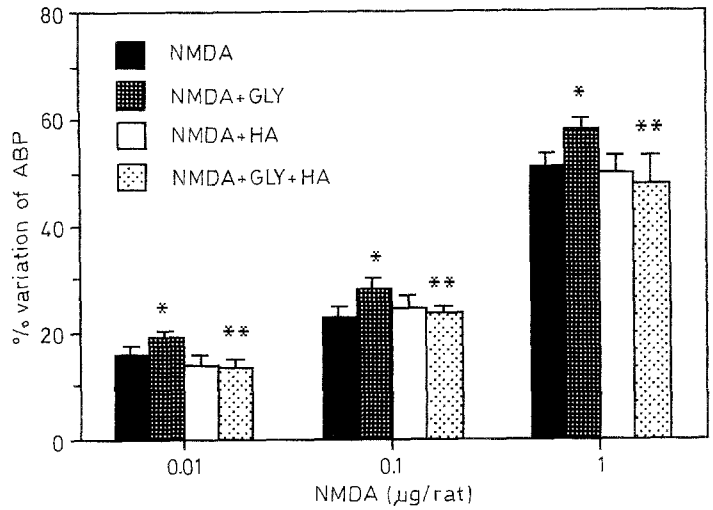

Fig. 1. Percentage variation of arterial blood pressure (*ABP*) after NMDA microinjections (from 0.01 to 1 μg/rat) in the 3rd ventricle (*icv*) in freely moving rats pretreated with glycine (*GLY*) (1 μg/rat *icv*) and/or HA-966 (*HA*) (1 μg/rat *icv*). Experimental groups of 5 animals were used. Significant differences are shown by asterisks (* $p < 0.05$ vs. NMDA; ** $p < 0.05$ vs. NMDA + GLY) determined by Student's *t*-test

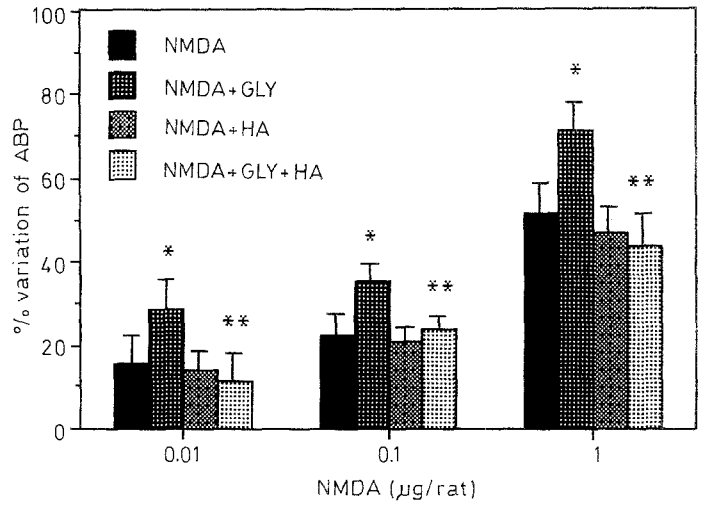

Fig. 2. Percentage variation of arterial blood pressure (*ABP*) after NMDA microinjections (from 0.01 to 1 μg/rat) in the 3rd ventricle (*icv*) in freely moving rats pretreated with glycine (*GLY*) (10 μg/rat *icv*) and/or HA-966 (*HA*) (10 μg/rat *icv*). Experimental groups of 5 animals were used. Significant differences are shown by asterisks (* $p < 0.05$ vs. NMDA; ** $p < 0.05$ vs. NMDA + GLY) determined by Student's *t*-test

rats. The pressor effect was associated, only at the highest dose, with behavioural modifications (jumping, rearing, teething and running). The glycine pretreatment (1 and 10 μg/rat 3rd ventricle), significantly and in a dose-dependent manner, increased the NMDA hypertension (Fig. 1 and 2); moreover it slightly reduces the NMDA behavioural effects in freely moving rats. Glycine caused no arterial blood pressure modifications, and induced a dose-dependent sedation.

HA-966 (an antagonist of the glycine site on NMDA receptor) administration (1 and 10 μg/rat *icv* 5 min before glycine), significantly and in dose-dependent manner, antagonized glycine effects on NMDA hypertension. Alone HA-966 neither modified arterial blood pressure and behaviour, nor antagonized NMDA hypertension (Fig. 1 and 2).

Control injections, carried out with the same volume of the solvent used to dissolved the drugs, produced not significant changes in arterial blood pressure and behaviour.

Discussion

Glycine is the main inhibitory neurotransmitter in the posterior regions of the brain and spinal cord, where its strychnine-sensitive receptors appear to be largely concentrated (Young and Snyder, 1973). However, neurochemical and neurophysiological studies on the glycine in the forebrain have yielded new data on glycine's role (Raiteri et al., 1990; Vamvakides, 1989; White et al., 1989). In fact, in rat hippocampus Pittaluga et al. (1990) and Raiteri et al. (1990) found both strychnine-inhibited and non-inhibited glycine-modulated NMDA receptors. Moreover, Schmidt et al. (1990) also found non-competitively inhibited glycine receptors to strychnine in rat hippocampus. Our results confirm that the NMDA-receptors present in the nuclei near the 3rd ventricle as the antero-dorsal medial hypothalamus and the ventromedial hypothalamic area are involved in the cardiovascular function and that they are positively modulated *in vivo* by glycine. In fact, NMDA induced an increase in arterial blood pressure that was potentiated by glycine pretreatment. The glycine effect is due to its specific and direct activity on its NMDA recognition site because it was antagonized by HA-966, a selective antagonist of the glycine site on NMDA receptor. These findings agree with recent evidence, showing the double role of the amino acid glycine in supraspinal regions of the central nervous system, acting both at the classical strychnine-sensitive receptors and at the site coupled to the NMDA receptor complex (Raiteri et al., 1990). On the contrary, the behavioural effects induced by NMDA were reduced after the glycine pretreatment. These results are in agreement with those of Salt et al. (1989) who demonstrated that glycine blocks excitatory responses in rat thalamus *in vivo*. Moreover, our data differ from the findings of Koek et al. (1990) since the glycine antagonist HA-966 blocked NMDA-induced convulsions in *icv* administered mice. They are also in contrast with Toth and Lajtha (1989) in that the motor effects of the NMDA injection into the rat striatum were not blocked by glycine. Our results suggest that the well known inhibitory nature of glycine reduced the NMDA elicited: jumping, rearing, teething and running and that NMDA receptors, positively modulated by glycine, are present in parts of the hypothalamus which regulate the cardiovascular apparatus. *In vivo* modifications on the effects of NMDA induced by glycine suggest that this may have different action sites which modulate the cardiovascular and behavioural effects of NMDA. In conclusion, glycine could both activate the classical strychnine-sensitive receptors and the strychnine-resistent site coupled to the NMDA receptor complex, the former

inhibiting the excitatory behaviour, the latter specifically potentiating glutamatergic transmission mediated by NMDA subtype receptors.

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