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Defense reaction induced by a metabotropic glutamate receptor agonist microinjected into the dorsal periaqueductal gray of rats

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Abstract

The behavioral effects of trans-(±)-1-amino-1,3-cyclopentanedicarboxylic acid (t-ACPD), a metabotropic glutamate receptor (mGluR) agonist, or 0.9% (w/v) saline, injected into the dorsal periaqueductal gray (DPAG), was investigated. Male Wistar rats showed defense reactions characterized by jumps toward the top edges of the cages (saline = 0 *vs* t-ACPD = 6.0, medians $P<0.05$) and gallops (saline = 0 *vs* t-ACPD = 10.0, medians $P<0.05$) during the 60-s period after the beginning of the injection. In another experiment animals were placed inside an open arena for 5 min immediately after injection. Their behavior was recorded by a video camera and a computer program analyzed the videotapes. Eleven of fifteen rats injected with t-ACPD showed a short-lasting (about 1 min) flight reaction. No saline-treated animal showed this reaction ($P<0.0005$, chi-square test). The drug induced an increase in turning behavior ($P = 0.002$, MANOVA) and a decrease in the number of rearings ($P<0.001$, MANOVA) and grooming episodes ($P<0.001$, MANOVA). These results suggest that mGluRs play a role in the control of defense reactions in the DPAG.

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- Rats

It has been well established that the periaqueductal gray (PAG) matter is implicated in the control of vocalizations, nociception and defensive behavior (1). Considerable evidence suggests that excitatory amino acids (EAA) play a critical role in integrating somatic and autonomic reactions in the PAG (2). L-Glutamic acid is the principal EAA neurotransmitter in the mammalian nervous system, acting on multiple receptor types classified as ionotropic and metabotropic receptors (3). Most of these receptor subtypes are found in the dorsolateral subdivision of the PAG (4). The metabotropic receptor family is linked by G-proteins to various second messenger systems. Eight subtypes have been cloned so far and they can be divided into three groups based on sequence homology, signal transduction system and pharmacology (5). Many studies have been performed to investigate the effects of agonists, antagonists or modulators of glutamatergic ionotropic receptors on defensive reactions (6,7). Only recently has the role of mGluR in these reactions begun to be investigated (8). The objective of this study was to examine the behavioral effects of trans-(±)-1-amino-1,3-cyclopentanedicarboxylic acid

(t-ACPD), a metabotropic glutamate receptor (mGluR) (class I or II) agonist (5), microinjected into the dorsolateral PAG (DPAG) of rats.

Male Wistar rats weighing 220-250 g were housed in pairs, with free access to food and water in a temperature-controlled room (24 ± 1°C) under a 12/12-h light/dark cycle (lights on at 7:00 a.m.) throughout the experiment. The rats were anesthetized with 2.5% 2,2,2-tribromoethanol (10 ml/kg, *ip*) and submitted to stereotaxic surgery to implant a stainless steel guide cannula (0.7 mm O.D.) aimed at the DPAG (coordinates: 1.9 lateral to the lambda and 4.0 mm below the surface of the skull at an angle of 16° with the sagittal plane). The cannula was attached to the bone with stainless steel screws and acrylic cement, and a stylet was introduced into it to prevent obstruction.

Seven days after surgery, unrestrained animals housed in a Plexiglas cage (29 x 19 x 34 cm) received microinjections of saline (0.3 µl) or t-ACPD (30 nmol/0.3 µl). The dose employed (30 nmol) was based on a report by Leyva et al. (9) and on a pilot study from our laboratory showing no effect of a 10-nmol dose. t-ACPD was dissolved in 0.1 M NaOH and the pH adjusted to 7.2 by adding 0.1 N HCl. Animals were microinjected with a thin dental needle (0.3 mm O.D.) which extended 1 mm below the cannula tip. A volume of 0.3 µl was injected over 30 s using a Hamilton microsyringe controlled by an infusion pump (model 200, kdScientific, Boston, MA, USA). After the experimental session, the rats were sacrificed and the injection sites were determined histologically according to the atlas of Paxinos and Watson (10). Only data from animals that received the injections inside the DPAG were used for analysis.

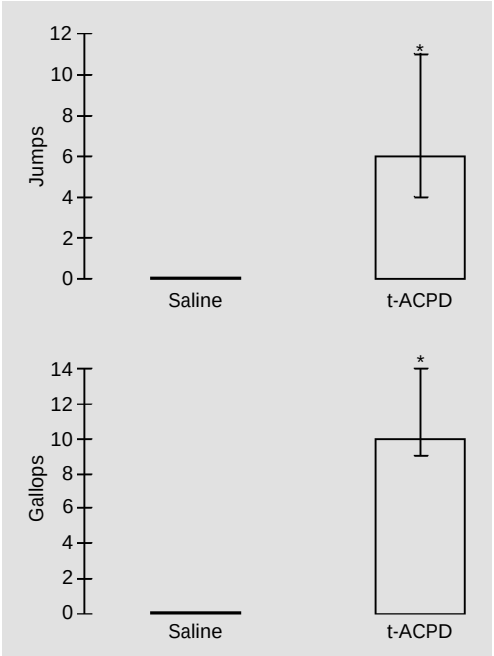

Figure 1 - Behavioral effects of saline (N = 4) or trans-(±)-1-amino-1,3-cyclopentanedicarboxylic acid (t-ACPD; 30 nmol; N = 5) microinjected into the dorsal periaqueductal gray of rats. The number of jumps (upper panel) and gallops (lower panel) was recorded for 1 min from the beginning of the injection period. The columns represent the medians and the vertical bars the interquartile intervals. *P<0.05 compared to control (Mann-Whitney U-test).

Animal behavior was videotaped for 1 min from the beginning of the injection and the number of jumps toward the top edges of the cage and gallops (short and frantic runs) was recorded. The results were analyzed by the non-parametric Mann-Whitney U-test.

t-ACPD induced a short-lasting (about 1 min) spontaneous defense reaction with exophthalmia, piloerection, escape attempts and runnings. There was a significant increase in the number of jumps and gallops ($P<0.05$, Mann-Whitney U-test, Figure 1). No animal showed any sign of seizure activity.

In a second experiment, animals received the same microinjections as described above. They were, however, hand-held during all the injection period. The injection needle remained in place for an additional 20-30 s to prevent reflux. Immediately after the end of this period, the rats were placed inside the open arena and the behavior was recorded with a videocamera (VHS movie, HNS-15B, Sony) placed 3.5 m above the arena for 5 min. The open circular arena (72 cm in diameter with 50-cm high transparent walls) was located in a sound-attenuated room illuminated with an indirect fluorescent light. Before each trial the floor was cleaned with an alcohol-water solution (7:3). Histological verification of the injection sites was performed as described above.

Videotapes were later examined on a video (VR756/78, Philips) connected to a microcomputer and the distance traveled by the animal was determined by the Ethovision software (version 1.9; Noldus, Wageningen, The Netherlands). Other behaviors, including frequency of grooming, rearings, turnings (360° circles), or number of defecation boli, were recorded manually by the operator.

The presence of an escape reaction during the injection was analyzed by a chi-square test and the number of defecation boli by a Student *t*-test. The remaining data were analyzed by repeated measures multivariate analysis of variance (MANOVA), using time (1 to 5 min) as a within-subjects factor and drug (saline or t-ACPD) as a between-subjects factor. Differences between treatments during a given one-minute period were analyzed by the *t*-test for independent observations.

Microinjection of saline elicited no noticeable behavioral effect. On the other hand, 11 of 15 rats that received t-ACPD into the DPAG showed an immediate defense reaction, which started during the 30-s injection and lasted for at least 1 min ($P<0.0005$, chi-square test). After this brief behavioral activation, the animals showed a decrease in grooming ($F_{1,25} = 13.84$, $P<0.001$) and rearing ($F_{1,25} = 15.14$, $P<0.001$) frequency, and an increase in turnings ($F_{1,25} = 11.89$, $P = 0.002$, Figure 2). No main effect was found in the distance traveled or in the number of defecation boli ($P>0.05$; data not shown). There was a main effect of time on rearing ($F_{4,22} = 15.9$, $P<0.001$), distance traveled ($F_{4,22} = 18.33$, $P<0.001$) and grooming ($F_{4,22} = 3.57$, $P = 0.02$).

A large body of evidence has shown that ionotropic glutamate receptors are involved in the control of defense reactions in the DPAG (11). However, few studies have investigated the behavioral effects of mGluR agonists. In a previous study, t-ADA, a proposed selective class I mGluR agonist, showed only minor effects on rat behavior in an open arena (12). However, this study employed *icv* administration, preventing

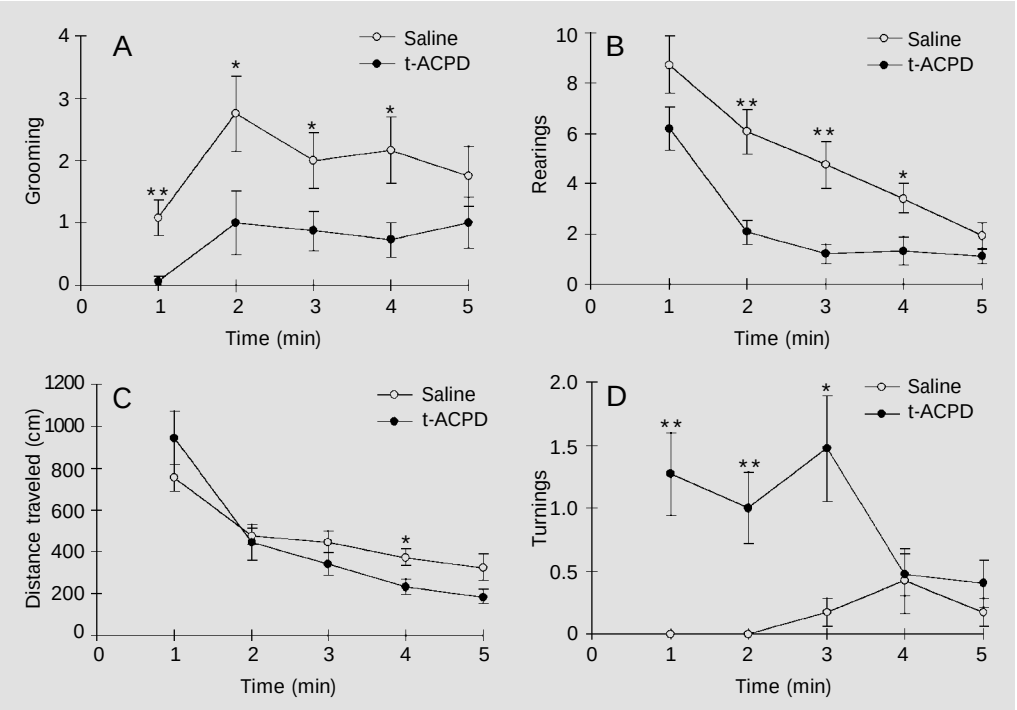

Figure 2 - Behavioral effects of saline (N = 12) or trans-(±)-1-amino-1,3-cyclopentanedicarboxylic acid (t-ACPD; 30 nmol; N = 15) microinjected into the dorsal periaqueductal gray of rats placed in an open arena. The number of grooming episodes (A), rearings (B), distance traveled in cm (C), and turnings (D) was recorded for 5 min. The points represent the means, and vertical bars the SEM. *P<0.05, **P<0.01 compared to control (Student *t*-test).

comparison with our data.

The present data show that t-ACPD, an mGluR agonist, induced a short-lasting flight reaction similar to that obtained with EEA or electrical stimulation (6,13). This defense response was followed by behavioral changes such as an increase in the number of turnings and a decrease in the number of rearings and grooming episodes. Aversive stimuli such as an electric footshock can induce rotational behavior in rats (14) and it has been suggested that turnings may be part of the defensive repertoire of these animals (15). Rearings are often used to evaluate exploratory activity (16) and a decrease in exploratory drive has been observed in rats submitted to stressful events (17). Many studies suggest that grooming behavior is increased by a mild aversive stimulus; however, the response varies with the degree of fear and the habituation to the stressful situation (18). The decrease in the number of rearings and grooming probably does not reflect any locomotor deficit since there was no difference in the distance traveled by the groups. These behavioral changes, therefore, may be reflecting an anxiogenic effect of t-ACPD.

Leyva et al. (9) showed that mGluR activation by t-ACPD (30 nmol) injection into the DPAG of rats had a depressor effect on blood pressure in anesthetized animals. Although we have not measured cardiovascular parameters in our study, defense reactions are usually accompanied by increases in blood pressure (2,6). It is possible that these apparent contradictory results are due to preferential actions of t-ACPD on various types of mGluR. In a recent report by the same group (19), a selective mGluR I agonist injection into the DPAG of mice increased the latency of the nociceptive reaction in the hot plate test while mGluR II and III agonists caused hyperalgesia. Anesthetic utilization may also have interfered with the response obtained by Leyva et al. (9).

Toxic effects of t-ACPD microinjected directly into the cerebral ventricle have been previously reported (20). However, Maione et al. (19) employed a higher dose than ours in the DPAG of mice without reporting any neurotoxic effects. Moreover, rats in which t-ACPD (30 nmol/0.3 µl) injection produced a defensive response received another microinjection of the same compound 48 h later, showing similar effects (data not shown). The reproducibility of the behavioral effects suggests that the drug did not produce significant neurotoxicity, at least within this period.

In conclusion, our results suggest that mGluRs play a role in the control of defense reactions. Further analyses, with subtype selective agonists or antagonists, are needed to clarify the involvement of different mGluR subtypes in defense reactions mediated by the DPAG.

Acknowledgments

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