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Behavioural Effects Induced by Microinjection of L-BOAA Into the Ventrolateral PAG Matter of the Mouse

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MAIONE, S., L. BERRINO, J. LEYVA, V. DE NOVELLIS, M. PALLOTTA AND F. ROSSI. *Behavioural effects induced by microinjection of L-BOAA into the ventrolateral PAG matter of the mouse.* PHARMACOL BIOCHEM BEHAV **50**(3) 453–455, 1995. – L-BOAA (1 μg/mouse), microinjected into the ventrolateral periaqueductal gray (PAG) matter, induced a strong reaction of forward avoidance (running) for 20–30 s in 18% of the mice and immobility for 7 ± 2 min in 72% of the mice from a total of 68 treated animals. Effects were also observed for grooming and clonus in 6% and 4% of the mice, respectively. Duration of L-BOAA-induced immobility was significantly ($p < 0.05$) reduced by a pretreatment with CNQX (0.5 μg/mouse), a selective antagonist of AMPA glutamergic subtype receptors, but not by a pretreatment with 2-APV (0.5 μg/mouse), a selective antagonist of NMDA glutamergic subtype receptors, nor by 2-AP3 (0.5 μg/mouse), a weak antagonist of metabotropic glutamergic subtype receptors. AMPA (0.05 μg/mouse), also microinjected into ventrolateral PAG, induced the same pattern of behavioural effects as L-BOAA. Forward avoidance, grooming, and clonus induced by L-BOAA or AMPA were also significantly antagonised by a pretreatment with CNQX (data not shown).

L-BOAA Ventrolateral PAG Behaviour Mouse

β-*N*-OXALYLAMINO-L-ALANINE (L-BOAA) is a non-structural amino acid that is present in the seeds of *Lathyrus sativus* L. (chickling pea). It has been suggested that L-BOAA may participate in the genesis of selective damage to cortico-spinal tract motor neurones and is thought to induce neurolathyrism (3,9,10,12).

Studies in vivo demonstrated that administration of non-convulsive doses of L-BOAA to primates causes a disease of upper motoneurones (9). Ross et al. (7) demonstrated in cerebral cortical slices of the mouse that micromolar concentrations of L-BOAA modify normal cell bodies morphology (postsynaptic vacuolisation and widespread neuronal degeneration) and, 2 years later, reported that L-BOAA in vitro mainly binds non-NMDA glutamergic subtype receptors (8). More recently, Willis et al. evaluated neurotoxic effects of L-BOAA in vivo, at the level of hippocampus, and confirmed the involvement of non-NMDA glutamergic subtype receptors. In particular, they concluded that L-BOAA-induced neurotoxicity would be mainly mediated by AMPA receptors than by KA or NMDA glutamergic receptors (13).

Thus, although L-BOAA seems to be a new and powerful analogue of glutamate that occasionally may be introduced with diet, information is lacking about the possible behavioural changes induced by L-BOAA when directly administered into the brain. Our previous studies demonstrated that glutamate participates in modulating cardiovascular function and defensive behaviour at the level of midbrain periaqueductal gray (PAG) matter (6). The PAG area is the most caudal region of the CNS by which an affective defence reaction can be obtained (4,5). We carried out the present study to evaluate in mice the major categories of behavioural effects produced by a nonconvulsant dose of L-BOAA when microinjected into the ventrolateral PAG matter.

METHOD

Subjects

Male Swiss-Webster mice (25–30 g) were used. Animals were housed in a group of five per cage and maintained at 20

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± 2°C and humidity of 50 ± 10% in a constant 12L : 12D (lights on at 0700 h). Food and water were available ad lib.

Surgical Preparation and Treatment

To carry out direct intracerebral administrations of drugs or respective vehicles (saline or dimethyl sulfoxide/saline 0.05%, v/v), a stainless steel guide cannula was fixed to the skull (flat positioned) with dental zinc cement. This guide cannula was implanted, 2 days before experimentation, above the ventrolateral PAG area under ketamine anaesthesia (100 mg/kg, IP). We employed a David Kopf stereotaxic apparatus and applied coordinates obtained in our laboratory (measured from bregma in mm: AP: −7.8; L: 0.2; V: 1.4). The day of the experiment each animal was placed in a Plexiglas cage (20 × 10 × 13 cm) and allowed to move freely for 15–20 min. Microinjections into lateral-ventral PAG area were conducted with a stainless steel fine cannula (o.d. 0.6 mm), connected by a polyethylene tube to a Hamilton 1-μl syringe, and carefully inserted through guide cannula. A volume of 100 nl drug solution, or vehicle, was injected. We observed the effects evoked by unilateral microinjections of L-BOAA (1 μg/mouse) or α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA, 0.05 μg/mouse) in mice pretreated (5 min before) or not with DL-2-amino-5-phosphono valeric acid (2-APV, 0.5 μg/mouse), a selective antagonist of the glutamergic NMDA subtype receptors; 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX, 0.5 μg/mouse), a selective antagonist of the glutamergic non-NMDA subtype receptors; 2-amino-3-phosphono-propionic acid (2-AP3, 0.5 μg/mouse), a weak antagonist of the glutamergic metabotropic subtype receptors. Each animal was observed for a period of 15 min during which we have highlighted the main behavioural modifications. At the end of the experiment, a volume of 100 nl of neutral red (0.1%) was injected intracerebrally 5 min before sacrificing the mouse with high dose of pentobarbital (200 mg/kg, IP). The brain was removed and immersed into saturated formalin solution for 2 days. The injection site was ascertained by using two consecutive sections (40 μm), one stained with cresyl violet to identify nuclei and the other one unstained to determine the dye diffusion. Only those mice that the microinjected site was located within the lateral-ventral PAG matter were used for data computation.

Statistical Analysis

The duration of immobility produced by L-BOAA or AMPA, alone or after 2-APV, CNQX, or 2-AP3 pretreatment, was analysed by analysis of variance (ANOVA) followed by the Newman-Keuls test (11). The level of significance was considered at $p < 0.05$.

Drugs

The following drugs were used: β-*N*-oxalylamino-L-alanine (L-BOAA), α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA), 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX), 2-amino-3-phosphono-propionic acid (2-AP3) (RBI, Natick, MA), 2-amino-5-phosphonovaleric acid (2-APV) (Sigma Chemical Co., St. Louis, MO), and ketamine hydrochloride (C. H. Boehringer Ingelheim, Florence, Italy).

RESULTS

Microinjection of 100 nl of saline or dimethyl sulfoxide/saline (0.05%, v/v) into the ventrolateral PAG matter did not induce apparent behavioural changes. L-BOAA (1 μg/mouse), microinjected into the same area immediately (2–5 s) induced a strong reaction of forward avoidance (running) for 20–30 s and immobility for 7 ± 2 min, respectively, in 18% and 72% of the total 68 treated animals (Fig. 1). Effects were also observed for grooming and clonus in 6% and 4% of the animals, respectively (Fig. 1). However, with higher doses (5 and 10 μg/mouse) L-BOAA, in a dose-related manner, induced wild running and tonic-clonic seizures, often followed by death of the animal (data not shown). L-BOAA-induced immobility was significantly ($p < 0.05$) reduced by a pretreatment with CNQX (0.5 μg/mouse), a selective antagonist of AMPA glutamergic subtype receptors, but not by its vehicle dimethyl sulfoxide/saline (0.05%, v/v) (Fig. 2). The duration of immobility induced by L-BOAA was not changed by a pretreatment with 2-APV (0.5 μg/mouse), a selective antagonist of *N*-methyl-D-aspartate (NMDA) glutamergic subtype receptors, nor by 2-AP3 (0.5 μg/mouse), a weak antagonist of metabotropic glutamergic subtype receptors (Fig. 2). During L-BOAA-induced immobility, we also observed exophthalmos and apparent leg muscular stiffness (data not shown). AMPA (0.05 μg/mouse), also microinjected into the ventrolateral PAG, induced the same pattern of behavioural effects observed after L-BOAA (Fig. 1). Forward avoidance, grooming, and clonus induced by L-BOAA and AMPA were significantly antagonised by a pretreatment with CNQX (data not shown).

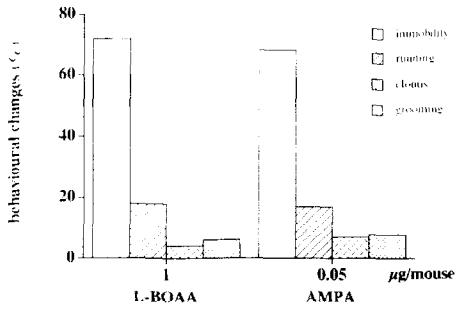


FIG. 1. Behavioural modifications (immobility, running, grooming, and clonus), expressed in percentage of responsive animals, induced by L-BOAA (1 μg/mouse) or by AMPA (0.05 μg/mouse) directly administered into the ventrolateral periaqueductal gray (PAG) matter of freely moving mice (25–30 g). Each group was 20–25 animals.

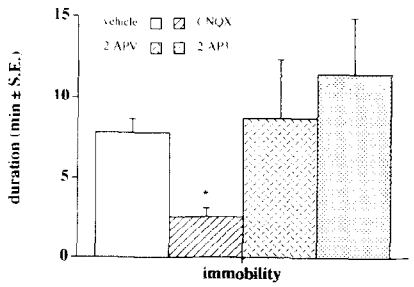


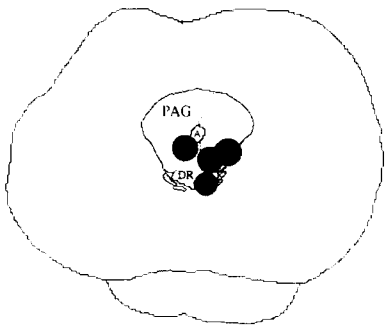
FIG. 2. Duration (min ± SE) of immobility induced by L-BOAA (1 μg/mouse) microinjected into the ventrolateral periaqueductal gray (PAG) matter of freely moving mice (25–30 g) and pretreated or not with 0.5 μg/mouse of DL-2-amino-5-phosphonovaleric acid (2-APV), 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX), or 2-amino-3-phosphonopropionic acid (2-AP3). The duration of immobility was statistically evaluated by ANOVA (*$p < 0.05$ with respect to saline pretreatment). Each group was 20–25 animals.

DISCUSSION

Previous studies, in vitro and in vivo, that have been carried out to understand L-BOAA mechanisms of action mainly evaluated its powerful neurodegenerative action (2,8). These studies showed an important implication of this neurotoxin for the genesis of motor system disorders (i.e., neurolathyrism and amyotrophic lateral sclerosis) and demonstrated that L-BOAA induces neuronal loss by activation of non-NMDA glutamergic subtype receptors.

The current study analysed the possible behavioural effects induced by L-BOAA when microinjected directly into the midbrain PAG matter. There have been two features of this area that have solicited interest among scientists: nociception control and modulation of behavioural changes, which are generally defined as defensive behaviour (4,5). In particular, following ventrolateral PAG microinjection of L-BOAA, two major categories of behavioural effects have been observed during a nonsocial test: forward avoidance (running) or immobility (18% and 72%, respectively). These results agree with previous studies demonstrating that chemical stimulation of ventrolateral PAG area mainly generates immobility (1). Due to the fact that immobility may allow animals to recover after stress, immobility as well may be considered a basic feature of a defensive reaction. Because all L-BOAA-induced behavioural changes were antagonised with a selective non-NMDA receptor antagonist, our data further confirm that this vegetable neurotoxin primarily activates non-NMDA glutamergic subtype receptors.

Although the immobility described in the rat by Bandler and coworkers (1) after stimulation of ventrolateral PAG is mainly characterised by relaxation, in this study we observed immobility and a muscle tone increase (1–3 min) with L-BOAA treatment. After this first phase, with an apparent muscular stiffness of the legs, all animals evolved to muscle relaxation for some more minutes (2–9 min). It is difficult to explain such a biphasic effect by L-BOAA on muscle tone. It is not suitable to hypothesise an involvement of nuclei close to ventrolateral PAG to explain this effect, because the length of the horizontal axis of mouse PAG is about 1.85 mm and the vertical axis is 1.65 mm. In fact, it is currently recognised that the theoretical radius of the sphere of a 1-μl volume is about 1.240 mm, indicating that our injections (only 100 nl of volume) were made inside the PAG (Fig. 3), also confirmed histologically. Therefore, our results further support the involvement of L-BOAA for neurolathyrism and also indicate that non-NMDA receptor stimulation at the level of the ventrolateral PAG, and not only at the level of cortico-spinal tract motor neurons, may be involved in generating neurolathyrism muscular stiffness.



Bregma -7.8 mm

FIG. 3. Schematic representation of a coronal section of mouse periaqueductal gray (PAG) matter showing localisation of the microinjection sites. Stereotaxic coordinates obtained in our laboratory (measured from bregma in mm: AP: −7.8; L: 0.2; V: 1.4) have been applied. DR: dorsal raphe; A: aqueduct.

In conclusion, this preliminary study demonstrates that, in vivo, L-BOAA neurotoxic effects are mainly mediated by activation of non-NMDA glutamergic subtype receptors and that PAG matter may be involved in these effects.

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