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Activation of dorsal periaqueductal gray by glycine produces long lasting hyponociception in rats without overt defensive behaviors

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ABSTRACT

Electrical or glutamate stimulation of the dorsal periaqueductal gray matter (DPAG) of rats induces overt defensive behavior, such as freezing or flight, and hyponociception, while glycine and D-serine, a specific NMDA/GLY_B-site ligand, produced only subtle defensive behavior related to risk assessment and avoidance from the open arms in the elevated plus-maze test. In order to verify whether the GLY_B site in the DPAG could also be involved in hyponociception, glycine (GLY; 10, 20, 50, and 80 nmol/0.3 µl) and (+/−)-3-amino-1-hydroxy-2-pyrrolidone (HA966; 10 nmol/0.3 µl), a GLY_B-site antagonist, were microinjected in rats submitted to the radiant heat-induced tail-flick test. GLY increased tail-flick latencies in a dose-dependent way. This hyponociceptive effect was completely reversed by co-administration with HA966. GLY given in the deep layer of superior colliculus did not produce changes in tail-flick latencies. Therefore, the results suggest that the activation of GLY_B receptors in the DPAG is also involved in the hyponociception elicited by this brain area.

Introduction

The analgesic effect induced by electrical stimulation of the brainstem in animals has indeed raised the possibility of treatment of chronic pain in humans. However, the procedure is often accompanied by side effects such as strong fear-like emotions (Nashold et al., 1969; Amano et al., 1978; Young, 1989), an undesirable side-effect that limits its clinical application. Rat dorsal periaqueductal gray matter (DPAG) stimulation also induces behavioral responses related to aversive situations and hyponociception (Fardin et al., 1984) that may mimic the equivalent emotional state reported by humans. Based on the results obtained using *N*-Methyl-D-Aspartate (NMDA), kainate, and also by quisqualate microinjection into the PAG, several types of glutamate receptors might mediate hyponociception, as well as the overt defensive behavior such as, jumping, running, and vocalization (Jacquet, 1988; Jensen and Yaksh, 1992). The NMDA-type receptor raises most interest since its remarkable pharmacology (Cotman et al., 1995) stimulates more detailed investigation on how glutamatergic synapses can be modulated to improve the analgesic effect. The NMDA receptor ionophore has a high Ca^{2+} permeability which is controlled in a voltage-dependent manner by extra-cellular Mg^{2+} (Unwin, 1993). The ionophore comprises five subunits with interdependent recognition sites for NMDA/glutamate, glycine (GLY_B), polyamines and blockers of ligand-gated Ca^{2+} channels (Yoneda and Ogita, 1991; Unwin, 1993; Cotman et al., 1995). The high-affinity strychnine-insensitive binding site for glycine, the GLY_B site (Yoneda and Ogita, 1991) is a prerequisite for NMDA channel opening (Bourne and Nicoll, 1993; Yoneda and Ogita, 1991). This function seems to be assured by in vivo cerebrospinal fluid concentrations of glycine, but the true amino acid concentration at the synaptic cleft is unknown (Bourne and Nicoll, 1993). Recent investigations have shown that glycine concentration in PAG seems to be inversely correlated to nociceptive paw stimulation in rats (Maione et al., 2000), suggesting that lower glycine may facilitate nociception. Glycine microinjection into the PAG does not produce similar overt defensive behaviors to those induced by glutamate, however it has been shown that glycine and D-serine, a specific GLY_B-site ligand (Kemp and Leeson, 1993), microinjected into the DPAG enhance the aversion to the open arms in the elevated plus-maze test (Schmitt et al., 1995; De-Souza et al., 1998). The aim of this study was to verify whether the kind of activation of the DPAG produced by glycine can also produce hyponociception in the radiant heat tail-flick test.

Materials and methods

Animals

Eighty seven male Wistar rats (250–350 g) housed in a temperature-controlled room (21 ± 1 °C) under a 12/12 light/dark cycle were used in the present study. Food and water were available *ad libitum*. All behavioral testing was performed between 7:00 a.m. and 2:00 p.m. Animal care and handling procedures were in accordance with the

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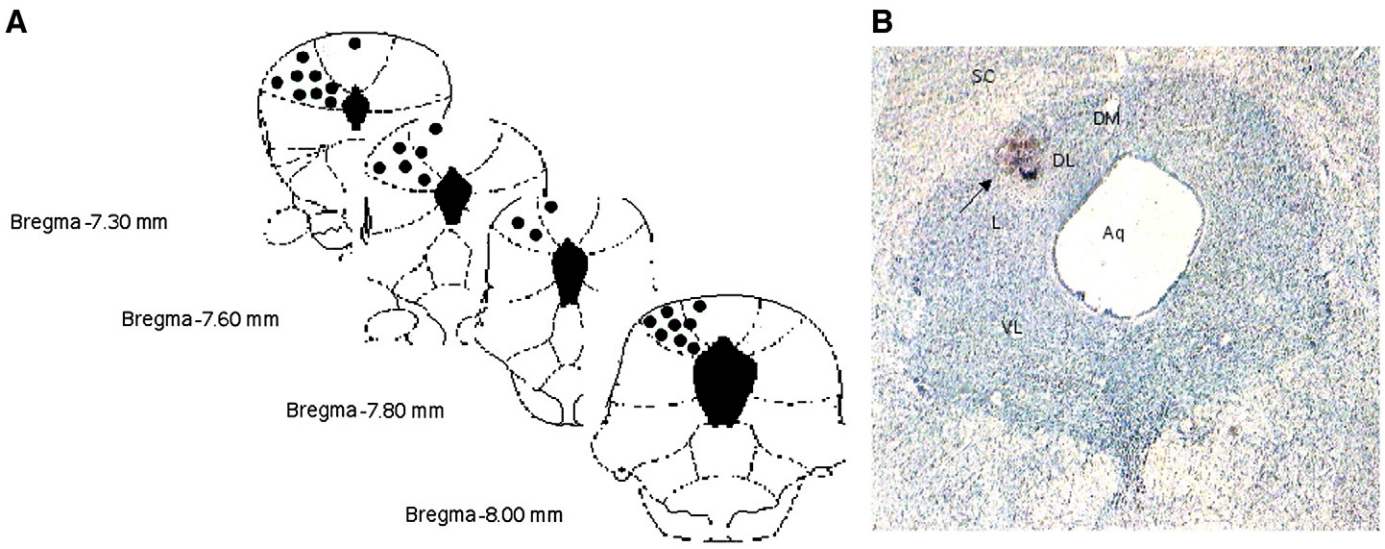

Fig. 1. (A) Diagrams of rat coronal sections, redrawn from the Paxinos and Watson rat brain atlas (Paxinos and Watson, 1998). The microinjection sites (black dots) are labelled by the Evans Blue injection in the DPAG. The number of points in the figures is less than the total number of rats used because of several site overlaps. (B) Photomicrograph of a slide of the PAG from a representative subject showing an injection site (arrowheads) into the DPAG column section. Section corresponds to −8.00 mm from bregma in the Paxinos and Watson atlas (Paxinos and Watson, 1998). DM, dorso-medial; DL, dorso-lateral; L, lateral; VL, ventro-lateral; Aq, aqueduct; SC, superior colliculus.

ethical guidelines of the International Association for the Study of Pain (IASP, 1983), and also approved by the local Ethical Committee for Animal Research.

Surgical and microinjection procedures

For intracerebral administration of drugs, a stainless steel guide cannula (o.d.=0.7 mm) was stereotaxically implanted in the dorsal periaqueductal gray (DPAG) area, seven days before the experiment, and under anesthesia with 1.5 ml/kg of a solution containing 20 mg/ml of xylazine (Rompum®, Bayer, Brazil), plus 100 mg/ml of ketamine (Dopalem®, Vetbrands, Brazil). Stereotaxic coordinates (AP=−7.6 and ML=+1.9 from bregma; DV=−2.0 from skull and at an angle of 22° from the sagittal plane) were from the Paxinos and Watson rat brain atlas (Paxinos and Watson, 1998). At the end of the surgery, rats received a 0.2-ml injection of veterinary pentabiotic (Fort Dodge, Brazil), i.m., and were placed in a thermal-controlled cage to prevent hypothermia, until complete anesthetic recovery.

Microinjections were made through a thin dental needle (0.3 mm o.d.) which extended 3.25 mm beyond the guide cannula's tip. The needle was connected to a 5.0-µl Hamilton microsyringe by PE-10 polyethylene tubing, and a total volume of 0.3 µl was injected over a 60-s period. The animals randomly received a single DPAG microinjection of glycine (GLY; 10, 20, 50, and 80 nmol) or vehicle (phosphate-buffered physiological saline). The GLY_B receptor antagonist, (+/−)-3-amino-1-hydroxy-2-pyrrolidone (HA966; 10 nmol) was applied alone or combined with glycine. The animals were injected only once, and the algesimetric test initiated after 5 min.

At the end of the experiments, the animals were deeply anesthetized by an i.p. injection of a xylazine/ketamine mixture (2 ml/animal) and intracardialy perfused with physiological saline (NaCl 0.9%) followed by 10% formalin solution. Evans Blue (0.5%, 0.3 µl) was applied through the guide cannula to dye the injection site. Entire brains were removed and stored in 10% formalin for later histological analysis. Frozen sections (50 µm) were obtained using a cryostat, mounted in glass slides and stained using the Giemsa (Sigma, USA) method for microscopic identification of the injection site. Only those rats whose microinjection site was located within the DPAG were used for analysis (Fig. 1). As shown in Fig. 1, histological analysis confirmed that the majority of the microinjection sites were in the caudal portions of the DPAG (i.e. 7.3–8.0 mm from bregma).

Tail-flick test

A 25-W heat source apparatus (Ugo Basile, Model 7360) was directed towards the tail at 1 cm from the caudal tip. The heating slope was calibrated to evoke baseline tail-flick latencies (TFL) of 5–6 s. The cut-off time was 21 s in order to prevent tissue damage. Three, 10 min apart TFLs were taken 60 min before DPAG microinjection (baseline control). After DPAG stimulation, TFLs were taken at 5, 15, 25, 35, 45, 55 and 65 min.

Drugs and vehicles

Glycine (Sigma, USA) and HA966 (Tocris, USA) were dissolved in a phosphate-buffered saline (PBS) solution.

Statistical analysis

All statistical analyses were carried out using Graphpad Prism version 3. All values presented are mean±S.E.M. (standard error of mean) of 5–10 rats. Each group comprised 5–10 animals. Multiple comparisons were

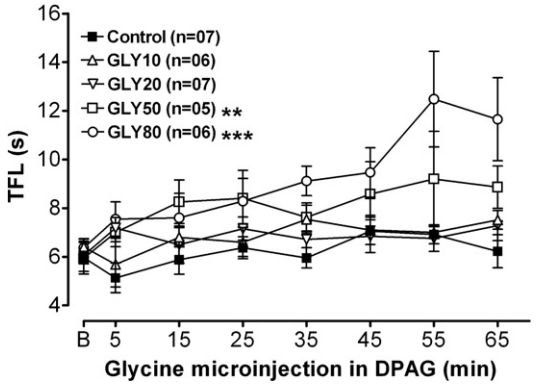

Fig. 2. Hyponociceptive effect induced by glycine microinjections into the DPAG. Glycine (GLY) at doses of the 10, 20, 50 and 80 nmol/0.3 µl/site was applied into the DPAG in rats. The radiant heat-induced tail-flick was tested after 5 min, and every 10 min thereafter for a period of 65 min. Data are shown as mean±S.E.M. of the tail-flick latencies in seconds (TFL, s). Control animals received only PBS (0.3 µl/site). ** $P<0.01$; *** $P<0.001$ (ANOVA for repeated measures followed by Tukey's test).