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Metabotropic and ionotropic glutamate receptors mediate opposite effects on periaqueductal gray matter

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Abstract

Microinjections, into the dorso-lateral periaqueductal gray matter, of *N*-methyl-D-aspartic acid (NMDA, 0.07–7 nmol/rat) significantly ($P < 0.01$) increased arterial blood pressure in a dose-related manner. Pretreatment, 5 min before NMDA (7 nmol/rat), in the same area with 2-amino-5-phosphonovaleric acid (2-APV, 5 nmol/rat), a selective antagonist of NMDA receptors, significantly ($P < 0.01$) reduced NMDA-induced arterial hypertension. *trans*-(±)-1-Amino-1,3-cyclopentanedicarboxylic acid (*t*-ACPD, 6–30 nmol/rat), an agonist of metabotropic glutamate receptors (mGlu receptors), significantly ($P < 0.01$) decreased arterial blood pressure when microinjected into the dorsal-lateral periaqueductal gray matter. Pretreatment, 5 min before *t*-ACPD (30 nmol/rat), in the same area with L-2-amino-3-phosphono-propionate (L-AP-3, 30 nmol/rat), a putative antagonist of the mGlu receptors, was not able to prevent *t*-ACPD-induced hypotension. Microinjections of L-AP-3 (30 nmol/rat) induced a hypotension similar to the one obtained with *t*-ACPD at the dose of 6 nmol/rat. From these data we can suggest that mGlu receptors act inversely to the NMDA receptors in the dorso-lateral periaqueductal gray area and that L-AP-3 is a partial agonist rather than an antagonist of mGlu receptors within the periaqueductal gray area.

Keywords: NMDA receptor; mGlu receptor; NMDA (*N*-methyl-D-asparate); *t*-ACPD (*trans*-(±)-1-amino-1,3-cyclopentanedicarboxylic acid); L-AP-3 (L-2-amino-3-phosphono-propionate); Periaqueductal gray matter

1. Introduction

Periaqueductal gray matter is defined as being made up of neural cell columns projecting along the aqueduct in a rostrocaudal direction (Bandler et al., 1991). This midbrain area controls nociception (Jensen and Yaksh, 1992), defensive behaviour (Depaulis et al., 1989), and respiratory and cardiovascular functions (Lovick, 1992). Our previous study (Maione et al., 1992) demonstrated that NMDA glutamatergic receptors may modulate vasopressor neurons of the dorsolateral periaqueductal gray matter. Because glutamatergic receptors are divided into ionotropic (iGlu receptors) and metabotropic (mGlu receptors) (Collingridge and Lester, 1989; Schoepp and Conn, 1993), and mGlu receptors have been found in the periaqueductal gray area (Catania et al., 1994), the aim of this research was to study mGlu receptor involvement in the modulation of vasopressor neurons in the dorso-lateral periaqueductal gray area.

2. Materials and methods

2.1. Animals

Male Sprague-Dawley rats, weighing 250–300 g, were housed at constant temperature (21 ± 1°C) and relative humidity (60%), under a regular light/dark schedule (light 7.00–19.00 h). Food and water were freely available. Animal care was in compliance with Italian regulations on protection of animals used for experimental and other scientific purposes (D.M. 116/92), as well as with the EEC regulations (O.J. of E.C. L358/1 18/12/1986).

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2.2. Surgical procedure

On the day of the experiment, each animal was anaesthetised with urethane (1.2 g/kg i.p.). We chose this anaesthetic because its effect lasts for at least 2–3 h. We tested the level of anaethesia periodically (25–30 min) during the course of the experiment by touching the cornea with a spatula. The possible presence of the neuroreflex indicated the need for an additional amount of urethane. Polyethylene catheters (PE 50) were placed in a femoral artery for direct measurement of arterial blood pressure, with a pressure transducer 52-9966, and display on a Harvard Universal oscillograph 50-9323 (Harvard Apparatus, Edenbridge, Kent, UK). Immediately after surgery, each rat was placed on a homeothermic temperature control blanket (Harvard Apparatus, Edenbridge, Kent, UK), and its head was fixed into a stereotaxic frame (David Kopf Instruments, Tujunga, CA, USA) for the direct intracerebral administration of *N*-methyl-D-aspartate (NMDA, 0.07–7 nmol/rat), 2-amino-5-phosphonovaleric acid (2-APV, 5 nmol/rat), (±)-1-aminocyclopentane-1,3-dicarboxylic acid (*t*-ACPD, 6, 12 and 30 nmol/rat) and L-2-amino-3-phosphono-propionate (L-AP-3, 30 nmol/rat) into the dorso-lateral periaqueductal gray matter. The intracerebral microinjections were carried out with multibarrel glass micropipettes (FHC, Brunswick, ME, USA) with an outside tip diameter of 40–50 μm pulled with a vertical pipette puller (David Kopf Instruments, Tujunga, CA, USA). The coordinates of the atlas of Paxinos and Watson (1986) (measured in mm from bregma AP: −7.8; L: 0.5; V: 4.5) were used. A control volume of 200 nl of saline or the same volume of drug solution was injected over a period of 5 s. After each experiment, the stereotaxic coordinates of the injection sites were checked histologically. A volume of 200 nl of methylene blue (0.2%) was injected intracerebrally 5 min before killing the rat with an overdose of pentobarbital (200 mg/kg i.v.). The animal was perfused intra-cardially with 50 ml of phosphate buffer solution (PBS) followed by 50 ml of a 10% formalin solution in PBS. The brain was removed and immersed into saturated formalin solution for 2 days; the injection site was ascertained by using two consecutive sections (40 μm), one stained with cresyl violet to identify nuclei and the other one unstained to determine dye diffu-

Fig. 1. Photomicrograph of a coronal section of rat periaqueductal gray (PAG) matter showing localisation of the microinjection site (arrow). Stereotaxic coordinates (measured in mm from bregma: AP: −7.8; L: 0.5; V: 4.5), obtained from the atlas of Paxinos and Watson (1986), were applied. Scale bar = 300 μm.

sion. Only those rats whose microinjected site was located within the dorsal-lateral periaqueductal gray area (Fig. 1) were used for data computation.

2.3. Drugs

Drug solutions were freshly prepared, dissolved in saline, and filtered on the day of the experiment. The following drugs were used: *N*-methyl-D-aspartate (NMDA), DL-2-amino-5-phosphono valeric acid (2-APV), urethane (Sigma Chemical Co., St. Louis, MO, USA), (±)-1-aminocyclopentane-*trans*-1,3-dicarboxylic acid (*t*-ACPD) and L-2-amino-3-phosphono-propionate (L-AP-3) (RBI, Natick, USA).

2.4. Data analysis

The results are expressed as maximum blood pressure increase (mean ± standard error of the mean (S.E.M.)). Statistical analysis of the cardiovascular changes was performed by one-way analysis of variance (ANOVA) followed by a test for linear trend or the Student-Newman-Keuls test. Bartlett's test for homogeneity of variances was carried out before the ANOVA. $P < 0.05$ was considered as the level of significance.

3. Results

Injection of 200 nl saline into the dorso-lateral periaqueductal gray area did not modify basal systolic arterial blood pressure (95 ± 8 mm Hg). NMDA (0.07–7 nmol/rat) injected into the same area significantly ($P < 0.01$; slope = 15; $r^2 = 0.395$) increased arterial blood pressure in a dose-related manner (Fig. 2). Pretreatment, 5 min before NMDA (7 nmol/rat), in the same area with 2-APV (5 nmol/rat), a selective antagonist of NMDA receptors, significantly ($P < 0.01$) decreased NMDA-induced arterial hypertension (Fig. 2). 2-APV was not able per se to modify basal arterial blood pressure (Fig. 2). *t*-ACPD (6–30 nmol/rat), an agonist of metabotropic glutamate receptors (mGlu receptors), significantly ($P < 0.01$; slope = −2.6; $r^2 = 0.340$) decreased arterial blood pressure when microinjected into the dorso-lateral periaqueductal gray area (Fig. 3). Pretreatment, 5 min before *t*-ACPD (30 nmol/rat), in the same area with L-AP-3 (30 nmol/rat), a putative antagonist of the mGlu receptors, was not able to prevent *t*-ACPD-induced hypotension (Fig. 3). The microinjection of L-AP-3 (30 nmol/rat) alone caused a hypotension similar to the one obtained with *t*-ACPD at the dose of 6 nmol/rat (Fig. 3).

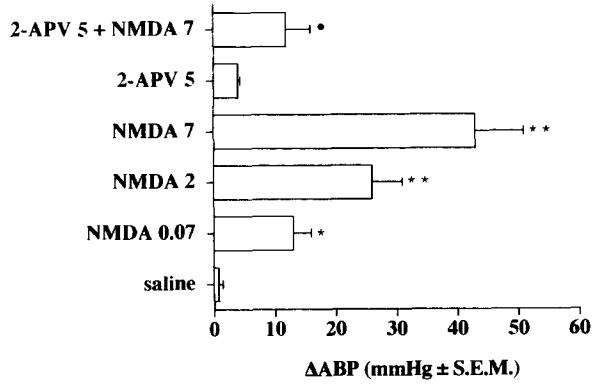

Fig. 2. Changes in arterial blood pressure (ΔABP) (mm Hg ± S.E.M.) induced by saline (200 nl) or NMDA (0.07, 2 and 7 nmol/rat). Pretreatment, 5 min before NMDA (7 nmol/rat) in the same area, with 2-APV (5 nmol/rat), a selective antagonist of NMDA receptors, decreased NMDA-induced arterial hypertension. The drugs were administered into the dorsal-lateral periaqueductal gray area of anaesthetised rats. Data are shown as the means ± S.E.M. ($n = 8$–10). Significant differences, determined by one-way analysis of variance (ANOVA) followed by a test for linear trend or Student-Newman-Keuls test: * $P < 0.05$ with respect to saline; ** $P < 0.01$ with respect to saline; • $P < 0.01$ with respect to NMDA 7 nmol.

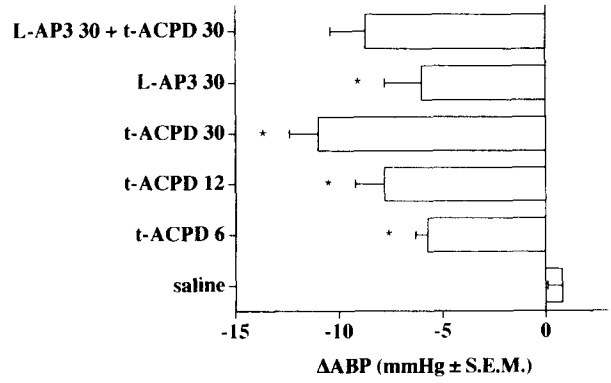

Fig. 3. Changes in arterial blood pressure (ΔABP) (mm Hg ± S.E.M.) induced by saline (200 nl) or *t*-ACPD (6, 12 and 30 nmol/rat). Pretreatment, 5 min before *t*-ACPD (30 nmol/rat), in the same area with L-AP-3 (30 nmol/rat), a putative antagonist of the mGlu receptors, did not decrease *t*-ACPD-induced arterial hypotension. The drugs were administered into the dorsal-lateral periaqueductal gray area of anaesthetised rats. Data are shown as the means ± S.E.M. ($n = 8$–10). Significant differences, determined by one-way analysis of variance (ANOVA) followed by a test for linear trend or Student-Newman-Keuls test: * $P < 0.01$ with respect to saline.

4. Discussion

The aim of this study was to evaluate the possible role of mGlu receptors in the control of vasopressor neurons within the dorso-lateral periaqueductal gray area. We have already demonstrated a significant hypertension induced by L-glutamate microinjections in this midbrain area secondary to activation of ionotropic NMDA subtype receptors (Maione et al., 1992). However, the current study showed that microinjection of

t-ACPD into the periaqueductal gray area induces a significant hypotension while NMDA induces an opposite effect. Recent molecular studies have established that the mGlu receptor family is a very heterogeneous class of glutamate receptors. Multiple types of mGlu receptors have been cloned (Schoepp and Conn, 1993) and it has been demonstrated that $mGlu_1$ and $mGlu_5$ receptor subtypes are coupled to phosphatidylinositol hydrolysis/Ca^{2+} signal transduction; $mGlu_2$, $mGlu_3$, $mGlu_4$ and $mGlu_6$ receptor subtypes appear to be coupled to inhibition of cAMP synthesis (Nakanishi, 1992). In fact, it has been reported that, in cerebellar granule cell neurons, $mGlu_2$ receptors and $mGlu_3$ receptors are able to inhibit L- and non-L-type Ca^{2+} channels via a pertussis toxin-sensitive G protein (Chavis et al., 1994).

In our case it could be possible that mGlu receptors are located presynaptically, where they produce presynaptic depression and block of glutamate release (the endogenous agonist) as recently demonstrated by Kemp et al. (1994). These authors showed that $mGlu_2$ receptor and/or $mGlu_3$ receptor agonists cause a presynaptically mediated depression of monosynaptic motoneuron excitation.

Alternatively, if $mGlu_1$ and $mGlu_5$ receptors (excitatory subtypes) are involved, a likely explanation could be that these receptors are located on the soma of a γ-aminobutyric acid (GABA)-ergic interneuron. The existence of such interneurons in the dorso-lateral periaqueductal gray area has recently been demonstrated in our laboratory (Maione et al., 1995). The activation of this interneuron blocks the pressor neurons in the dorso-lateral periaqueductal gray area.

Our results also show that L-AP-3 failed to modify the *t*-ACPD hypotensive effect. This result agrees with findings of our previous study (Vitagliano et al., 1994), that L-AP-3 did not antagonise *t*-ACPD-induced cardiorespiratory (apnoea and hypotension) effects in the nucleus of the solitary tract and subretrofacial nucleus. This effect has been also observed electrophysiologically by Lovinger et al. (1993) who demonstrated that the depression of population spike, or EPSP amplitude, induced by *t*-ACPD in rat neostriatum slices was not modified in the presence of the putative metabotropic receptor antagonist, L-AP-3 (1 mM).

Moreover, our results showed that L-AP-3 behaves as a partial agonist since microinjections of L-AP-3 mimic the *t*-ACPD-hypotensive effect. This is in agreement with other work carried out with the nucleus of the solitary tract and subretrofacial nucleus (Vitagliano et al., 1994). In conclusion, these preliminary data suggest that mGlu receptors act inversely to the NMDA receptors in the dorso-lateral periaqueductal gray area and that L-AP-3 is a partial agonist rather than an antagonist of mGlu receptors within the periaqueductal gray area.

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References

Bandler, R., P. Carrive and A. Depaulis, 1991, Emerging principles of organization of the midbrain periaqueductal gray matter, in: The Midbrain Periaqueductal Gray Matter, eds. A. Depaulis and R. Bandler (Plenum Press, New York) p. 1.

Catania, M.V., H. De Socarraz, J.B. Penney and A.B. Young, 1994, Metabotropic glutamate receptor heterogeneity in rat brain, Mol. Pharmacol. 45, 626.

Chavis, P., H. Shinozaki, J. Bockaert and L. Fagni, 1994, The metabotropic glutamate receptor types 2/3 inhibit L-type calcium channels via a pertussis toxin-sensitive G protein in cultured cerebellar granule cells, J. Neurosci. 14, 7067.

Collingridge, G.L. and A.J.R. Lester, 1989, Excitatory amino acid receptors in the vertebrate central nervous system, Pharmacol. Rev. 40, 145.

Depaulis, A., R. Bandler and M. Vergues, 1989, Characterization of pretentorial periaqueductal gray neurons mediating intraspecific defensive behaviour in the rat by microinjections of kainic acid, Brain Res. 486, 121.

Jensen, T.S. and T.L. Yaksh, 1992, The antinociceptive activity of excitatory amino acids in the rat brainstem: an anatomical and pharmacological analysis, Brain Res. 569, 255.

Kemp, M., P. Roberts, P. Pook, D. Jane, A. Jones, P. Jones, D. Sunter, P. Udvarhelyi and J. Watkins, 1994, Antagonism of presynaptically mediated depressant responses and cyclic AMP-coupled metabotropic glutamate receptors, Eur. J. Pharmacol. Mol. Pharmacol. 266, 187.

Lovick, T.A., 1992, Inhibitory modulation of the cardiovascular defence response by the ventrolateral periaqueductal gray matter in rats, Exp. Brain Res. 89, 133.

Lovinger, D.M., E. Tyler, S. Fidler and A. Merritt, 1993, Properties of a presynaptic metabotropic glutamate receptor in rat neostriatal slices, J. Neurophysiol. 69, 1236.

Maione, S., L. Berrino, S. Vitagliano, J. Leyva and F. Rossi, 1992, Interactive role of L-glutamate at the level of the periaqueductal gray area, for cardiovascular tone and stereotyped behaviour, Brain Res. 597, 166.

Maione, S., J. Leyva, M. Pallotta, L. Berrino, V. De Novellis and F. Rossi, 1995, Involvement of opioid receptors in *N*-methyl-D-aspartate-induced arterial hypertension in periaqueductal gray matter, Naunyn-Schmied. Arch. Pharmacol. 351, 87.

Nakanishi, S., 1992, Molecular diversity of glutamate receptors and implications for brain function, Science 258, 597.

Paxinos, G. and C. Watson, 1986, The Rat Brain in Stereotaxic Coordinates (Academic Press, San Diego).

Schoepp, D. and P.J. Conn, 1993, Metabotropic glutamate receptors in brain function and pathology, Trends Pharmacol. Sci. 14, 13.

Vitagliano, S., L. Berrino, A. Pizzirusso, M. D'Amico, V. Calderaro, S. Maione and F. Rossi, 1994, Metabotropic glutamate receptors are involved in the control of breathing at the medulla oblongata level of anaesthetized rats, Neuropharmacology 33, 859.