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Interaction between vanilloid and glutamate receptors in the central modulation of nociception

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Abstract

This study investigates the effect of microinjections of capsaicin in the periaqueductal grey matter of rats on nociceptive behaviour and the possible interactions with NMDA and mGlu receptors. Intra-periaqueductal grey microinjection of capsaicin (1–3–6 nmol/rat) increased the latency of the nociceptive reaction in the plantar test. This effect was prevented by pretreatment with capsazepine (6 nmol/rat), which had no effect per se on the latency of the nociceptive reaction. 7-(Hydroxyimino)cyclopropa[*b*]chromen-1α-carboxylate ethyl ester (CPCCOEt, 50 nmol/rat) and 2-Methyl-6-(phenylethynyl)pyridine (MPEP, 50 nmol/rat), antagonists of $mGlu_1$ and $mGlu_5$ receptors, respectively, completely blocked the effect of capsaicin. Similarly, pretreatment with DL-2-Amino-5-phosphonovaleric acid (DL-AP5, 5 nmol/rat) and riluzole (4 nmol/rat), an NMDA receptor antagonist and a voltage-dependent Na^+ channels blocker which inhibits glutamate release, respectively, completely antagonized the effect of capsaicin. However, pretreatment with (2*S*)-α-Ethylglutamic acid (30 nmol/rat) and (*RS*)-α-Methylserine-*O*-phosphate (MSOP, 30 nmol/rat), antagonists of group II and group III mGlu receptors, respectively, had no effects on capsaicin-induced analgesia. Similarly, pretreatment with *N*-(piperidin-1-yl)-5-(4-chlophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide (SR 141716A, 5 pmol/rat), a selective cannabinoid CB_1 receptor antagonist, did not affect the capsaicin-induced antinociception. In conclusion, this study shows that capsaicin might produce antinociception at the periaqueductal grey level by increasing glutamate release, which activates postsynaptic group I mGlu and NMDA receptors.

Keywords: Nociception; Capsaicin; Glutamate; Periaqueductal grey

1. Introduction

Capsaicin, a natural vanilloid responsible for the piquancy of red pepper, activates a recently cloned ligand-gated ion channel receptor named vanilloid VR1 (Caterina et al., 1997), which acts as integrator of painful stimuli on primary sensory neurons (Szallasi and Blumberg, 1999; Tominaga et al., 1998). The vanilloid VR1 receptor seems to date the only receptor to be activated by vanilloids, and the question whether anandamide functions as its physiological endogenous ligand on primary afferent neurons is still disputed (Zygmunt et al., 1999; Tognetto et al., 2001; Szolcsanyi, 2000). The point is whether anandamide ever reaches a sufficiently high concentration to physiologically activate these receptors. Moreover, recently, Hwang et al. (2000) have shown that different lipoxygenase derivatives have similar potency, but greater efficacy, than anandamide in activating vanilloid VR1 receptors. The presence of vanilloid VR1 receptors in the brain has been studied with an antibody and a complementary RNA probe by Mezey et al. (2000). The evidence that vanilloid VR1 receptors are also expressed in the central nervous system (CNS) may suggest their possible participation in the pathophysiology of some neurological disorders, including chronic pain syndrome. The brainstem pain modulatory circuitry includes the periaqueductal grey, which, together with other midbrain nuclei (i.e. raphe nuclei), constitutes the antinociceptive descending pathway. The periaqueductal grey projects to the rostral ventrolateral medulla, which, in turn, sends projections to the spinal cord dorsal horn (Fields et al., 1977; Fields and Basbaum, 1978; Cho and Basabaum, 1991), thus attenuating nociceptive signals originating in the periphery. The importance of periaqueductal grey in processing pain was recognized in

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1969, when it emerged that electrical stimulation of the periaqueductal grey produced analgesia in unanaesthetized animals (Reynolds, 1969). Several neurotransmitters participate in the periaqueductal grey control of pain: besides glutamate and γ-aminobutyric acid (GABA), opioids and cannabinoids produce analgesia within the periaqueductal grey by distinct and overlapping mechanisms (Lichtman et al., 1998; Maione et al., 1998a; Millan et al., 1987; Moreau and Field, 1986; Palazzo et al., 2001).

Although the effect of centrally administered capsaicin has been investigated previously in some brain areas (Koulchitsky 1998; Mazzone and Geraghty, 1999, 2000; Jancsò-Gabor et al., 1970; Hajos et al., 1987), to our knowledge, there is no study aimed at investigating its possible effect at the level of the periaqueductal grey. Moreover, to date, there is no evidence of vanilloid VR1 receptors within the periaqueductal grey, even if the dorsal raphe nucleus, an area which shows anatomical and functional similarity to the periaqueductal grey, showed positiveness to immunolabelling with an antibody to vanilloid VR1 receptors (Mezey et al., 2000). We considered, therefore, it interesting to evaluate the effect of intra-periaqueductal grey microinjection of capsaicin, alone or in combination with capsazepine, on thermoceptive sensitivity (plantar test). The possible interaction with glutamate ionotropic and metabotropic receptors, as well as with cannabinoid CB_1 receptors, was also considered in this study.

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; lights on 06.00 h) and environmental conditions (ambient temperature 20–22 °C, humidity 55–60%) for at least 1 week before the commencement of experiments. 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 for the protection of laboratory animals. All efforts were made to reduce both animal number and suffering during the experiments.

2.2. Surgical procedure

To carry out direct intracerebral administration of drugs or respective vehicle, dimethylsulfoxide (10%) in artificial cerebrospinal fluid, a stainless steel guide cannula (A-M System, Everett, USA) was fixed to the skull (flat position) with dental zinc cement. The cannula was implanted 2 days before the experiment above the dorso-lateral periaqueductal grey area under chloral hydrate (400 mg/kg i.p.) anaesthesia. We used a David Kopf stereotaxic apparatus (David Kopf Instrument, Tujunga, CA) to implant the guide cannula into the periaqueductal grey and the coordinates of the Atlas of Paxinos and Watson (1986) (A −7.5 mm and L +0.5 mm from bregma, V −4.75 mm below the dura) were applied. The inner end of the guide cannula was located 0.4–0.5 mm above the injection site. During this surgical procedure, the animal was positioned on a homothermic temperature control blanket (Harvard Apparatus, Edenbridge, Kent, UK). Microinjections into the periaqueductal grey were given by means of a stainless steel fine cannula (0.6 mm o.d.) connected by polyethylene tube to a 1-μl Hamilton syringe, and carefully inserted through to the guide cannula. A volume of 0.2 μl drug solution or vehicle was injected over a period of 5 s. At the end of the experiment, all animals were given a lethal dose of sodium pentobarbital (100 mg/kg). Absolute Blue (0.2 μl) was microinjected through the cannula to label the injection sites. Brains were removed for histological examination. The placement sites were identified on 20-μm serial coronal sections. Data from subjects in which the cannula tips were in the appropriate brain region were included in the statistical analysis.

2.3. Thermosensitivity

Changes in nociception were evaluated using a Plantar Test Apparatus (Ugo Basile, Varese, Italy). On the day of the

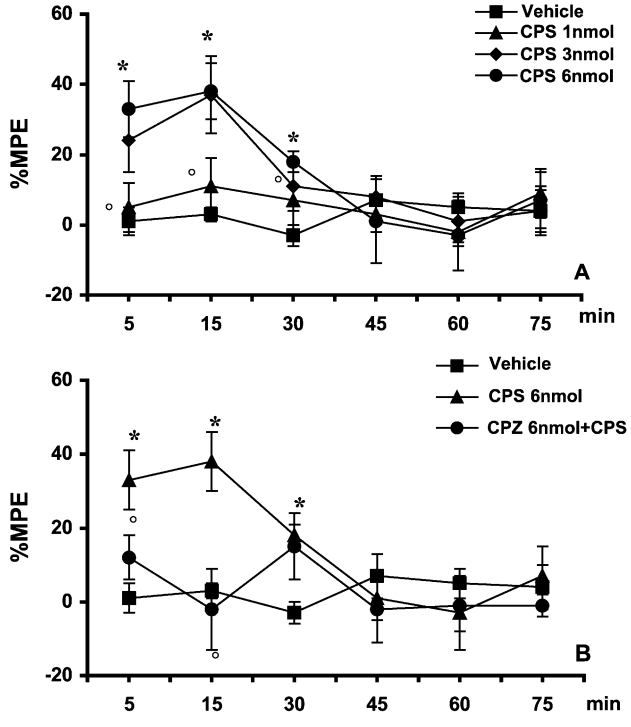

Fig. 1. Antinociception, calculated as percentage of the maximum possible effect (%MPE), after microinjection of (A) vehicle or capsaicin (CPS, 1–3–6 nmol/rat), and (B) vehicle, CPS (6 nmol/rat) or CPS (6 nmol/rat) in combination with capsazepine (CPZ, 6 nmol/rat) into the periaqueductal grey of rats. Each point represents the mean ± S.E. of 13–16 observations. (*) Indicates significant differences vs. vehicle and (°) significant differences vs. CPS 6 nmol/rat. *P* values < 0.05 were considered statistically significant.

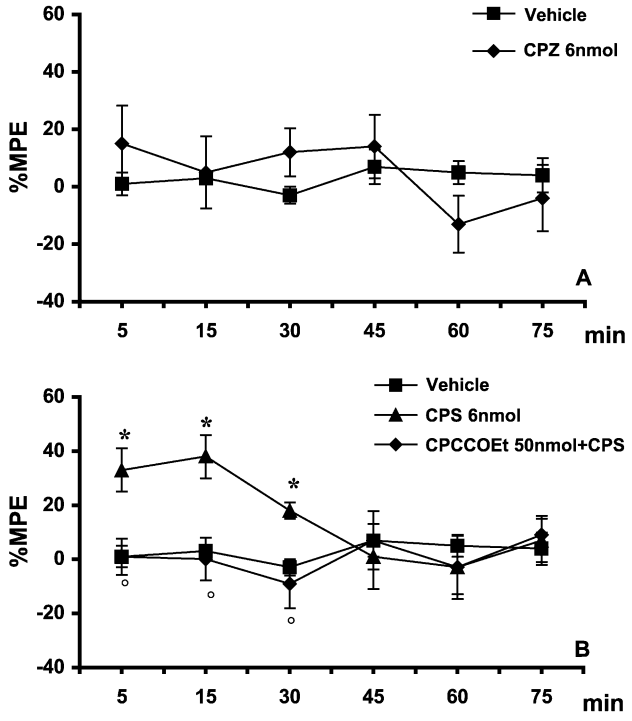

Fig. 2. Antinociception, calculated as percentage of the maximum possible effect (%MPE), after microinjection of (A) vehicle or capsazepine (CPZ, 6 nmol/rat) and (B) vehicle, capsaicin (CPS, 6 nmol/rat) or capsaicin in combination with 7-(Hydroxyimino)cyclopropa[*b*]chromen-1α-carboxylate ethyl ester (CPCCOEt; 50 nmol/rat) into the periaqueductal grey of rats. Each point represents the mean ± S.E. of 13–16 observations. (*) Indicates significant differences vs. vehicle and (°) significant differences vs. CPS 6 nmol/rat. *P* values <0.05 were considered statistically significant.

experiment, each animal was placed in plastic cages (22 × 17 × 14 cm, length × width × height) with a glass floor. After a 25- to 30-min habituation period, the plantar surface of the hind paw was exposed to a beam of radiant heat through the glass floor. The radiant heat source consisted of an infrared bulb (Osram halogen-bellaphot bulb 8 V, 50 W). A photoelectric cell detected light reflected from the paw and turned off the lamp when paw movements interrupted the reflected light. The paw withdrawal latency was automatically displayed to the nearest 0.1 s; the cut-off time was 25 s in order to prevent tissue damage. The latency of the nociceptive reaction was measured in seconds under basal conditions and at 5, 15, 30, 45, 60 and 75 min after drug(s) administration. Each rat served as its own control, the latency to response being measured both before and after drug administration. Pre-drug latency was the mean of five values for each animal, measured at 15-min intervals. Groups of 10–16 animals per treatment were used, with each animal being used for one treatment only. The dosages of the drugs used in the current study were chosen as follows: capsaicin and capsazepine in agreement with previous in vivo studies carried out at supraspinal sites (Hajos et al., 1987; Koulchitsky et al., 1994; Mazzone and Geraghty, 1999); antagonists of cannabinoid CB_1, metabotropic and NMDA glutamate receptors were selected after extensive testing in our laboratory of lower dosages which were ineffective per se in changing nociception but which were able to block the effect of the respective agonists (Maione et al., 1998a, 2000; Palazzo et al., 2001; Berrino et al., 2001).

2.4. Drugs

The following drugs were used: (*E*)-*N*-[(4-Hydroxy-3 methoxyphenyl)methyl]-8 methyl-6-nonenamide (capsaicin); *N*-[2-(4-Clorophenyl)ethyl]-1,3,4,5-tetrahydro-7,8-dihydroxy-2*H*-2-benzazepine-2-carbothioamide (capsazepine); 7-(Hydroxyimino)cyclopropa[*b*]chromen-1α-carboxylate ethyl ester (CPCCOEt); 2-Methyl-6-(phenylethynyl)pyridine (MPEP); (2*S*)-α-Ethylglutamic acid; (*RS*)-α-Methylserine-*O*-phosphate (MSOP); 2-Amino-6-trifluoromethoxybenzothiazole (riluzole) (Tocris Cookson, Bristol, UK); DL-2-Amino-5-phosphonovaleric acid (DL-AP5) (Sigma, Milano, Italy). *N*-piperidino-5-(4-chlorophenyl)-l-(2,4-dicloro-phenyl)-4-methyl-8-pyrazole-carboxamide (SR 141716A) was a gift from Sanofi Recherche. Capsaicin, capsazepine, CPCCOEt and SR141716A were dissolved in 10% dimethylsulfoxide in artificial cerebrospinal fluid (ACSF). All the other drugs were dissolved in ACSF.

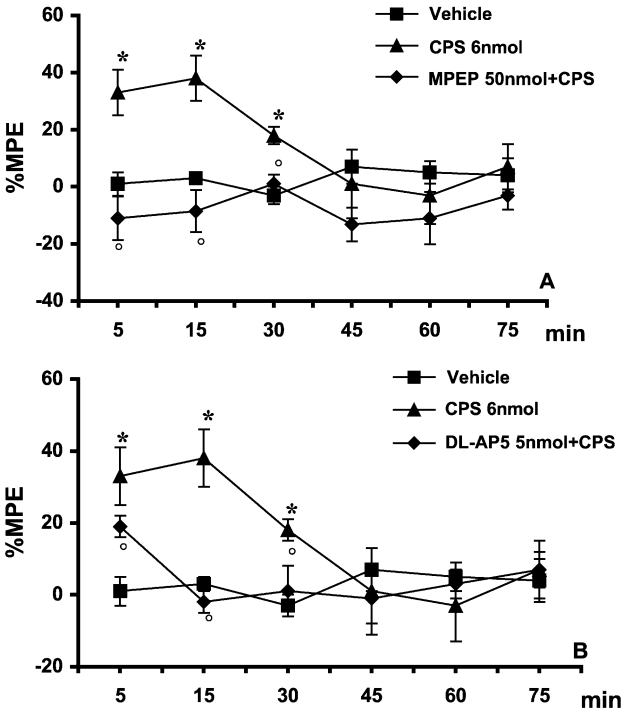

Fig. 3. Antinociception, calculated as percentage of the maximum possible effect (%MPE), after microinjection of (A) vehicle, capsaicin (CPS, 6 nmol/rat) or capsaicin in combination with 2-methyl-6-(phenylethynyl)pyridine (MPEP; 50 nmol/rat) and (B) vehicle, capsaicin (CPS, 6 nmol/rat) or capsaicin in combination with DL-2-Amino-5-phosphonovaleric acid (DL-AP5; 5 nmol/rat) into the periaqueductal grey of rats. Each point represents the mean ± S.E. of 12–14 observations. (*) Indicates significant differences vs. vehicle and (°) significant differences vs. CPS 6 nmol/rat. *P* values <0.05 were considered statistically significant.

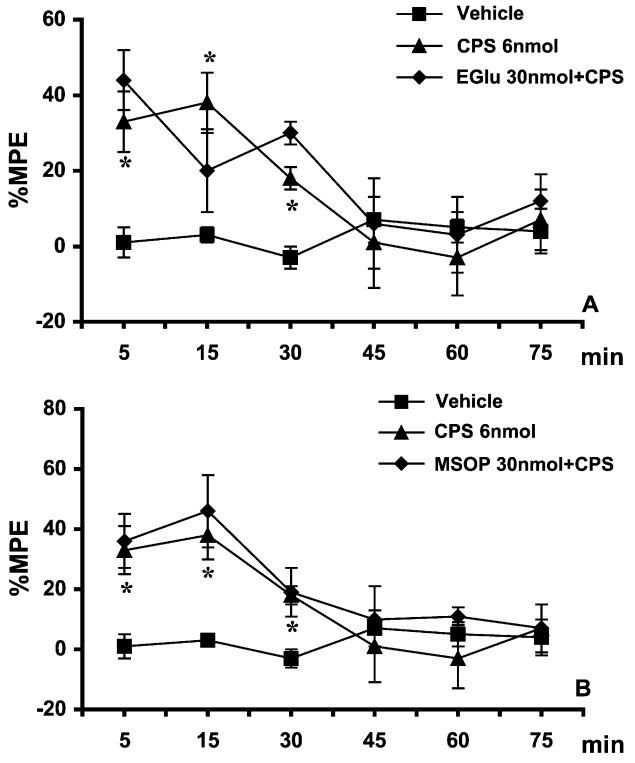

Fig. 4. Antinociception, calculated as percentage of the maximum possible effect (%MPE), after microinjection of (A) vehicle, capsaicin (CPS, 6 nmol/rat) or capsaicin in combination with ethylglutamic acid (EGlu; 30 nmol/rat), and (B) vehicle, capsaicin (6 nmol/rat) or capsaicin in combination with (*RS*)-α-Methylserine-*O*-phosphate (MSOP; 30 nmol/rat) into the periaqueductal grey of rats. Each point represents the mean ± S.E. of 12–13 observations. (*) Indicates significant differences vs. vehicle. P values <0.05 were considered statistically significant.

2.5. Data analysis

The results are expressed as a percentage of the maximum possible effect (%MPE), using the following formula:

$$\%\text{MPE} = \frac{(\text{test latency}) - (\text{control latency})}{(\text{cut} - \text{off time}) - (\text{control latency})} \times 100$$

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

3. Results

Microinjections of capsaicin (1–3–6 nmol/rat), a vanilloid VR1 receptor agonist, into the dorso-lateral periaqueductal grey produced a significant ($P<0.001$) increase in the latency of the nociceptive reaction of 37 ± 11% and 39 ± 8% with the dosages of 3 and 6 nmol/rat, respectively (Fig. 1A). The capsaicin-induced analgesia was prevented by capsazepine (6 nmol/rat), a selective vanilloid receptor antagonist (Fig. 1B). Capsazepine (6 nmol/rat) did not modify per se the latency of the nociceptive reaction (Fig. 2A). Pretreatment with CPCCOEt (50 nmol/rat), a $mGlu_1$ receptor antagonist, 5 min before capsaicin, elicited a blockage of the effect of the vanilloid (Fig. 2B). Likewise, microinjections of MPEP (50 nmol/rat), a selective $mGlu_5$ receptor antagonist and DL-AP5 (5 nmol/rat), a selective antagonist of NMDA glutamate receptor, 5 min before capsaicin, completely antagonized the antinociceptive effect induced by capsaicin (Fig. 3A and B). However, microinjection of either ethylglutamic acid (30 nmol/rat), a selective antagonist of group II mGlu receptors, or MSOP (30 nmol/rat), an antagonist of group III mGlu receptors, 5 min before capsaicin, did not antagonize the effect produced by capsaicin (Fig. 4A and B). Finally, riluzole (4 nmol/rat), a voltage-dependent Na^+ channel blocker which inhibits the release of glutamate, 5 min before capsaicin, prevented the effect of capsaicin (Fig. 5A). Riluzole (2–4 nmol/rat) per se did not change the latency of the nociceptive reaction (Fig. 5B). Pretreatment with SR1417 16A, 5 min before capsaicin (6 nmol/rat), did not change the capsaicin-induced antinociception (Fig. 6). To exclude cap-

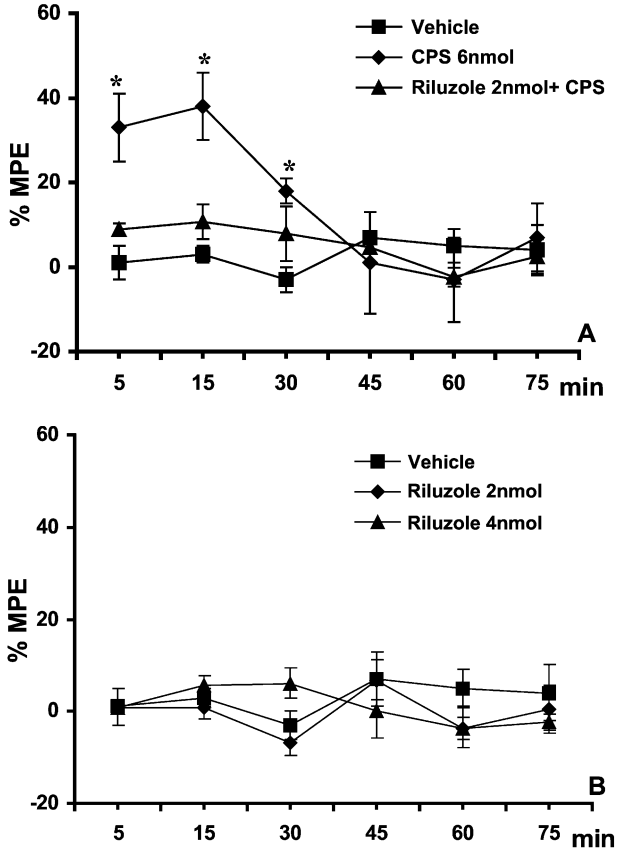

Fig. 5. Antinociception, calculated as percentage of the maximum possible effect (%MPE), after microinjection of (A) vehicle, capsaicin (CPS, 6 nmol/rat) or capsaicin in combination with riluzole (2 nmol/rat) and (B) vehicle or riluzole (2–4 nmol/rat) into the periaqueductal grey of rats. Each point represents the mean ± S.E. of 12–13 observations. (*) Indicates significant differences vs. vehicle. P values <0.05 were considered statistically significant.

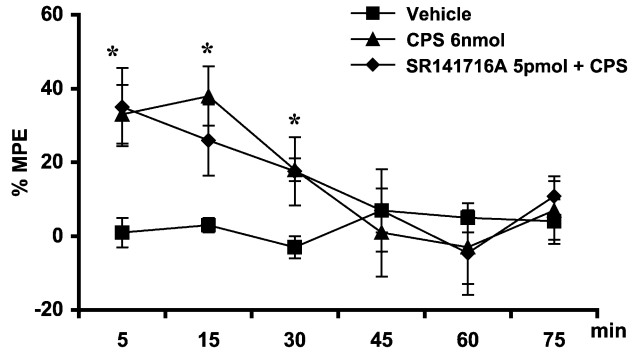

Fig. 6. Antinociception, calculated as percentage of the maximum possible effect (%MPE), after microinjection of (A) vehicle, capsaicin (CPS, 6 nmol/rat) or capsaicin in combination with SR141716A (5 pmol/rat) into the periaqueductal grey of rats. Each point represents the mean ± S.E. of 12–13 observations. (*) Indicates significant differences vs. vehicle. *P* values <0.05 were considered statistically significant.

saicin diffusing to other sites (i.e. raphe nuclei), microinjections of capasaicin were intentionally carried out 1 mm away from the periaqueductal grey. These latter injections were not able to significantly modify the latency of the nociceptive reaction (data not shown). In spite of the evidence that the periaqueductal grey is a key site in integrating the aversive behaviour in this current study, we did not observe any characteristic behavioural changes in response to capsaicin microinjections at the dosages used.

4. Discussion

Capsaicin activates specific vanilloid receptors on sensory nerve endings, triggering cation influx, action potential firing, transmission of information to the CNS, and the subsequent sensation of burning pain and discomfort (Bevan and Szolcsanyi, 1990; Holzer, 1991; Szolcsanyi, 1993). To date, there is little information on the role of vanilloid VR1 receptors at supraspinal sites. Capsaicin was able to modify blood pressure, temperature response, sympathetic nerve activity, heart rate and respiration when injected into the ventral medulla (Koulchitsky, 1998; Osaka et al., 2000; Seller et al., 1997; Koulchitsky et al., 1994). When injected into the commissural nucleus of the solitary tract, capsaicin reduced the respiratory frequency (Mazzone and Geraghty, 1999, 2000). Moreover, in the hypothalamus, capsaicin produced hypothermia (Jancsò-Gabor et al., 1970) and, in the locus coeruleus, it caused a marked activation of the firing rate of neurons (Hajos et al., 1987). There is evidence of a significant expression of vanilloid VR1 receptors in some other areas of the brain including cortex, limbic system, striatum, hypothalamus and thalamus. Even though significant levels of vanilloid VR1 receptors were not observed in the periaqueductal grey (Mezey et al., 2000), we found in this study that intra-periaqueductal grey microinjections of capsaicin produced a significant increase in the latency of the nociceptive reaction to thermal stimuli. Capsaicin does not bind to cannabinoid CB_1 receptors (Di Marzo et al., 1998) and, to date, there is no evidence that capsazepine binds to cannabinoid CB_1 receptors. Since capsazepine completely blocked the capsaicin-induced antinociception, this effect was probably due to vanilloid VR1 receptor stimulation. Our data so far represent pharmacological evidence that vanilloid VR1 receptors might be expressed at level of the periaqueductal grey, as well as in other brain regions.

The reason for this discrepancy between morphological and our pharmacological data could be due to the presence of different vanilloid VR1 receptor isoforms or to a low density of vanilloid VR1 receptors in this area, probably within a specific class of terminals. Indeed, capsaicin-sensitive glutamatergic terminals have been reported by Sasamura et al. (1998) in the hypothalamus, some of them expressing vanilloid VR1 receptors, whose stimulation elicits glutamate release, an effect mediated via vanilloid VR1 receptors and blocked by capsazepine. To date, there is no evidence that capsaicin or capsazepine are able to bind to multiple glutamatergic receptors. However, since it has been shown that capsaicin interacts with the glutamatergic system (Sasamura et al., 1998; Kawamata et al., 2001), in this study, we examined whether selective antagonists of glutamate receptors, as well as riluzole, a voltage-dependent Na^+ channel blocker which inhibits the release of glutamate, could modify the antinociceptive response to intra-periaqueductal grey microinjections of capsaicin.

Pretreatment with CPCCOEt and MPEP completely blocked the capsaicin-induced analgesia. This confirms that periaqueductal grey group I mGluRs play an important role in the modulation of pain and seem to be necessary for capsaicin-induced analgesia. The involvement of $mGlu_5$ in nociceptive processes emerged from the discovery of the $mGlu_5$ protein and mRNA at several levels of the somatosensory pathways (Walker et al., 2001a,b; Valerio et al., 1997; Vidnyanszky et al., 1994; Romano et al., 1995). There is evidence that $mGlu_5$ receptors have a role in thermal hyperalgesia or acute nociception both at spinal (Dogrul et al., 2000; Bordie and Ugolini, 2000) and supraspinal levels (Palazzo et al., 2001).

Similarly to MPEP and CPCCOEt, DL-AP5, an antagonist of NMDA glutamate receptors, blocked the capsaicin-induced antinociception. This allowed us to think that postsynaptic glutamate receptors ($mGlu_1$, $mGlu_5$ and NMDA) have a critical role in the capsaicin-induced effect. The possibility that NMDA and $mGlu_{1/5}$ work "in series", i.e. the participation of these receptors is necessary for the nociceptive response, was shown in our previous study (Berrino et al., 2001). In this last study, blockade of postsynaptic, but not presynaptic, glutamate receptors was relevant for the appearance of the late nociceptive phase in the formalin test. The lack of effect of group II and group III mGlu receptor-selective antagonists, ethylglutamic acid and MSOP, respectively, was observed also in the current study. Pretreatment with these drugs did not antagonize the capsaicin-induced antinociception. This suggests that while

these receptors, which are mainly distributed presynaptically (Bradley et al., 1996; Shigemoto et al., 1997), modulate the release of many neurotransmitters (Maione et al., 1998b; Cartmell and Schoepp, 2000), they do not seem to be involved in capsaicin-induced analgesia at the periaqueductal grey level. This could be a consequence of their presynaptic fine-tuning of neuronal activity rather than direct postsynaptic neuron activation. These data support the idea that intra-periaqueductal grey-administered capsaicin may generate analgesia by increasing the release of glutamate. Further confirmation of this possibility emerged following pretreatment with riluzole, a voltage-dependent Na^+ channel blocker that inhibits glutamate release, which antagonized the capsaicin-induced effect. However, it has been shown that peripheral noxious stimuli may increase endocannabinoid release in the periaqueductal grey (Walker et al., 1999), but there is no evidence at the moment that local administration of capsaicin in the periaqueductal grey can increase the extracellular concentration of endocannabinoids locally. Indeed, the blockade of cannabinoid CB_1 receptors by SR141716A did not modify the capsaicin-induced antinociception. Moreover, the dosage of SR141716A used in this study was not able to modify per se the nociceptive latency whereas a higher dosage could (Palazzo et al., 2001).

In conclusion, this preliminary study shows a pharmacological interaction between NMDA, $mGlu_1$ and $mGlu_5$ receptors and capsaicin-induced antinociception at the level of the periaqueductal grey. As shown in other CNS regions (Sasamura et al., 1998; Valtschanoff et al., 2001), it is possible that vanilloid VR1 receptors are expressed also at the level of periaqueductal grey, and, possibly, on glutamatergic terminals or dendrites. Moreover, owing to the complex organization of the periaqueductal grey, where a huge population of GABAergic interneurons are tonically active and where opioids negatively modulate these same interneurons (Moreau and Field, 1986; Maione et al., 1995), more insight is needed to clarify the mechanisms of capsaicin-induced antinoception and its interaction with postsynaptic metabotropic and NMDA glutamate receptors.

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