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Modulation of tonic immobility in guinea pig PAG by homocysteic acid, a glutamate agonist

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ABSTRACT

Tonic immobility (TI) is an innate defensive behavior elicited by physical restriction and postural inversion, and is characterized by a profound and temporary state of motor inhibition. The participation of the periaqueductal gray matter (PAG) in TI modulation has previously been described. In addition, the excitatory amino acids (EAA) are important mediators involved in the adjustment of several defensive responses produced by PAG. In the present study, we investigated the effect of microinjection of the EAA agonist DL-homocysteic acid (DLH) and the *N*-methyl-D-aspartate (NMDA) receptor antagonist (MK-801) into the ventrolateral and dorsal PAG over the duration of TI in guinea pigs. Microinjection of 15 nmol/0.2 µl of DLH into the ventrolateral PAG (vlPAG) and 30 nmol/0.2 µl of DLH into the dorsal PAG (dPAG) promoted an increase and decrease in TI duration, respectively. These responses were blocked by prior microinjection of the NMDA receptor antagonist, MK-801 (3.6 nmol/0.2 µl) at the same site. Microinjection of MK-801 alone into the vlPAG and dPAG did not alter the duration of TI episodes. These results suggest that NMDA receptors are involved in the modulation of TI in both the vlPAG and dPAG. In addition, PAG excitatory amino acids modulate the TI response via columnar organization of the PAG. In this manner, the vlPAG facilitates TI modulation whereas dPAG has an inhibitory role in TI.

1. Introduction

Tonic immobility (TI) is an innate defensive response characterized by a temporary state of profound akinesis and a relative lack of responsiveness to external stimuli. This response is triggered in many vertebrate and invertebrate species by different types of sensory stimuli, mainly those from prolonged physical contact with predators [1,2]. Generally, TI is elicited in extreme conditions, when the captured prey expresses its limitation without loss of control of the situation [1,2]. The lack of movement caused by TI frequently interrupts the predator attack and consequently promotes a condition where the seized prey may be able to escape [3].

Studies carried out in our laboratory showed the participation of periaqueductal gray matter (PAG) in the modulation of the TI response. This participation is distinct between the longitudinal columns. The cholinergic stimulation of dorsal PAG (dPAG) decreases TI duration, while the same stimulation in the ventrolateral PAG (vlPAG) increases the TI response [4].

Some research has associated the vlPAG with modulation of passive strategies of defensive reactions, whereas the dPAG is associated with the mediation of active forms of defense to cope with threats [5,6]. In this context, administration of excitatory amino acids (EAA), specifically the DL-homocysteic acid (DLH), into distinct regions of the rat and cat PAG changes somatomotor and autonomic responses [7–10]. According to Carrive [5] and Bandler and Shipley [6], stimulation of the vlPAG of rats and cats by DLH microinjection causes opioid-mediated antinociception, quiescence, bradycardia, hypotension and a decreased responsiveness to the environment (hyporeactivity). In contrast, EAA microinjection into the dPAG produces reactions associated with fight and flight [5,6,11].

Some studies have demonstrated the presence of a high density of glutamatergic receptors and neurotransmitters in the PAG of rats and cats [12–14], suggesting that EAA are involved in the PAG modulation of behavioral activities. Nonetheless, the participation of EAA in the mediation of TI responses has still not been demonstrated. The present study investigated the effects of injecting agonists (DLH) and antagonists (MK-801) into the ventrolateral and dorsomedial/dorsolateral (dorsal) PAG upon the TI duration in guinea pig, with the aim of contributing to the understanding of the role of EAA in modulation of the TI response.

2. Materials and methods

2.1. Animals

Adult male guinea pigs (*Cavia porcellus*) weighing 400 to 500 g were obtained from the animal care facility of the Faculty of Medicine of Ribeirão Preto (FMRP). The animals (n=90) were kept in Plexiglas wall cages (56×37×39 cm, five animals per cage) in a room

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maintained at 24±1 °C, on a 12-h light cycle, with free access to water and food. The experiments were carried out in compliance with the recommendations of Brazilian Association for Laboratory Animal Science (COBEA), with the approval (Proc.no. 051/2005) of the Ethical Committee for Animal Experimentation of the Ribeirão Preto School of Medicine, University of São Paulo. All efforts were made to minimize animal suffering.

2.2. Tonic immobility recordings

In the present study, each animal was submitted to five control maneuvers of TI induction and the duration of the episodes was recorded. Induction of TI was carried out by holding the animal around the thorax, quickly inverting it and pressing it down into a V-shaped plywood trough (30×17×17 cm). The pressure applied by the hands of the experimenter was proportional to the resistance offered by the animal to the restraining maneuver. When the animal stopped moving, the experimenter slowly withdrew their hands and a chronometer was activated to measure the duration (in seconds) of the response, which ended when the animal resumed the upright position. If the animal did not become motionless within 60 s, the episode was recorded as having zero duration. For group analysis, the mean of five episodes per animal was considered. It was established that, in one and the same trial group, the animals serve as their own controls, that is, the mean of TI episodes in the control situation was compared with the mean of the sham and of the trial situation.

2.3. Surgical procedures

One day after the control TI episode, the animals were anesthetized by intramuscular injection of 40 mg/kg ketamine plus 5 mg/kg xylazine, and placed in a stereotaxic apparatus (David–Kopf Instruments, USA) with the mouthpiece 21.4 mm below the interauricular line, and a guide cannula (14 mm long and 0.6 mm outer diameter, prepared from a hypodermic needle) was implanted into the PAG.

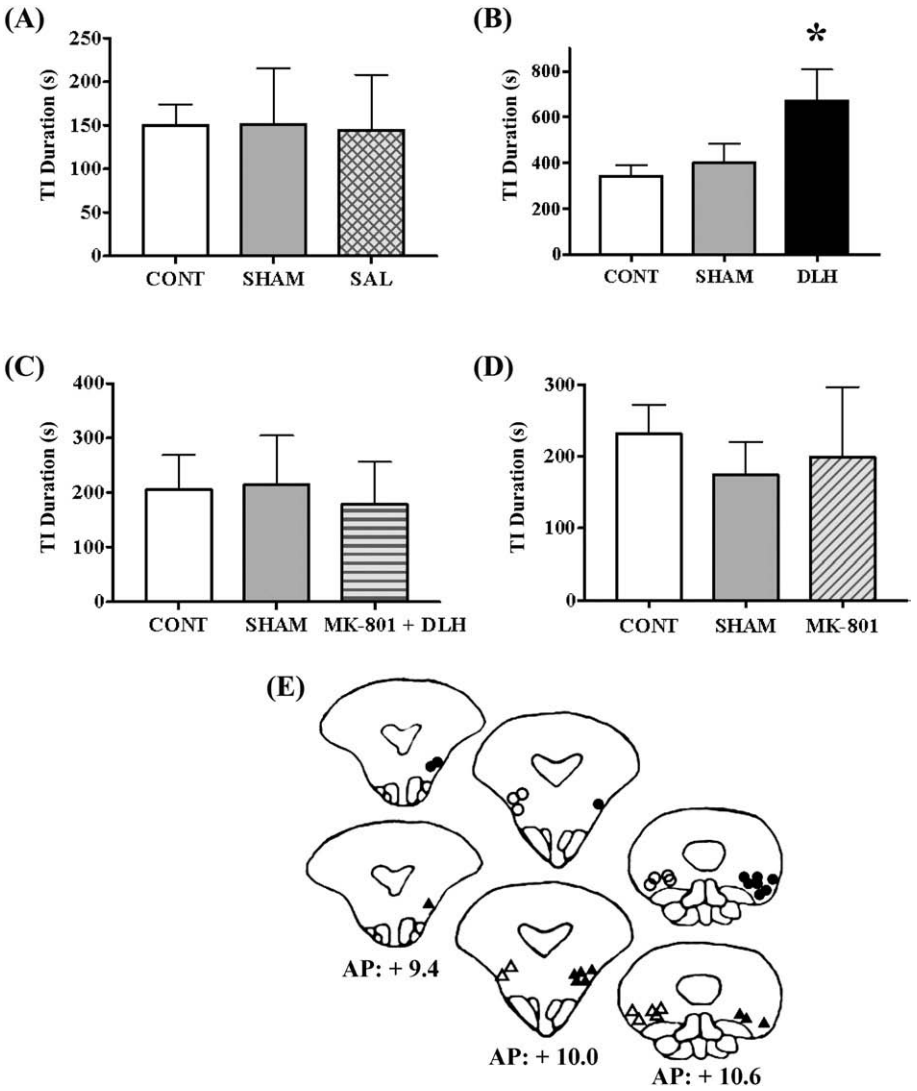

Fig. 1. Duration of tonic immobility (TI) episodes. Mean±S.E.M. TI duration under control conditions (CONT), after surgery (SHAM) and after microinjection of different drugs into the ventrolateral periaqueductal gray matter (vlPAG). (A) TI duration after saline (SAL) microinjection (0.2 µl; $n=7$); (B) TI duration after DLH microinjection (15 nmol /0.2 µl; $n=10$); (C) TI duration ($n=7$) after MK-801 microinjection (3.6 nmol /0.2 µl) and prior DLH microinjection (15 nmol/0.2 µl); (D) TI duration after MK-801 microinjection (3.6 nmol /0.2 µl; $n=9$). *$p<0.05$ compared to CONT and SHAM. (E) Schematic drawing of frontal sections obtained at representative levels of the guinea pig periaqueductal gray matter. The open circles (○) represent the sites where saline solution was microinjected; the filled circles (●) represent the sites where DLH (15 nmol) was microinjected; the open triangles (△) represent the sites where MK-801 + DLH were microinjected; the filled triangles (▲) represent the sites where MK-801 (3.6 nmol) was microinjected. All microinjections were made on the right side, however DLH and MK-801 are illustrated on the left side for clarity.