Psychopharmacology (1991) 103:91–94

Psychopharmacology

Anxiolytic effect in the elevated plus-maze of the NMDA receptor antagonist AP7 microinjected into the dorsal periaqueductal grey

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Received May 3, 1990

Abstract. In order to localise the often reported anxiolytic action of N-methyl-D-aspartate (NMDA) receptor antagonists, 2-amino-7-phosphonoheptanoic acid (AP7) was injected into the dorsal periaqueductal grey (DPAG) of rats exposed to the elevated plus-maze model of anxiety. Doses of 0.2, 2 and 20 nmol AP7 caused a dose-dependent increase in the percentage of open arm entries, the effect of the last two doses being significantly different from control. A non-significant tendency to increase the percentage of time spent on the open arms of the maze was also noticed. In contrast, the total number of entries into either the open or enclosed arms was not affected. Injections of AP7 localized outside the DPAG were ineffective. Therefore, microinjection of AP7 into the DPAG caused a selective anxiolytic effect in the elevated plus-maze. It may be suggested that the DPAG is a site of the anxiolytic action of NMDA antagonists reported following systemic administration.

Key words: Excitatory amino acids – NMDA receptor antagonist – Intracerebral injection – Dorsal periaqueductal grey – Elevated plus-maze – Anxiolytic effect

Experimental evidence gathered over the last decade has established excitatory amino acids (EA), such as glutamate and aspartate, as neurotransmitters in the mammalian central nervous system (CNS), likely to be involved in several important physiological and pathophysiological processes. At least three types of EA receptors, named after their preferential agonists N-methyl-D-aspartate (NMDA), quisqualate and kainate have been identified (for reviews see Cavalheiro et al. 1988).

As regards behavioural functions, anxiolytic effects have been reported in several animal models of anxiety following administration of both competitive and non-competitive antagonists of the NMDA receptor (Brandão et al. 1980; Wenger 1980; Clineschmidt et al. 1982; Bennett and Amrick 1986; Stephens et al. 1986; Porter 1989). However, since only peripheral injection has been used, the site of the anxiolytic action of these compounds has not been localized.

Among the brain structures likely to be involved in anxiety (for a recent review see Graeff 1990), the dorsal part of the midbrain periaqueductal grey (DPAG), a region involved in defensive/aversive behaviour, has been under systematic study in our laboratories. In summary, it has been shown that microinjection into the DPAG of drugs that either enhance or mimic the inhibitory neurotransmitter gamma-aminobutyric acid (GABA), here including benzodiazepine anxiolytics, as well as of drugs that facilitate serotonergic neurotransmission, such as the serotonin (5–HT) uptake inhibitor zimelidine and the 5–HT autoreceptor blocker propranolol, decrease the aversive consequences of DPAG electrical stimulation. Therefore, neurons in the DPAG controlling aversion seem to be under the inhibitory modulation of GABA and 5–HT (for reviews see Graeff et al. 1986 and Graeff 1987, 1988). In contrast, endogenous EA may stimulate the same neurons, as indicated by experimental results showing that microinjection into the rat DPAG of a quisqualic receptor antagonist, glutamate diethyl ester (GDEE), attenuated behavioural and neurovegetative defence reactions elicited by electrical stimulation of either the DPAG or the medial hypothalamus (Carobrez 1987; Graeff et al. 1988).

Using the elevated plus-maze, an animal model of anxiety that has been validated behaviourally, physiologically and pharmacologically (Pellow et al. 1985), Audi et al. (1989) demonstrated an anxiolytic effect of propranolol microinjected into the rat DPAG, indicating that this region is critical for the expression of fear/anxiety generated in the elevated plus-maze test. The last result prompted us to investigate whether microinjection of the NMDA antagonist 2-amino-7-phosphonoheptanoic acid (AP7) into the DPAG of the rat would cause an anxiolytic effect in the same animal model.

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Materials and methods

Subjects. Male Wistar rats weighing 250–300 g were housed individually and given free access to food and water throughout the experiment.

Surgery. Rats were anaesthetized with sodium pentobarbitone, IP, and implanted with a stainless steel guide cannula (o.d. 0.7 mm) aimed at the DPAG. The guide cannula was introduced 1.9 mm lateral to the right side of lambda at an angle of 22° with the sagittal plane, until the cannula tip was 4.0 mm below the surface of the skull. The cannula was attached to the bone with stainless steel screws and acrylic cement. A stiletto was introduced inside the guide cannula to prevent obstruction.

Apparatus. The apparatus consisted of a plus-shaped maze elevated 50 cm from the floor and comprising two opposite open arms, 50 × 10 cm, crossed at right angle by two arms of the same dimensions enclosed by 40-cm high walls, having an open roof (Pellow et al. 1985). In addition, a 1-cm high edge made of plexiglass surrounded the open arms to avoid falls.

Drug. AP7 (2-amino-7-phosphonoheptanoic acid; Ciba Geigy) was dissolved in isotonic saline for injections.

Procedure. Five to seven days after the surgery, the rats were randomly assigned to four treatment groups. The control group received saline injection, while the remaining groups were injected with the doses of 0.2, 2 and 20 nmol AP7, respectively. All experiments were performed between 8:00 and 13:00 hours.

For intracerebral injections, a thin dental needle (0.3 o.d.) was introduced through the guide cannula until its tip was 1 mm below the cannula end. A polyethylene catheter (PE 10) was interposed between the upper end of the dental needle and the microsyringe. A volume of 0.5 µl was injected in 30 s using a Hamilton (USA) 10 µl microsyringe. The movement of an air bubble inside the PE 10 tubing confirmed drug flow.

In order to increase maze exploration, each rat was left inside a wooden arena (60 × 60 × 35 cm) for a 5-min period, starting 5 min after the injection, before being placed in the centre of the plus-maze facing the same enclosed arm and allowed to explore for 5 min. An observer scored the number of entries and the time spent on open or enclosed arms, respectively. The maze was cleaned with an alcohol-water solution (1:10) after each trial.

Analysis of results. From the above measures, the total number of entries (open + closed) as well as the percentage of open arms entries (100 × open/total), and of time spent on the open arms (100 × open/(open + closed)) were calculated. These values were analysed by a completely randomized single-factor analysis of variance (ANOVA). Comparisons between individual groups and control were made with the Dunnett test.

Histology. After behavioural testing rats were sacrificed under deep anaesthesia and their heads perfused through the left ventricle of the heart with isotonic saline followed by 10% formalin solution. After that, the dental needle was inserted through the guide cannula and a 0.5 µl microinjection of Evans blue was made. The brains were removed and frozen sections of 75 µm were placed on a glass slide coated with a thin layer of gelatine and later stained with neutral red. The stained sections were examined with light microscope at low magnification. Injection sites were localized in diagrams of König and Klippel's (1963) rat brain atlas.

Results

Localization of injection sites

As shown in Fig. 1, most of the injections were made within the borders of the DPAG. All rats injected with

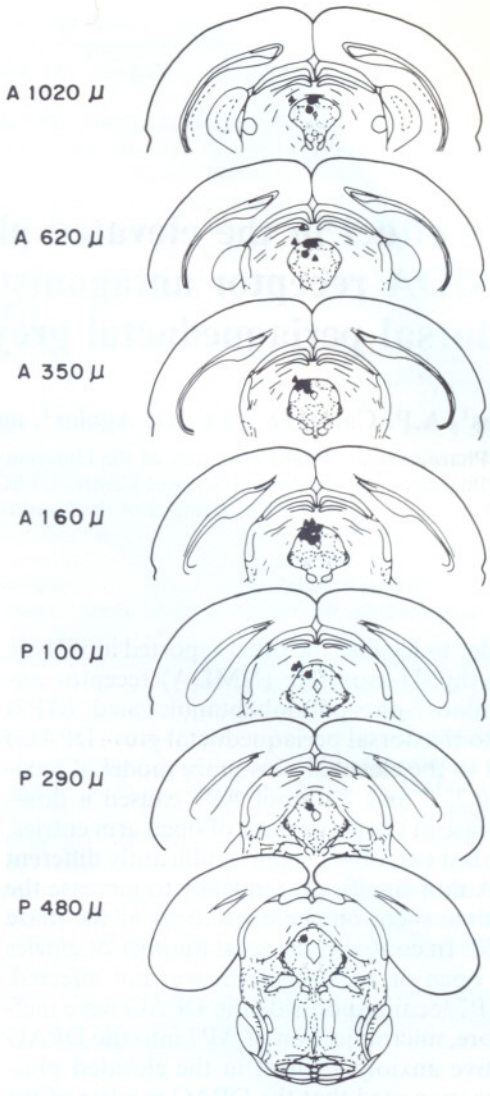

Fig. 1. Localization of injection sites inside diagrams from König and Klippel's rat brain atlas. *Circles* represent sites inside and *triangles* sites outside the dorsal periaqueductal grey, including one in the midbrain aqueduct. Figures represent the atlas coordinates, anterior (*A*) and posterior (*P*) to the interaural line

AP7 at sites localized outside the DPAG were removed from the original groups and joined together in an additional group (OUT) for analysis. From the ten rats of this group, three were injected with 0.2, three with 2, and four with 20 nmol AP7.

Effect of AP7 on maze exploration

The injection of AP7 into the DPAG raised the percentage of open arm entries in a dose-dependent way (Fig. 2). ANOVA showed an overall effect of drug treatment [$F(4,32) = 5.3$; $P = 0.002$]. Post-hoc comparisons revealed that only the effects of 2.0 and 20 nmol on the percentage of open arm entries were significantly different from control ($P < 0.05$ and $P < 0.01$, respectively).

As may be seen in Fig. 2, there was a tendency of the percentage of time spent on the open arms to increase as

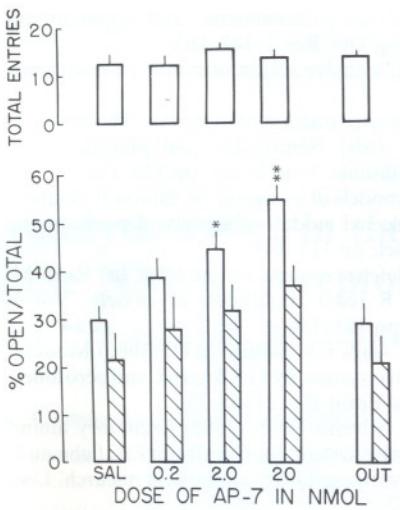

Fig. 2. Anxiolytic effect of AP7 microinjected into the dorsal periaqueductal grey (DPAG) of rats on the elevated plus-maze. Columns represent the mean and bars the SEM of six to ten rats. In the upper part of the figure, wide *open columns* refer to the total number of entries made onto either the open or the enclosed arms of the maze. In the lower part of the figure *open columns* represent the percentage of entries onto the open arms while *hatched columns* refer to the percentage of time spent on the open arms. *Asterisks* signal significant differences from control (*SAL*) at $P<0.05$ (*) and $P<0.01$ (**) detected by the Dunnett's t test. *OUT* labels the group of rats injected with different doses of AP7 at sites localized outside the DPAG (see Fig. 1)

a function of the dose of AP7 injected. Nonetheless, this trend did not reach significance level [$F(4,32)=1.3$; $P>0.05$].

ANOVA did not evidence a significant effect of drug treatment on total arm entries [$F(4,32)=0.6$, $P>0.05$].

Discussion

The present results clearly show that microinjection of AP7 into the DPAG of rats placed on the elevated plus-maze increased the relative number of open arm entries in a dose-dependent way, without affecting the total number of arm entries. The percentage of time spent on the open arms also tended to increase with the dose of AP. Since open arm and total arm exploration are viewed as indexes of fear/anxiety and general activity, respectively (Pellow et al. 1985), it may be concluded that the NMDA antagonist caused a selective anxiolytic effect in the elevated plus-maze test following its injection into the DPAG.

An anxiolytic profile of NMDA receptor antagonists administered systemically has been evidenced in several animal models of anxiety. Thus, AP7 increased punished locomotion in the four-plate test and open arm exploration in the elevated plus-maze, both effects being measured in mice (Stephens et al. 1986). The same drug released lever-pressing behaviour suppressed by foot-shock punishment in rats (Bennett and Amrick 1986), and similar anticonflict effects of another competitive NMDA receptor antagonist, CPP, as well as of four non-competitive antagonists, phenylcyclidine (PCP), ketamine, (+)-5-methyl-10,11-dihydro-5H-dibenzo[a,d]-cyclohepten-5,10-imine (MK–801) and etoxadrol, have been described in pigeons as well as in rats (Brandão et al. 1980; Wenger 1980; Clineschmidt et al. 1982; Porter et al. 1989). Since in the present results doses of AP7 far lower than those formerly administered peripherally (3–160 mg/kg in the aforementioned studies) caused an anxiolytic effect in the elevated plus-maze when microinjected into the DPAG, but not in sites outside its borders, this region may be a site of the anxiolytic action of NMDA antagonists.

The above suggestion is compatible with experimental evidence reviewed elsewhere (see, e.g., Graeff 1984, 1987, 1988) implicating the DPAG in anxiety as well as in anxiolytic drug action. In addition, there are reported results indicating that EA neurotransmission participates in the regulation of defensive/aversive behavior by the DPAG. Indeed, microinjection of EA receptor agonists into the DPAG has been shown to induce affective-defence reactions in cats as well as freezing or flight behaviour accompanied by blood pressure rise in rats (Bandler 1984; Krieger and Graeff 1985). Moreover, pretreatment with the quisqualic receptor antagonist GDEE not only blocked the defence reaction induced by microinjection of glutamate into the rat DPAG, but also attenuated the same response evoked by electrically stimulating either the DPAG itself or the medial hypothalamus. The last results led to the suggestion that descending nerve fibres containing EA, either originating in or running through the medial hypothalamus and projecting onto the DPAG, mediate defensive/aversive behaviour (Carobrez 1987; Graeff et al. 1988). Substantiating this proposal are reported studies using retrograde transport, autoradiographic and immunohistochemical techniques evidencing that important projections to the central grey come from the dorsomedial and ventromedial hypothalamic nuclei (Beart et al. 1988; Beitz 1989). Furthermore, synaptic responses evoked from the ventromedial hypothalamus and electrophysiologically recorded in the periaqueductal grey were reduced by microelectrophorectically administered EA antagonists blocking either NMDA or non-NMDA receptors (Beart et al. 1988).

As presently shown with AP7, microinjection of propranolol into the DPAG also caused a selective anxiolytic effect in the elevated plus-maze test (Audi et al. 1989). Therefore, the kind of anxiety generated by this aversive situation is susceptible to pharmacological intervention localized in the DPAG, highlighting the participation of this region for its elaboration. Nevertheless, that additional brain structures may also be involved is indicated by reported results showing that microinjection of buspirone into the dorsal hippocampus of the rat caused an anxiolytic effect in this experimental model (Kostowski et al. 1989).

From the evidence discussed above it may be suggested that the anxiolytic effects of NMDA receptor antagonists administered systemically are due to impairment of the EA-mediated neurotransmission in the DPAG that activates neuronal groups commanding de-

fensive/aversive behaviour. Nevertheless, the circulating drugs may also affect other regions of the CNS involved in anxiety.

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