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Research report

L-NOARG-induced catalepsy can be influenced by glutamatergic neurotransmission mediated by NMDA receptors in the inferior colliculus

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HIGHLIGHTS

- The catalepsy induced by L-NOARG can be enhanced by intracollicular microinjection of NMDA.
- Intracollicular microinjection of AP7 attenuated the catalepsy induced by L-NOARG ip.
- Glutamatergic neurotransmission into the inferior colliculus can modulate the catalepsy induced by a NOS inhibitor.
- Possible involvement of the glutamate-mediated mechanisms into the inferior colliculus in the sensorimotor and sensorilimbic gating.

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ABSTRACT

The inferior colliculus (IC), a midbrain structure that processes acoustic information of aversive nature, is distinguished from other auditory nuclei in the brainstem by its connections with structures of the motor system. Recent evidence relating the IC to motor behavior shows that glutamate-mediated mechanisms in the neural circuits at the IC level modulate haloperidol-induced catalepsy. It has been shown that N^G-nitro-L-arginine (L-NOARG), inhibitor of enzyme nitric oxide synthase (NOS), can induce catalepsy after intraperitoneal (ip), intracerebroventricular or intrastriatal administration. The present study examined whether the catalepsy induced by L-NOARG (ip) can be influenced by collicular glutamatergic mechanisms and if a NO-dependent neural substrate into the IC plays a role in this immobility state. L-NOARG-induced catalepsy was challenged with prior intracollicular microinjections of glutamate NMDA receptor antagonists, AP7 (20 or 40 nmol/0.5 μl), or of the NMDA receptor agonist N-methyl-D-aspartate (NMDA, 30 nmol/0.5 μl). Catalepsy was evaluated by positioning both forepaws of the rats on an elevated horizontal wooden bar and recording the time for which the animal maintained this position. The results showed that intracollicular microinjection of AP7 previous to systemic injections of L-NOARG (90 mg/kg) significantly attenuated the catalepsy. Conversely, intracollicular microinjection of NMDA increased the time of catalepsy when administered 10 min before systemic L-NOARG (10 or 45 mg/kg). The microinjection of L-NOARG (50 or 100 nmol) directly into the IC was not able to induce catalepsy. These findings suggest that glutamate-mediated mechanisms in the neural circuits of the IC modulate L-NOARG-induced catalepsy and participate in the regulation of motor activity.

1. Introduction

Nitric oxide (NO) is a short-lived, highly diffusible free radical gas and is recognized as a messenger molecule in the nervous system, where it is synthesized from L-arginine by a nitric oxide synthase (NOS) [1,2]. NO acts as a signaling molecule in the central nervous system and has been related to several physiological or pathological conditions [3,4]. Several lines of evidence suggest that NO modulates motor behavior, probably by interfering with dopaminergic, serotonergic, cholinergic and glutamatergic neurotransmission in the striatum [5]. Rats and mice treated with various NOS inhibitors show problems with fine motor control [6–11]. Systemic or intrastriatal administration of N^G-nitro-L-arginine (L-NOARG), an inhibitor of NOS, induces catalepsy [11–14].

The inferior colliculus (IC) is primarily involved in the processing of auditory information, but it is also in a position to send auditory information to motor centers that undoubtedly participate in behaviors such as prey catching, predator avoidance, or orientation to a novel stimulus or communication sound [15]. The IC has

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a massive projection to the cerebellum via pontine gray suggesting that auditory information is provided to neural networks that coordinate timed movements [16–18]. In addition, there is also evidence that motor systems project to the IC. Projections from the substantia nigra [19] and from the globus pallidus to the IC have been reported [20]. There is also physiological evidence suggesting that the responses of neurons in the IC can be modulated by motor activity [21].

It is interesting to observe that in the subcortical auditory pathway, the highest levels of NOS are found in the IC. Two studies in rat have described the distribution of neurons that express NOS in the IC [22,23]. In each case the presence of NOS was identified by staining for β-nicotinamide adenine dinucleotide phosphate diaphorase (NADPH-d), which in aldehyde fixed tissue, represents the activity of NOS [24,25].

More recently, we demonstrated that intracollicular administration of glutamate receptor antagonists (AP7 and MK-801) and agonist (NMDA) influences antipsychotic haloperidol-induced catalepsy in rats, with the antagonists attenuating the catalepsy and the agonist potentiating it [26]. These findings suggest that glutamatergic neural substrates in the IC modulate the state of immobility characterizing catalepsy.

In spite of these findings, the possible involvement of the IC with the cataleptic effect of NOS inhibition has not yet been investigated. The objective of the present study was to investigate the participation of the NO neural substrate in the IC in this immobility state and to determine if catalepsy induced by NOS inhibition could be modulated by collicular glutamatergic mechanisms.

2. Materials and methods

2.1. Animals

Male Wistar rats from the animal facility of the State University of Campinas (UNICAMP) weighing 220–260 g at the beginning of the experiments were housed in Plexiglas-walled cages under a 12 h/12 h light/dark cycle (lights on at 07:00 h) at 23 ± 1 °C, with food and water available ad libitum. The experiments were conducted during the light phase of the light/dark cycle (between 14:00 and 18:00 h). All experiments were performed in accordance with the recommendations of the Brazilian Society for Neuroscience and Behavior (SBNeC), which are based on the guidelines of the American National Institute of Health for the Care and Use of Laboratory Animals. All efforts were made to minimize the number of animals used and their suffering.

2.2. Surgery

Animals were anesthetized with xylazine/ketamine (200 mg/kg and 100 mg/kg respectively) ip and fixed in a stereotaxic frame (David Kopf Instruments, Tujunga, CA, USA). A stainless steel guide cannula (outer diameter, 0.6 mm; inner diameter, 0.4 mm) was vertically introduced unilaterally and aimed at the IC using the following coordinates, with bregma serving as the reference: anterior/posterior, −8.8 mm; medial/lateral, 1.5 mm; and dorsal/ventral, 3.5 mm [27]. A stylette inside the guide cannula prevented cannula occlusion. All subjects were allowed to recover for 7 days after surgery and had ad libitum access to food and water.

2.3. Drugs

N^G-nitro-L-arginine (L-NOARG, Sigma) was dissolved in a 0.01 N HCl solution. The drugs 2-amino-7-phosphonoheptanoic acid (AP7; Ciba-Geigy Corp, Basel, Switzerland) and NMDA (Sigma–Aldrich, St. Louis, MO, USA) were all dissolved in physiological saline. All intracollicular microinjections were performed in a volume of 0.5 μl, and systemic injections of drugs were administered in a volume of 1 ml/kg. Controls received an equivalent volume of physiological saline or vehicle. Drug solutions were freshly prepared before administration.

2.4. Microinjection procedure

After removal of the stylette, the microinjections were performed with a thin dental needle made of stainless steel (Mizzy, São Paulo, Brazil; 30 gauge, outer diameter, 0.3 mm) that was introduced through the guide cannula until its tip protruded 1 mm. This infusion needle was connected by a polyethylene tube to a 10 μl Hamilton syringe, and 0.5 μl of the vehicle or drug solution was injected directly into the IC over 1 min under the control of an infusion pump (Model BI2000, Insight Instruments, Ribeirão Preto, Brazil). The needle was left in place for an additional minute after injection to allow for diffusion. All animals used in this study were naive and received only a single drug microinjection.

2.5. L-NOARG-induced catalepsy

Catalepsy was evaluated by carefully positioning both forepaws of the animal on a horizontal wooden bar at a height 8 cm above the floor, while their hind paws remained on the floor [28]. A cataleptic animal will maintain this position without stepping down. The time in seconds (s) during which the animal maintained this position was recorded (300 s maximum). This measure of catalepsy strength was made at 10, 30, 60, 90 and 120 min after intraperitoneal administration or 60, 120, 180 and 240 min after intracollicular administration of L-NOARG. The experimental session was recorded with a video-recording system and analyzed by two different observers that were not aware of the pharmacological treatment. The experiments were conducted in a quiet room.

The following experiments were performed: *Experiment 1*; to assess the effects of NMDA glutamate receptor antagonists, the rats received a prior microinjection of AP7 (20 or 40 nmol/0.5 μl; AP7/L-NOARG groups; $n = 9$ to each group), or physiological saline (0.5 μl; Sal/L-NOARG group; $n = 10$) directly into the IC, followed 10 min later by an intraperitoneal injection of L-NOARG (90 mg/kg). Control groups received AP7 (20 or 40 nmol/0.5 μl; AP7/Vehicle groups; $n = 8$ and 9, respectively), or physiological saline (0.5 μl; Sal/Vehicle group; $n = 11$) injected directly into the IC, followed 10 min later by an intraperitoneal injection of vehicle (1 ml/kg). *Experiment 2*; to investigate the effects of the glutamate receptor agonist, NMDA (30 nmol/0.5 μl; NMDA/L-NOARG10 and NMDA/L-NOARG45 groups; $n = 9$ and 10, respectively;) or physiological saline (1 ml/kg; Sal/L-NOARG10 and Sal/L-NOARG45 groups; $n = 9$ and 8, respectively) was microinjected into the IC 10 min before L-NOARG administration (10 or 45 mg/kg). The dose of NMDA (30 nmol) used in this experiment was based on results from previous studies [26] and the lower doses of L-NOARG were based upon a dose–response curve, previously obtained in pilot studies (data not shown). These doses of L-NOARG were sufficient to induce mild catalepsy and avoid a ceiling effect, since enhanced catalepsy was to be expected after NMDA agonist microinjection in the IC. Additional groups received NMDA (30 nmol/0.5 μl; NMDA/Vehicle group; $n = 11$) or physiological saline (0.5 μl; Sal/Vehicle group; $n = 7$) followed in 10 min by an injection of vehicle (1 ml/kg, ip). *Experiment 3*; to investigate if NO synthesis' inhibition in the IC could induce catalepsy, L-NOARG (50 or 100 nmol/0.5 μl L-NOARG groups; $n = 9$ and 8, respectively) or vehicle (1 ml/kg Vehicle group; $n = 9$) were microinjected directly into this structure. These doses and the time of observation (60, 120, 180 and 240 min) were chosen based on results from previous studies [12,14,29].

2.6. Histology

At the end of the behavioral experiments the animals were deeply anesthetized with xylazine/ketamine (300 mg/kg and 200 mg/kg, respectively) ip and perfused through the left ventricle. The blood was washed out with physiological saline followed by 200 ml ice-cold 4% (w/v) paraformaldehyde in 0.1 M sodium phosphate buffer, pH 7.3. The midbrains were quickly removed and immersed for 4 h in fresh fixative at 4 °C. After fixation, the brains were frozen and 50 μm serial brain sections were cut using a microtome. The sections were stained with methylene blue in order to locate the positions of the cannula tips, according to the atlas by Paxinos and Watson's [27]. Data from rats with cannula tips located outside the IC were not included in the statistical analysis.

2.7. Statistical analysis

The latency to step down from the bar was analyzed using the nonparametric Kruskal–Wallis H-test. Comparisons between treatment groups for the measurements conducted at each time-point (10, 30, 60, 90 and 120 min after intraperitoneal or 60, 120, 180 and 240 min after intracollicular L-NOARG administration) were made using the Bonferroni post hoc test. Values of $P < 0.05$ were considered statistically significant.

3. Results

The sites of microinjections were mainly localized in the central nucleus of the IC, but some were also localized to the cortical dorsal nucleus and external cortical nucleus of the IC (Fig. 1).

Fig. 2 shows that the L-NOARG (90 mg/kg) alone induced catalepsy, and that this catalepsy was indeed attenuated by previous intracollicular microinjection of both doses of AP7 in each time (10 min: $H = 12.48$, $P < 0.05$; 30 min: $H = 24.16$, $P < 0.0001$; 60 min: $H = 25.51$, $P < 0.0001$; 90 min: $H = 28.29$, $P < 0.0001$; 120 min: $H = 27.19$, $P < 0.0001$). It also shows that the IC microinjection of the antagonist AP7 had no effect alone, i.e., when vehicle was