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Group I metabotropic glutamate receptors modulate glutamate and γ-aminobutyric acid release in the periaqueductal grey of rats

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Abstract

In this study, we investigated the effects of group I metabotropic glutamate (mglu) receptor ligands on glutamate and γ-aminobutyric acid (GABA) extracellular concentrations at the periaqueductal grey level by using in vivo microdialysis. An agonist of group I mglu receptors, (*S*)-3,5-dihydroxyphenylglycine [(*S*)-3,5-DHPG, 1 and 2 mM], as well as a selective agonist of $mglu_5$ receptors, (*RS*)-2-chloro-5-hydroxyphenylglycine (CHPG, 2 and 4 mM), both increased dialysate glutamate and GABA concentrations. 7-(Hydroxyimino)cyclopropa-[*b*]-chromen-1α-carboxylate ethyl ester (CPCCOEt, 1 mM), a selective $mglu_1$ receptor antagonist, and 2-methyl-6-(phenylethynyl)pyridine (MPEP, 0.5 mM), a selective $mglu_5$ receptor antagonist, perfused in combination with DHPG, antagonized the effect induced by DHPG on the extracellular glutamate and GABA concentrations. MPEP (0.5 mM), perfused in combination with CHPG, antagonized the increased glutamate and GABA extracellular levels induced by CHPG. MPEP (1 mM) decreased the extracellular concentrations of glutamate but did not modify the dialysate GABA concentrations. Moreover, as the intra-periaqueductal grey perfusion of (*RS*)-3-(2-carboxypiperazin-4-yl)-propyl-1-phosphonic acid [(*RS*)-CPP, 100 μM], a selective *N*-methyl-D-aspartate (NMDA) glutamate receptor antagonist, did not change the extracellular concentrations of glutamate, this suggests that the MPEP-induced decrease in glutamate is not a consequence of NMDA receptor blockade. These data show that group I mglu receptors in the periaqueductal grey may modulate the release of glutamate and GABA in awake, freely moving rats. In particular, $mglu_5$, but not $mglu_1$, receptors seem to be functionally active on glutamate terminals.

Keywords: mglu receptor; Amino acid neurotransmitter; Microdialysis; Periaqueductal grey

1. Introduction

Glutamate plays a critical role as excitatory neurotransmitter in the central nervous system, and its effects are mediated by activation of ionotropic and metabotropic receptors (Nakanishi et al., 1998). Metabotropic glutamate (mglu) receptors are a heterogeneous family of G-protein-coupled receptors linked to multiple second messengers and modulation of ion channel function in the nervous system (Conn and Pinn, 1997; Knöpfel et al., 1995). Their classification into three groups (groups I–III) was determined by similarities in coupling mechanisms, molecular structure, homology of sequence and pharmacology (Nakanishi, 1994). Group I receptors ($mglu_1$ and $mglu_5$, and their splice variants) are positively linked to phospholipase C and, therefore, their activation results in increased phosphoinositide turnover. Localization of $mglu_1$ and $mglu_5$ receptors on the postsynaptic terminal seems to be under the regulation of a small family of "Homer" proteins (Brakeman et al., 1997; Ciruela et al., 1999). These glutamate receptors not only modulate the function of glutamatergic neurons but also are able to change the activity of inhibitory neurons via excitatory glutamatergic inputs to these neurons. Biochemical studies in a number of preparations consistently indicate that activation of groups II and III mglu receptors leads to

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suppression of the release of excitatory and inhibitory amino acids, whereas group I enhances the release of glutamate and γ-aminobutyric acid (GABA) (Cartmell and Schoepp, 2000). A specific role for $mglu_1$ and $mglu_5$ receptors in nociceptive processing has long been demonstrated in the dorsal horn by pharmacological, immunohistochemical and in situ hybridisation (Berthele et al., 1999; Bond and Lodge, 1995; Dickenson et al., 1997; Fisher and Coderre, 1996; Jia et al., 1999). The control of spinal cord nociception is subject to supraspinal, neuronal centres, including the midbrain periaqueductal grey, the medullary nucleus raphe magnus and the adjacent medullary reticular formation (Basbaum and Fields, 1984; Duggan and Griersmith, 1979; Liebeskind et al., 1973). Since 1969, when it was demonstrated that stimulation of the periaqueductal grey produced analgesia, many studies have shown that this effect is the result of complex processes mediated by the periaqueductal grey (Reynolds, 1969). Besides glutamate and opioids, several other neurotransmitters in the periaqueductal grey participate in the control of nociception (Behbehani and Fields, 1979; Millan et al., 1987). Among these, GABA and glycine seem to play a crucial role in the processing of pain within this area (Maione et al., 1999, 2000; Moreau and Fields, 1986). In the current study, we analysed the possible participation of $mglu_1$ and $mglu_5$ glutamate receptors in the control of glutamate and GABA release in periaqueductal grey matter. In particular, we considered it interesting to explore whether periaqueductal grey functionally counteracting neurotransmissions, like glutamate and GABA, would be modulated at the same time by group I mglu subtype receptors. In our opinion, a better understanding of these processes might provide further insight into the pathophysiology of pain syndromes and possibly in changes in the functioning of the endogenous antinociceptive pathway. Previous studies in fact assessed the in vivo modification of glutamate or GABA (although not in the periaqueductal grey) induced by either stimulation or blockade of group I mglu receptors in some areas of the central nervous system (Battaglia et al., 2001; Cartmell and Schoepp, 2000; Cozzi et al., 1997, 2002; Herrero et al., 1992; Pellegrini-Giampietro et al., 1999). Although these previous findings confirmed the difficulty of determining the source of basal glutamate or GABA, they consistently found that group I mglu receptor stimulation increased both glutamate and GABA release. In particular, the group I mglu receptor-mediated increase in activity of GABAergic inputs could ultimately result in decreased excitatory neurotransmission.

Therefore, as the presence of mglu receptors in the periaqueductal grey has been shown by autoradiographic, immunostaining and pharmacological studies, and no study to date has investigated their possible role in the modulation of glutamate and GABA release at that level (Azcue et al., 1997; Catania et al., 1994; Leyva et al., 1995; Maione et al., 1998a, 2000), we evaluated this possibility in awake, freely moving rats.

2. Materials and methods

2.1. Animals

Male Wistar rats (250–300 g) were housed three per cage under controlled illumination (12:12-h light/dark cycle; light on 06.00 h) and environmental conditions (ambient temperature, 20–22 °C; humidity, 55–60%) for at least 1 week before the experiments started. Rat chow and tap water were available ad libitum. The experimental procedures were approved by the Animal Ethics Committee of the Second University of Naples. Animal care was in compliance with Italian (D.L. 116/92) and EEC (O.J. of E.C. L358/1 18/12/86) regulations on the protection of laboratory animals. All efforts were made to minimise animal suffering and to reduce the number of animals used.

2.2. Microdialysis procedure

Brain microdialysis experiments were performed in awake and freely moving rats as described previously (Biggs et al., 1992). In brief, rats were anaesthetised with chloral hydrate (400 mg/kg, i.p.) and stereotaxically implanted with concentric microdialysis probes, which were constructed, as previously described, into the ventrolateral periaqueductal grey using coordinates A: −7.5 mm from bregma, L: +0.5 mm and V: 7.7 mm below the dura (Hutson et al., 1985; Maione et al., 1998b; Paxinos and Watson, 1986). Following a postoperative recovery period of approximately 18 h, dialysis was commenced with an artificial cerebrospinal fluid (ACSF, composition in millimolar: KCl, 2.5; NaCl, 125; $MgCl_2$, 1.18; $CaCl_2$, 1.26). ACSF (pH 7.2) was perfused at a rate of 0.8 μl/min using a Harvard Apparatus infusion pump (mod. 22), and following an initial 60 min equilibration period (two discarded samples), 12 consecutive 30-min dialysate samples were collected. Rats received selective mglu receptor agonists, alone or in combination with the corresponding antagonists, directly through the dialysis probe (30-min perfusion). On completion of each experiment, rats were anaesthetised with pentobarbitone sodium and their brains were perfused-fixed via the left cardiac ventricle with heparinised paraformaldehyde saline (4%). Brains were removed 120 min following fixation, and coronal sections were cut in order to verify probe placements. Dialysates were analysed for amino acid content using a high-performance liquid chromatography (HPLC) method. The system comprised two Gilson pumps (mod. 303), a C18 reverse-phase column, a Gilson refrigerated autoinjector (mod. 231) and a Gilson fluorimetric detector (mod. 121). Dialysates were precolumn derivatised with *o*-phthaldialdehyde (10 μl dialysate + 10 μl *o*-phthaldialdehyde), and amino acid conjugates were resolved using a gradient separation. The detection limit of GABA and glutamate in 10-μl samples was about 0.5–1 and 2–3 pmol, respectively. The mobile phase consisted of two compo-

nents: (A) 50 mM sodium dihydrogen orthophosphate, pH 5.5, with 20% methanol and (B) 100% methanol. Gradient composition was determined with an Apple microcomputer installed with Gilson gradient management software, and the mobile phase flow rate was maintained at 1.0 ml/min. Data were collected by a Dell Corporation PC system 310 interfaced to the detector via a Drew data collection unit.

2.3. Immunohistochemistry

Animals were anaesthetised with sodium pentobarbitone and perfused with 4% paraformaldehyde, 1% glutaraldehyde in 0.1 M phosphate buffer, pH 7.4. Brains were removed, and transverse sections (60 μm thick) of mesencephalon were cut using a vibratome. After preincubation with normal goat serum, the sections were incubated overnight at 4 °C with either anti-mglu$_1$ or anti-mglu$_5$ polyclonal antibodies (Upstate Biotechnology, Lake Placid, NY). After two washes with phosphate buffer solution, the sections were incubated with a biotinylated secondary antibody by using the avidin–biotin peroxidase procedure (Vector Laboratories, Burlingame, CA) and diaminobenzidine as chromogen. The sections were then treated with 1% osmium, stained with uranyl acetate, dehydrated in graded solutions of acetone and embedded in araldite. Ultrathin sections were cut and analysed using a Philips CM10 electron microscope. The negative controls were made by omitting the primary antibodies and using nonimmune antisera, which resulted in complete absence of staining.

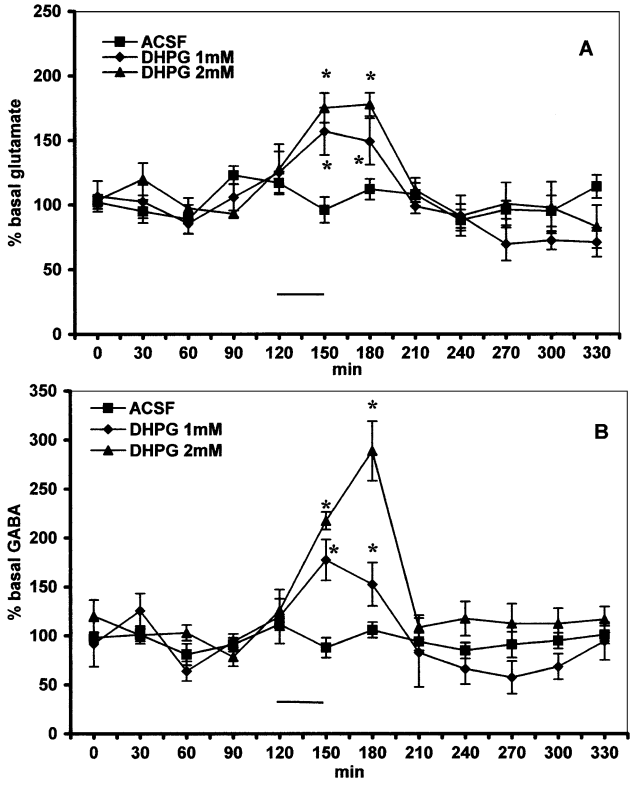

Fig. 1. Effect of artificial cerebrospinal fluid (ACSF) (A and B) or (*RS*)-3,5-DHPG (DHPG, 1 and 2 mM) on periaqueductal grey matter dialysate levels of glutamate (A) and GABA (B). The bars indicate the period of (*RS*)-3,5-DHPG perfusion. Data (five to eight rats per group) are means ± S.E.M. of amino acid extracellular concentrations expressed as percentages of the basal values. The mean basal extracellular GABA and glutamate levels (not corrected for probe recovery) were 6.1 ± 0.7 and 32 ± 5 pmol/10 μl of dialysate (means ± S.E.M.), respectively. *Significant difference versus the vehicle. P values <0.05 were considered statistically significant.

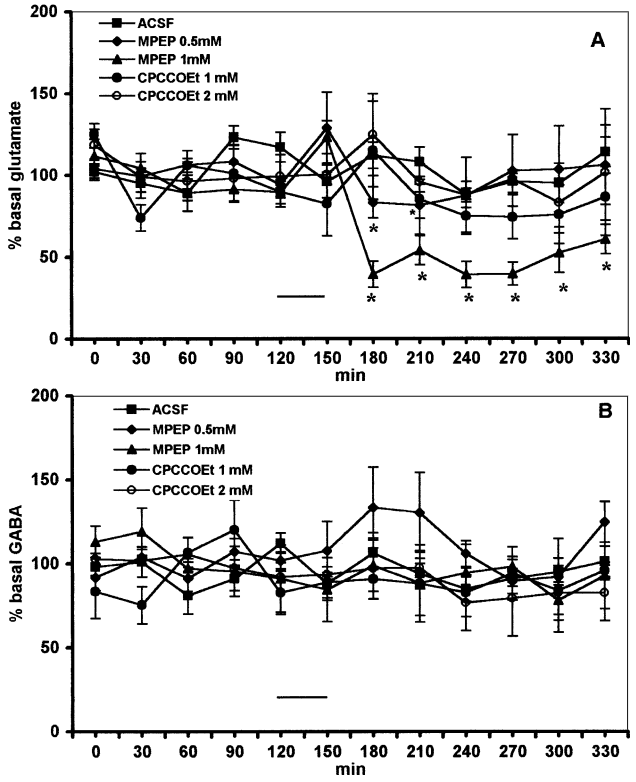

Fig. 2. Effect of artificial cerebrospinal fluid (ACSF) (A and B) CPCCOEt (1 and 2 mM) or MPEP (0.5 and 1 mM) on periaqueductal grey matter dialysate levels of glutamate (A) and GABA (B). The bars indicate the period of MPEP or CPCCOEt perfusion. Data (six to eight rats per group) are means ± S.E.M. of amino acid extracellular concentrations expressed as percentages of the basal values. The mean basal extracellular GABA and glutamate levels (not corrected for probe recovery) were 5.9 ± 0.7 and 33 ± 6 pmol/10 μl of dialysate (means ± S.E.M.), respectively. *Significant difference versus the vehicle. P values <0.05 were considered statistically significant.

2.4. Drugs

The following drugs were used: (*S*)-3,5-dihydroxyphenylglycine [(*S*)-3,5-DHPG], (*RS*)-2-chloro-5-hydroxyphenylglycine (CHPG), 7-(hydroxyimino)cyclopropa-[*b*]-chromen-1α-carboxylate ethyl ester (CPCCOEt), 2-methyl-6-(phenylethynyl)pyridine (MPEP), tetrodotoxin and (*RS*)-3-(2-carboxypiperazin-4-yl)-propyl-1-phosphonic acid [(*RS*)-CPP] (Tocris Cookson, Bristol, UK). CPCCOEt was dissolved in 10% dimethylsulphoxide in ACSF (pH 7.2). All the other drugs were dissolved in ACSF with final pH of 7.2.

2.5. Statistics

Statistical analysis of the data was performed by analysis of variance (ANOVA) followed by the Student–Newman–Keuls multiple comparisons test. Differences were considered significant at $P < 0.05$.

3. Results

The mean basal extracellular GABA, glutamate and glutamine levels in the periaqueductal grey (not corrected for probe recovery of 28 ± 6%, 32 ± 4% and 30 ± 7% for GABA, glutamate and glutamine, respectively) were 6.4 ± 0.4, 28 ± 5 and 422 ± 18 pmol/10 μl of dialysate (means ± S.E.M.), respectively. These values are in accordance with those obtained in our previous studies and in other laboratories (Renno et al., 1992; Maione et al., 1999, 2000). Each animal was used once only, and the reported basal values of glutamate, GABA and glutamine are the mean concentrations obtained from all experiments. Intra-periaqueductal grey perfusion of tetrodotoxin (1 μM) decreased the extracellular levels of glutamate and GABA (48 ± 6% and 53 ± 7% for glutamate and GABA, respectively), but not glutamine (data not shown). Intra-periaqueductal grey perfusion of (*S*)-3,5-DHPG (1 and 2 mM), a selective agonist of group I mglu receptors, increased the dialysate glutamate (156 ± 18% and 177 ± 10%, respectively) and GABA (177 ± 23% and 288 ± 20%, respectively) concentrations (Fig. 1). CPCCOEt (2 and 4 mM), as well as its vehicle (dimethylsulphoxide, 10% in ACSF), did not modify per se dialysate glutamate and GABA levels (Fig. 2). MPEP (0.5–1 mM) decreased, in a concentration-dependent manner, the dialysate glutamate concentrations (− 58 ± 7%) without affecting GABA concentrations (Fig. 2). Intra-periaqueductal grey perfusion of (*RS*)-CPP (100 μM), a selective *N*-methyl-D-aspartate (NMDA) receptor antagonist, did not modify the extracellular levels of glutamate (data not shown). CPCCOEt (1 mM), a selective $mglu_1$ receptor antagonist, and MPEP (0.5 mM), a selective $mglu_5$ receptor antagonist, perfused in combination with (*S*)-3,5-

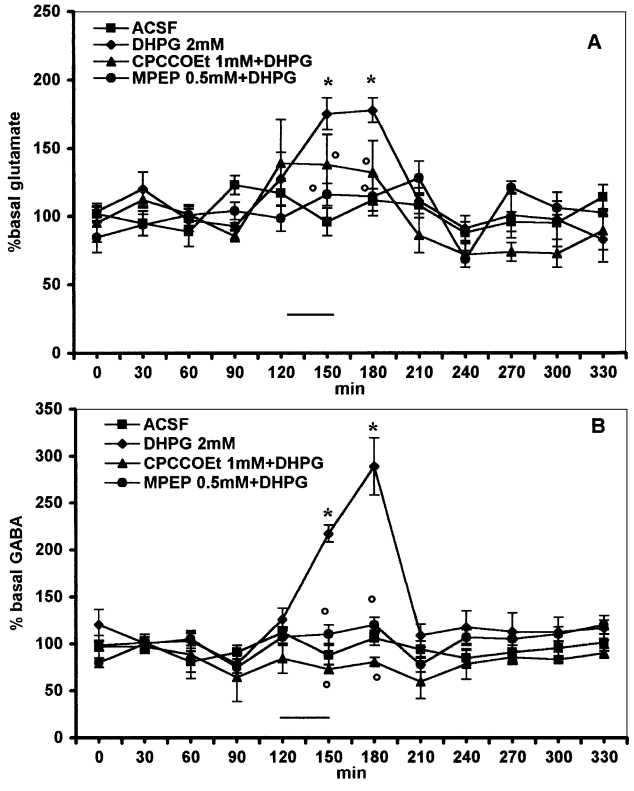

Fig. 3. Effects of artificial cerebrospinal fluid (ACSF) (A and B) (*RS*)-3,5-DHPG (DHPG, 2 mM), alone or in combination with CPCCOEt (1 mM) or MPEP (0.5 mM), on periaqueductal grey matter dialysate levels of glutamate (A) and GABA (B). The bars indicate the period of drug perfusion. Data (five to eight rats per group) are means ± S.E.M. of amino acid extracellular concentrations expressed as percentages of the basal values. The mean basal extracellular GABA and glutamate levels (not corrected for probe recovery) were 6.9 ± 0.8 and 29 ± 6 pmol/10 μl of dialysate (means ± S.E.M.), respectively. *Significant difference versus the vehicle. °Significant difference versus DHPG 2 mM. *P* values < 0.05 were considered statistically significant.

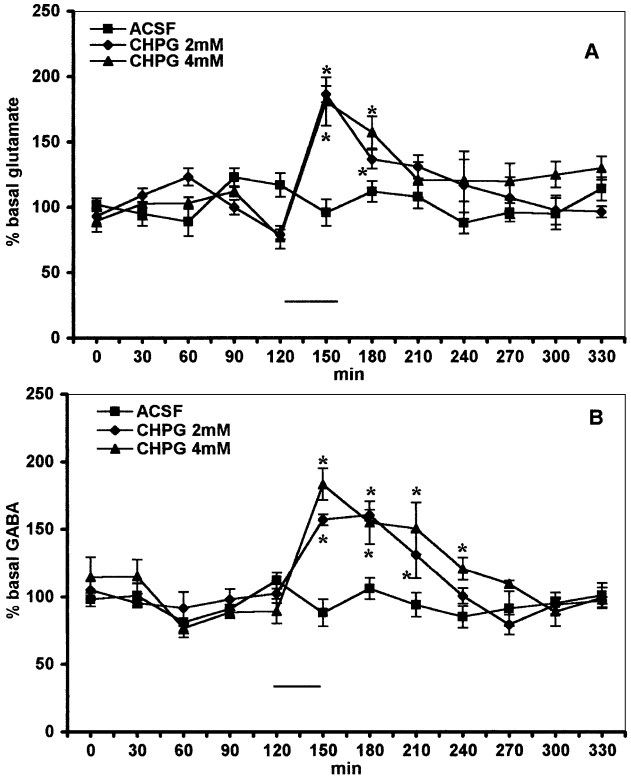

Fig. 4. Effect of artificial cerebrospinal fluid (ACSF) (A and B) or CHPG (2 and 4 mM) on periaqueductal grey matter dialysate levels of glutamate (A) and GABA (B). The bars indicate the period of CHPG perfusion. Data (five to eight rats per group) are means ± S.E.M. of amino acid extracellular concentrations expressed as percentages of the basal values. The mean basal extracellular GABA and glutamate levels (not corrected for probe recovery) were 6.6 ± 0.8 and 26 ± 7 pmol/10 μl of dialysate (means ± S.E.M.), respectively. *Significant difference versus the vehicle. *P* values < 0.05 were considered statistically significant.

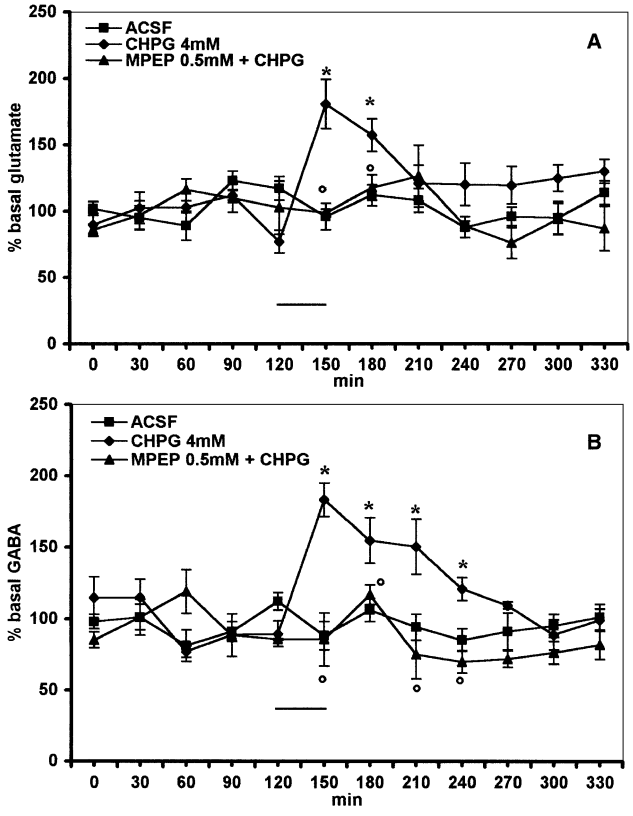

Fig. 5. Effects of artificial cerebrospinal fluid (ACSF) (A and B) or CHPG (4 mM), alone or in combination with MPEP (0.5 mM), on periaqueductal matter dialysate levels of glutamate (A) and GABA (B). The bars indicate the period of drug perfusion. Data (five to eight rats per group) are means ± S.E.M. of amino acid extracellular concentrations expressed as percentages of the basal values. The mean basal extracellular GABA and glutamate levels (not corrected for probe recovery) were 6.9 ± 0.5 and 30 ± 40 pmol/10 μl of dialysate (means ± S.E.M.), respectively. *Significant difference versus the vehicle. °Significant difference versus CHPG 4 mM. P values <0.05 were considered statistically significant.

DHPG, antagonized the (*S*)-3,5-DHPG-induced increase in extracellular concentrations of glutamate and GABA (Fig. 3). CHPG (2 and 4 mM), a selective agonist of $mglu_5$ receptors, increased the dialysate glutamate (186 ± 5% and 180 ± 18%, respectively) and GABA (160 ± 5% and 183 ± 11%, respectively) concentrations (Fig. 4). With (*S*)-3,5-DHPG or CHPG, the changes in amino acid extracellular values were greatest for GABA. MPEP (0.5 mM), a selective $mglu_5$ receptor antagonist, perfused in combination with CHPG (4 mM), antagonized the CHPG-induced increase in glutamate and GABA extracellular concentrations (Fig. 5). The extracellular concentrations of glutamine never changed following treatment with tetrodotoxin (1 μM), (*S*)-3,5-DHPG, CHPG, CPCCOEt and MPEP (data not shown). At the ultrastructural level, immunostaining for $mglu_1$ and $mglu_5$ receptors was mainly localized in the dendrites of periaqueductal grey neurons (Fig. 6); however, some perikarya showed weak $mglu_5$ positivity. Many of the $mglu_1$- and $mglu_5$-positive dendrites received synapses containing round vesicles.

4. Discussion

There is an evidence that ionotropic and metabotropic glutamate receptors are expressed on both neural synaptic and astrocytic processes (Gallo and Ghiani, 2000). Although glutamate released from neurons can activate glutamate receptors on glia to cause changes such as (i) transmitter uptake into glial cells, (ii) modulation of K^+ conductances and (iii) release of neuroactive substances from glia that can modulate synaptic transmission (Vernadakis, 1996; Araque et al., 1999), there is no way in this study to distinguish between glial or neural dialysate amino acids either before or after mglu receptor stimulation. Nevertheless, the changes in periaqueductal grey glutamate or GABA levels may deeply affect nociceptive perception, as this midbrain area is part of the endogenous antinociceptive system (Gebhart et al., 1984). In this study, the possible synaptic nature of periaqueductal grey dialysate glutamate and GABA seems confirmed in part

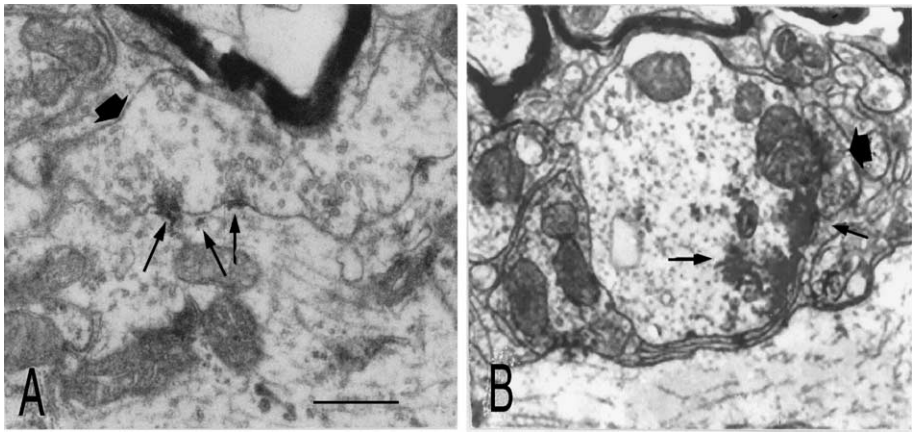

Fig. 6. Photomicrograph of $mglu_1$ (A)- and $mglu_5$ (B)-positive dendrites in the periaqueductal grey. The arrows indicate reaction product. The arrowhead indicates the axon terminal. Bar: 1 μm.

by the fact that tetrodotoxin almost halved their extracellular concentrations. This finding suggests that almost 40% of extracellular GABA or glutamate in the periaqueductal grey may function as neurotransmitter. Moreover, this study provides pharmacological evidence in vivo that intra-periaqueductal grey perfusion of (*S*)-3,5-DHPG, an agonist of group I mglu receptors, and CHPG, a selective agonist of $mglu_5$ receptors, leads to an increase in glutamate and GABA extracellular concentrations. Contrary to what was observed with glutamate and GABA, the extracellular concentration of glutamine never changed with either (*S*)-3,5-DHPG or CHPG. This confirms that glutamine is not synaptically released and is mainly important in amino acid metabolic pathways (Maione et al., 2000). With (*S*)-3,5-DHPG or CHPG treatment, the change in amino acid extracellular levels was greatest for GABA. The postsynaptic nature of group I mglu receptors, as well as the huge population of GABAergic interneurons in the periaqueductal grey (possibly expressed at the somato-dendritic level), may underlie this difference (Lujan et al., 1996; Moreau and Fields, 1986). The selective $mglu_1$ receptor antagonist CPCCOEt was used in combination with (*S*)-3,5-DHPG in order to discriminate which mglu subtype receptor was involved. This antagonist was found to antagonize the effect of (*S*)-3,5-DHPG on both glutamate and GABA extracellular values. Since CHPG also increased extracellular concentrations of glutamate and GABA, the involvement of $mglu_5$ receptors was suggested. Compared to (*S*)-3,5-DHPG, CHPG induced a smaller increase in glutamate and GABA levels, and this may be the consequence of its lower potency (Doherty et al., 1997). Also, CHPG did not generate a concentration-dependent change in the extracellular levels of these amino acids, presumably because very similar concentrations (2 and 4 mM) of this drug were perfused. Intra-periaqueductal grey treatment with MPEP, a selective $mglu_5$ receptor antagonist, perfused in combination with CHPG, further confirmed the involvement of $mglu_5$ receptors in the modulation of glutamate and GABA release. Moreover, $mglu_5$ receptors seem to exert a tonic control of glutamate release at the periaqueductal grey level as their blockade with MPEP reduced per se the extracellular concentrations of this amino acid. The possible involvement of NMDA receptors in this effect was ruled out by the fact that (*RS*)-CPP, a selective NMDA receptor antagonist, did not decrease the glutamate extracellular level in the periaqueductal grey. The blockade of $mglu_1$ receptors with CPCCOEt had no effect either on glutamate or GABA extracellular level. In agreement with previous studies (Cozzi et al., 2002; Lorrain et al., 2002; Maione et al., 1998b; Neugebauer et al., 1999; Pintor et al., 2000), the concentration of mglu receptor ligands used in this study was higher than their in vitro EC/IC_{50} (millimolar versus micromolar) values. The fact that micromolar concentrations were ineffective may be possibly due to (i) the relatively low probe recovery (20–30%), (ii) their uptake and metabolism by glial and neural cells or (iii) drug diffusion from probe site. We suppose that due to these reasons, the actual drug concentrations reaching cerebral tissue resemble those used in the in vitro studies (Schoepp et al., 1999). Since MPEP decreased extracellular concentrations of glutamate in the periaqueductal grey, a role of presynaptic $mglu_5$ receptors may be suggested in this response. In agreement with this concept, intracerebral microdialysis revealed a presynaptic mglu receptor-mediated enhancement of [^{3}H]L-glutamate and endogenous transmitter release from the rat striatum and nucleus tractus solitarius (Jones et al., 1998a; Patel and Croucher, 1998). This is in agreement with a recent study by Thomas et al. (2000, 2001), showing that presynaptically located $mglu_5$ receptors positively modulate neuronal glutamate release in the rat forebrain in the presence of selective agonists. We recently showed that microinjections of MPEP into the periaqueductal grey of rat reduced the latency of the nociceptive reaction in the plantar test (Palazzo et al., 2001). This suggests that glutamate may physiologically modulate the endogenous antinociceptive pathway since decreased $mglu_5$ receptor activation may generate a persistent hyperalgesic effect. However, it also has been suggested that $mglu_5$ receptors serve as autoreceptors to inhibit synaptic transmission in the hippocampal area CA1 (Manzoni and Bockaert, 1995). Moreover, $mglu_1$ and $mglu_5$ receptors may exert opposite effects in primate spinothalamic tract neurons since (*S*)-3,5-DHPG potentiated and CHPG decreased the responses to brief innocuous and noxious mechanical stimulation (Neugebauer et al., 1999). In agreement with a previous neuroanatomical study (Azcue et al., 1997), we showed in this study that $mglu_5$, as well as $mglu_1$, immunoreactivity was located in cell bodies and dendritic processes. Axon terminals making synaptic contact with both $mglu_1$- and $mglu_5$-immunoreactive dendrites contained round clear vesicles. Therefore, in the light of these morphological findings, we can rule out the possibility that dialysate glutamate levels were modulated presynaptically by $mglu_5$ receptors.

In addition, this study suggests that group I mglu receptors regulate GABA release in the periaqueductal grey of awake, freely moving rat. This is shown by the finding that CPCCOEt and MPEP, selective antagonists of $mglu_1$ and $mglu_5$ receptors, respectively, antagonized the (*S*)-3,5-DHPG- and CHPG-induced increase in dialysate GABA levels. Previous studies showed either an augmentation of KCl-evoked GABA release or an increased release of this amino acid by selective mglu agonists in slices of rat striatum (Wang and Johnson, 1995; Wang et al., 1996). Also, 1*S*,3*R*-ACPD and the selective group I mglu receptor agonists (*S*)-3,5-DHPG and quisqualate increased KCl-evoked [^{14}C]GABA release in superfused slices of rat nucleus tractus solitarius or potentiated increases in [^{14}C]GABA release induced by NMDA in

striatal slices (Jones et al., 1998a,b; Hanania and Johnson, 1999). The role played by $mglu_1$ and $mglu_5$ receptors in the periaqueductal grey on the physiology of GABA release is far from established in awake, freely moving rat. There is evidence that microinjections of selective GABA receptor antagonists in the periaqueductal grey induce analgesia by decreasing the tonic GABAergic function (Moreau and Fields, 1986). In this study, we reported that group I mglu receptors may positively modulate the release of GABA, which could be expected to generate hyperalgesia by inhibiting the antinociceptive pathway. However, this is in contrast with our previous observation that both acute and persistent nociceptive behaviours were decreased by intra-periaqueductal grey microinjection of group I mglu receptor agonists (Maione et al., 1998a, 2000). It is possible that the variations in the extracellular levels of these amino acids may be a result of the complex network activity in the periaqueductal grey in vivo. Under our conditions, generalised stimulation of group I postsynaptic mglu receptors in the periaqueductal grey may cause sustained activation of the output antinociceptive pathways. This may prevail over the other modulatory effects in the periaqueductal grey (i.e. the positive modulation of GABA release) and possibly mask the fine-tuning. The precise antinociceptive mechanisms induced by the selective $mglu_{1/5}$ agonists in the periaqueductal grey, in spite of the fact that GABA levels were increased, are unclear, and further investigation is needed to throw new light on the relationship between periaqueductal grey-induced analgesia and GABA and mglu receptors. However, it is worth noting that mglu receptor activation might either down-regulate or up-regulate inhibitory postsynaptic currents in the nucleus of the tractus solitarius and spinal cord in the rat (Glaum and Miller, 1993). Furthermore, activation of group I mglu receptors was found to depress $GABA_A$-mediated inhibitory postsynaptic currents (IPSCs) in slices of rat midbrain dopaminergic neurons (Bonci et al., 1997). The fact that group I mglu receptors modulate the release of excitatory and inhibitory neurotransmitters in the periaqueductal grey in the same direction may be suggestive of the physiological fine-tuning of these two counteracting neurotransmissions. As in other brain areas like the pallidum or substantia nigra (Hanson and Smith, 1999; Hubert et al., 2001), it is possible that group I mglu receptors facilitate the release of GABA at extra synaptic sites or symmetric synapses in the periaqueductal grey. This mglu receptor-mediated GABA release might be physiologically relevant to prevent excessive glutamate accumulation and, therefore, limit possible excitotoxic effects of high concentrations of glutamate.

In conclusion, this study provides evidence that $mglu_1$ and $mglu_5$ receptors may control the release of glutamate and GABA within the periaqueductal grey matter. In particular, $mglu_5$, but not $mglu_1$, receptors seem to be functionally active on postsynaptic terminals in this midbrain area, where they tonically modulate the endogenous antinociceptive pathway.

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