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Protection by NMDA receptor antagonists against seizures induced by intracerebral administration of 4-aminopyridine

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Abstract

The effects of NMDA receptor antagonists on the convulsant action of the administration of 4-aminopyridine in the rat lateral cerebral ventricle (i.c.v. injection) and motor cerebral cortex (i.cx. injection) were studied. 4-Aminopyridine administration in both regions induced various preconvulsive symptoms, such as salivation, tremors, chewing and rearing, followed by continuous clonic convulsions and, only after i.c.v. injection, running fits and generalized tonic convulsions. This behavioral pattern appeared 5–9 min after administration of 4-aminopyridine and persisted for 100–150 min. 4-Aminopyridine also generated epileptiform electroencephalographic (EEG) discharges characterized by isolated spikes, poly-spikes and spike-wave complexes, which began some seconds after administration of the drug and were present for more than 2 h. The NMDA receptor antagonists (±)-3-(2-carboxy-piperazin-4-yl)-propyl-1-phosphonic acid (CPP), (±)-2-amino-7-phosphono-heptanoic acid (AP7) and (+)-5-methyl-10,11-dihydro-5*H*-dibenzo[*a*,*d*]cyclohepten-5,10-imine hydrogen maleate (MK-801) clearly protected against some of the behavioral alterations induced by i.c.v. 4-aminopyridine, particularly the tonic convulsions, but were less effective against those produced by i.cx. 4-aminopyridine. These antagonists also delayed the appearance of EEG epileptiform discharges, reduced its amplitude, frequency and duration, and blocked their propagation to other cortical regions after i.cx. 4-aminopyridine. These results, together with previous data showing that 4-aminopyridine stimulates the release of glutamate in vivo, suggest that an excessive glutamatergic neurotransmission involving NMDA receptors is implicated in 4-aminopyridine-induced seizures.

Keywords: 4-Aminopyridine; NMDA receptor antagonist; Seizure; Excitatory amino acid neurotransmission

1. Introduction

The convulsant action of the systemic administration of the K^+ channel blocker 4-aminopyridine in several species is well known (Schafer et al., 1973; Fragoso-Veloz et al., 1990). It has also been reported that the microinjection of the drug in the rat substantia nigra reticulata (Tapia and Flores-Hernández, 1990) or the CA1 region of the hippocampus (Fragoso-Veloz et al., 1990; Fragoso-Veloz and Tapia, 1992; Bagetta et al., 1992) produces electroencephalographic (EEG) seizures and intense motor alterations, including circling behavior, limbic type seizures and wet-dog shakes.

Although the mechanism of its convulsant action has not been determined, it is known that 4-aminopyridine stimulates the release of neurotransmitters, including glutamate, in several preparations such as the neuromuscular junction (Lundh, 1978; Thesleff, 1980), brain slices (Dolezal and Tucek, 1983; Hu et al., 1991) and synaptosomes (Tapia and Sitges, 1982; Tapia et al., 1985; Tibbs et al., 1989). Furthermore, we have recently found by a microdialysis procedure in vivo that glutamate is by far the amino acid predominantly released by 4-aminopyridine in the striatum, whereas tetraethylammonium, another K^+ channel blocker, has only a weak effect (Morales-Villagrán and Tapia, 1996). Therefore, and in view of the well established role of excitatory amino acid synapses in convulsive and excitotoxic mechanisms (Dingledine et al., 1990; Meldrum, 1992), it seems possible that the excess glutamate released may be involved in the excitatory effects of 4-aminopyridine.

In order to better understand the mechanism of 4-aminopyridine-induced hyperexcitation, and the possible role of excessive glutamatergic neurotransmission, in the present communication we have studied the behavioral and

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electroencephalographic effects of the intracerebroventricular and intracortical administration of this drug, as well as the protective action of several NMDA receptor antagonists.

2. Materials and methods

2.1. Materials

Adult male Wistar rats (200–250 g weight) were used throughout and handled in accordance with the Rules for Research in Health Matters (Mexico), with approval of the local Animal Care Committee. They were maintained at constant temperature (18–22°C) and humidity (40%) under controlled light-dark cycles (12/12 h) and with free access to food and water.

4-Aminopyridine and direct blue 15 were obtained from Sigma Chemical Co. (St. Louis, MO, USA). (±)-3-(2-Carboxypiperazin-4-yl)-propyl-1-phosphonic acid (CPP), (±)-2-amino-7-phosphonoheptanoic acid (AP7) and (+)-5-methyl-10,11-dihydro-5*H*-dibenzo[*a*,*d*]cyclohepten-5, 10-imine hydrogen maleate (MK-801) were from Research Biochemicals Int. (Natick, MA, USA).

2.2. Surgical procedures

Rats were anesthetized with halothane-oxygen mixture and secured in a Stoelting stereotaxic frame with the incisor bar positioned at −3.3 mm. They were implanted with guide cannulas (0.4 mm diameter) for drug injection into the lateral ventricle (i.c.v. administration) or on the motor cerebral cortex (i.cx. administration). Coordinates were: A 0.8, L 1.7 and V 3.5 for lateral ventricle and P 2.0, L 3.0 and V 1.5 for motor cerebral cortex, respectively (Paxinos and Watson, 1986). The cannula tip was located 1 mm above the injection zone, and it was sealed with a dummy injection cannula until the time of drug administration. For EEG recording in i.c.v.-injected rats, in addition to the cannula animals were bilaterally implanted with copper bipolar electrodes (0.25 mm diameter, 0.1 mm tip, 0.25 mm interelectrode distance) in the motor cerebral cortex (coordinates as above) and hippocampus (P 3.2, L 2.0 and V 3.5). For EEG recording after i.cx. administration, the cannula guide served as an electrode, in addition to bilaterally implanted epidural electrodes consisting of stainless steel screws fixed to the skull. The reference electrode was located over the frontal sinus. Screw electrodes were positioned at frontal A 4 and L 2, premotor A 0 and L 3, motor P 2 and L 3 and occipital P 5 and L 3 coordinates. The electrodes were attached to a multipin socket anchored to the skull with dental acrylic cement. Amplification and recording of electrical activity were carried out using a Grass polygraph (paper speed 15 mm/s). Electrical activity was monitored during 1 min every 5 min, starting 30 min before drug injection (basal record), until the epileptiform discharges had disappeared.

2.3. Drug administration

Some rats were not implanted with guide cannulas nor electrodes but were anesthetized and injected i.c.v. with 4-aminopyridine (75 nmol in 5 μl, infused in a period of 2–3 min) through a needle attached to a 10 μl syringe. The anesthesia was then discontinued and behavior was evaluated by continuous observation during 2–3 h (until motor abnormalities disappeared), as soon as the animals woke up (about 10 min after injection). Unanesthetized rats implanted with guide cannulas received 75 nmol of 4-aminopyridine i.c.v. through the cannulas, one week after surgery, and were used for EEG recording. The i.cx. 4-aminopyridine administration was in all cases through the guide cannula (75 nmol in 1 μl).

The following NMDA receptor antagonists were tested against both the i.c.v. and the i.cx. 4-aminopyridine administration, at the doses and routes of administration indi-

Table 1
Main behavioral effects of the i.c.v. administration of 4-aminopyridine (75 nmol) and protection by NMDA receptor antagonists

Symptom	4-Aminopyr. alone	+CPP	+AP7	+MK-801	+MK-801 i.p. (0.2 mg/kg)
Wet-dog shakes	0/13	6/6 [a]	7/7 [a]	6/6 [a]	7/7 [a]
(number)	(0)	(39 ± 9)	(122 ± 37)	(73 ± 5)	(129 ± 27)
Salivation	13/13	4/6 [b]	6/7	0/6 [a]	0/7 [a]
Clonic convuls.	12/13	6/6	7/7	5/6	4/7
(number)	(5.5 ± 1.2)	(5.0 ± 1.0)	(5.0 ± 1.2)	(3.6 ± 1.2)	(3.5 ± 1.7)
Tonic convuls.	7/13	0/6 [b]	1/7 [b]	0/6 [b]	0/7 [b]
(number)	(5.3 ± 1.8)	(0)	(3)	(0)	(0)
Running fits	13/13	0/6 [a]	0/7 [a]	0/6 [a]	0/7 [a]
(number)	(3 ± 1)	(0)	(0)	(0)	(0)
Status epilept.	9/13	0/6 [b]	0/7 [b]	0/6 [b]	0/7 [b]

With the exception of systemic MK-801 (last column), 10 nmol of each antagonist was coinjected i.c.v. with 4-aminopyridine. Figures are No. of rats showing the symptom/No. of rats treated. The mean total number of times that the symptom occurred in a single animal ± S.E.M. is shown in parentheses. [a] $P < 0.001$, [b] $P < 0.05$, when compared to control (Mann-Whitney U-test).

cated: CPP, 10 nmol, coinjected i.c.v. or i.cx. with 4-aminopyridine; AP7, 10 nmol coinjected i.c.v. or i.cx. with 4-aminopyridine; MK-801, 10 nmol coinjected i.c.v. or i.cx. with 4-aminopyridine and, furthermore, 0.2 mg/kg i.p. 30 min before 4-aminopyridine. These doses have been shown to be effective under similar experimental conditions (Fragoso-Veloz and Tapia, 1992; Belmar et al., 1995).

For intracerebral injections, the drugs were dissolved in isotonic saline at a slightly alkaline pH ($\approx$ 8.0, due to 4-aminopyridine) containing 6 mg/ml of direct blue 15 to locate the injection site. This dye did not produce any effect per se, nor affected the action of the drugs used. At the end of the experiment rats were decapitated and the brain was removed and sliced. The location of both the electrodes and injection sites were verified by gross histological examination.

3. Results

3.1. Intracerebroventricular injections

3.1.1. Behavioral observations

The i.c.v. administration of 75 nmol 4-aminopyridine produced a series of preconvulsive symptoms including hyperexcitability, tremors, head nodding, salivation, chewing and rearing. After these symptoms, all treated animals examined showed convulsive behavior characterized by bursts of forepaw clonic jerks and running fits. Furthermore, 69% of the animals showed continuous clonic convulsions (status epilepticus), which usually lasted for more than 30 min, during which generalized tonic convulsions frequently occurred (Table 1). The behavioral changes appeared at 9.3 $\pm$ 1.0 min after treatment and lasted for 147 $\pm$ 8.0 min (n = 13).

The NMDA receptor antagonists CPP, AP7 and MK-801 prevented or diminished the intensity of the behavioral alterations produced by i.c.v. 4-aminopyridine. The most notable protective effect was that status epilepticus, wild running and generalized tonic convulsions were prevented. All animals receiving the NMDA receptor antagonists showed numerous wet-dog shakes, which were not observed with 4-aminopyridine alone (Table 1) or with the antagonists alone (Fragoso-Veloz and Tapia, 1992; Belmar et al., 1995).

3.1.2. EEG recordings

The i.c.v. administration of 4-aminopyridine induced epileptiform discharges in all regions recorded, but were persistently much more intense in the ipsilateral motor

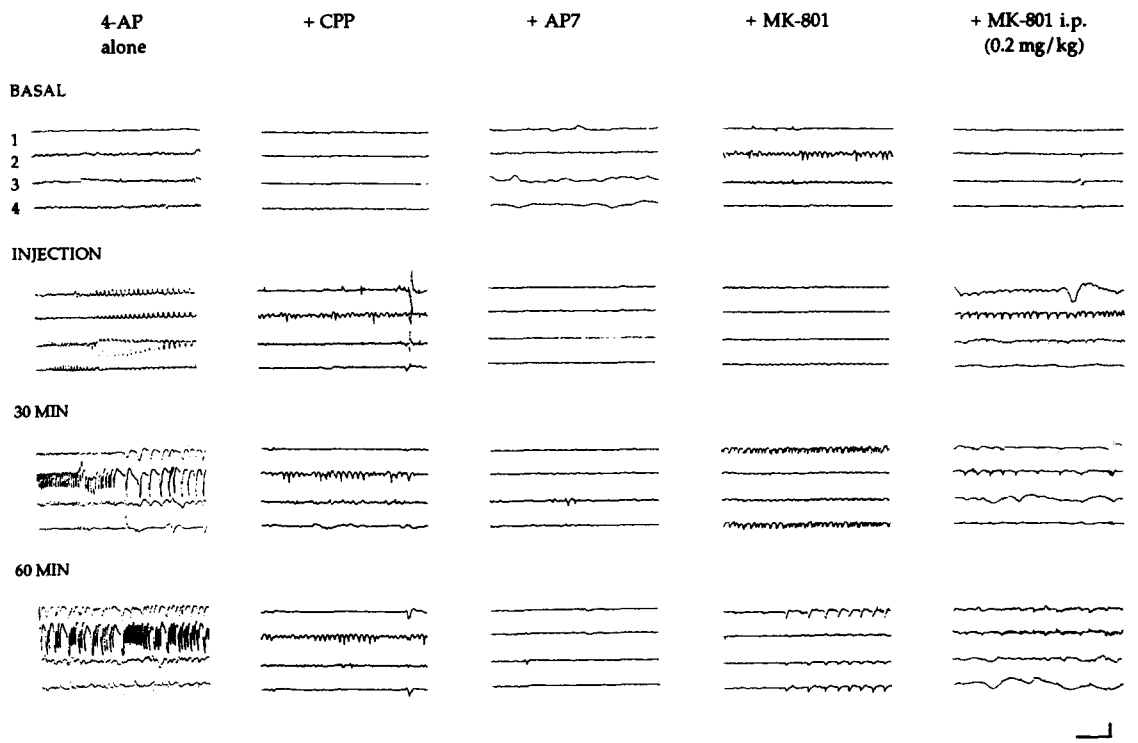

Fig. 1. Representative examples of the effect of the NMDA receptor antagonists on the discharges induced by the i.c.v. administration of 4-aminopyridine in the right lateral ventricle. Except for the last column (MK-801 administered i.p. 30 min before 4-aminopyridine), 10 nmol of the NMDA receptor antagonists were coinjected i.c.v. with 4-aminopyridine. Each set of records show the basal activity, the activity immediately after injection of 4-aminopyridine, and at 30 and 60 min afterwards, as indicated. In each set, records 1 and 2 are left and right motor cerebral cortex, respectively, and 3 and 4 left and right hippocampus, respectively. The convulsant action of 4-aminopyridine was observed in 4 rats. Protective effects by the antagonists similar to those shown were observed in 4 of 8 treated rats for CPP, 4 of 7 for AP7, 6 of 6 for i.c.v. MK-801, and 3 of 6 rats for i.p. MK-801. Scale: horizontal bar = 2 s; vertical = 840 μV.

cortex. The discharges appeared immediately, even before the end of the injection period, and were characterized by high frequency poly-spikes and spike-wave complexes which were of low amplitude at the beginning of the effect. Their amplitude increased with time, reaching a maximum at 30–60 min. During the first 10–20 min the average duration of discharges was 40 s with interictal periods of approximately one min. After about 60 min the discharges became continuous for periods longer than 4 h, even when the behavioral alterations had ceased, and then gradually disappeared.

A notable protection against 4-aminopyridine-induced EEG seizures was observed in most, but not all rats treated with the NMDA receptor antagonists. When protection occurred, a significant delay in the latency of appearance of the discharges was observed (25–150 s). Furthermore,

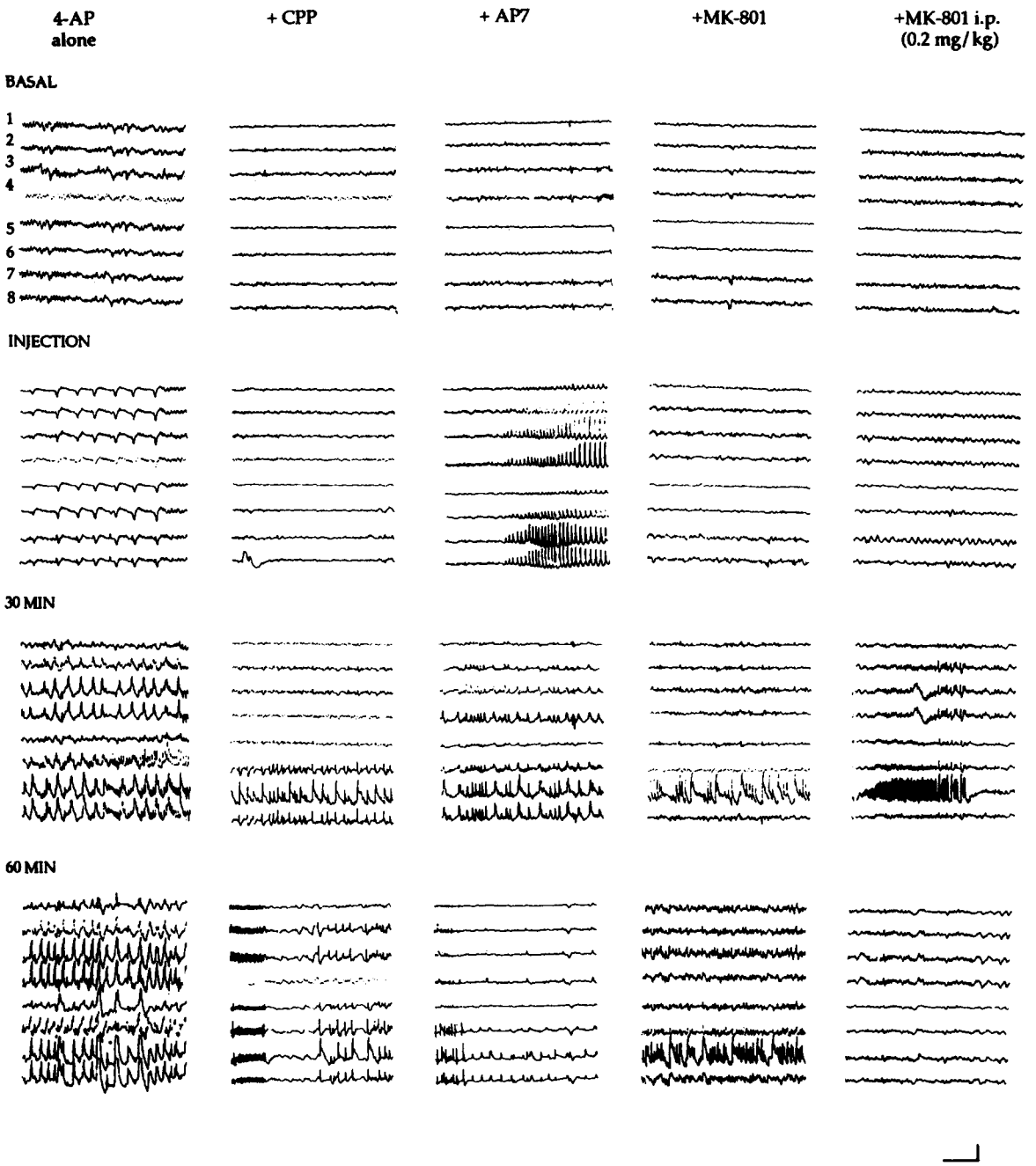

Fig. 2. Representative examples of the effect of NMDA receptor antagonists on the discharges induced by the i.cx. administration of 4-aminopyridine in the right cortex. Except for the last column (MK-801 administered i.p. 30 min before 4-aminopyridine), 10 nmol of the NMDA receptor antagonists were coinjected i.cx. with 4-aminopyridine. In each set of records, traces 1–4 correspond to the left frontal, premotor, motor and occipital cortex, respectively, and traces 5–8 to the equivalent right cortices. Other details as in Fig. 1. The convulsant action of 4-aminopyridine was observed in 4 rats. Protective effects by the antagonists similar to those shown were observed in 4 of 6 treated rats for CPP, 5 of 7 for AP7, 4 of 4 for i.cx. MK-801, and 4 of 6 rats for i.p. MK-801. Scale: horizontal bar = 2 s; vertical = 600 μV.

as exemplified in Fig. 1, the frequency, amplitude and duration of the discharges were also considerably reduced. In no case the discharges became continuous and their duration did not excede 20 s. As indicated in the legend to Fig. 1, with CPP, AP7 and i.p. MK-801 protection was observed in at least 50% of the animals, whereas with i.c.v. MK-801 protection was observed in all rats treated. However, even in the less protected rats, with all antagonists the spike amplitude decreased and the duration of the continuous discharge was reduced to less than 1.5 h, although the maximum spike frequency did not change.

3.2. Intracortical injections

3.2.1. Behavioral observations

The i.cx. administration of 4-aminopyridine produced a pattern of preconvulsive symptoms similar to that induced by the i.c.v. injection, except that weak contralateral circling behavior and occasional wet-dog shakes were also observed. An important difference was that running fits and generalized tonic convulsions did not occur and only 2/7 animals showed status epilepticus. Motor alterations appeared 4.4 ± 0.6 min after injection and lasted for 107 $\pm$ 6.5 min.

At the doses indicated in Table 1, neither of the NMDA receptor antagonists tested exerted a significant protection against the behavioral effects of i.cx. 4-aminopyridine, with the exception of salivation, which was observed in 6 of 7 rats treated with 4-aminopyridine alone, and in only 1 or 2 animals receiving any of the antagonists tested ($n = 6$, $P < 0.05$, Mann-Whitney U-test).

3.2.2. EEG recordings

Epileptiform discharges were observed in all electrode derivations, starting about 14 s after the i.cx. administration of 4-aminopyridine. The discharges were characterized by isolated spikes, repetitive spikes and spike-wave complexes. Discharge amplitude increased with time and reached a maximum at 30–60 min. Differently from the i.c.v. administration, the EEG epileptiform pattern was continuous from the time of its appearance and lasted for more than 2 h.

As indicated in the legend to Fig. 2, in contrast to the lack of protection against the i.cx. 4-aminopyridine behavioral effects, in at least 65% of the rats all NMDA receptor antagonists tested produced a notable delay in the latency of appearance of the discharge. Moreover, in these animals the discharges were not continuous but lasted for 12–14 s and were interrupted by interictal periods of about 12 s during the first 30 min. After this time, the duration of the interictal periods gradually increased with time, until the disappearance of the discharges. In addition, particularly during the first 30 min the propagation of the epileptiform activity to the contralateral hemisphere, and sometimes even to other ipsilateral regions, was prevented (Fig. 2).

4. Discussion

The present study shows that the i.c.v. administration of 4-aminopyridine results in intense EEG epileptic discharges, which appeared immediately after the injection and were followed after a few minutes by intense behavioral motor alterations, including status epilepticus and tonic generalized convulsions. In contrast, the i.cx. administration produced continuous EEG epileptiform activity and weaker behavioral effects, probably reflecting the more restricted site of action of 4-aminopyridine.

The three NMDA receptor antagonists tested, particularly MK-801, prevented or reduced the intensity of the hyperexcitability symptoms induced by intracerebral 4-aminopyridine. MK-801, at doses similar to those used in the present work, has also been shown to protect rats (Fragoso-Veloz and Tapia, 1992) and mice (Cramer et al., 1994) against seizures induced by the systemic administration of 4-aminopyridine. These results, therefore, indicate that activation of NMDA receptors play a role in the mechanism of its epileptogenic effect. Such activation probably arises from an excess of glutamate release produced by 4-aminopyridine, since we have recently found that the infusion of the drug through a microdialysis probe in vivo induces seizures and a massive release of glutamate into the extracellular space, whereas other amino acids are much less affected (Morales-Villagrán and Tapia, 1996). It is well known that in vitro 4-aminopyridine stimulates the basal (not depolarization-dependent) release of neurotransmitters in a Ca^{2+}-dependent manner (Lundh, 1978; Thesleff, 1980; Tapia and Sitges, 1982; Dolezal and Tucek, 1983; Tapia et al., 1985; Tibbs et al., 1989; Hu et al., 1991).

Other K^+ channel blockers, such as α-dendrotoxin or mast-cell degranulating peptide, also induce seizures when administered i.c.v. or into the hippocampus. However, in contrast to 4-aminopyridine, their effects are not antagonized by NMDA receptor antagonists (Gandolfo et al., 1989; Bagetta et al., 1992) and, conversely, some L-type Ca^{2+} channel blockers are good anticonvulsants against α-dendrotoxin but they fail to protect, or even potentiate, the effects of 4-aminopyridine (Gandolfo et al., 1989; Fragoso-Veloz et al., 1990; Cramer et al., 1994). This suggests that a generalized depolarizing action of 4-aminopyridine, which is probably shared by the other K^+ channel blockers, is not the main factor involved in its convulsant action. Furthermore, unlike 4-aminopyridine, tetraethylammonium, another K^+ blocker, did not produce seizures nor induced glutamate release when administered through dialysis probes (Morales-Villagrán and Tapia, 1996).

Although some data in vitro, in amygdala (Gean, 1990) and hippocampal (Perreault and Avoli, 1991) slices, have suggested an involvement of non-NMDA receptors in the epileptiform activity induced by 4-aminopyridine, in vivo the antagonists to these receptors did not significantly

protect against systemic or intrahippocampal 4-aminopyridine, in the rat (Fragoso-Veloz and Tapia, 1992) and in the mouse (Yamaguchi and Rogawski, 1992). Moreover, the anticonvulsant efficacy of a variety of drugs, especially the dihydropyridine Ca^{2+} channel antagonists, shows a very different pattern when tested against seizures induced by the non-NMDA receptor agonist kainic acid, as compared to those produced by 4-aminopyridine (Fragoso-Veloz et al., 1990; Yamaguchi and Rogawski, 1992; Cramer et al., 1994). Therefore, in intact animals the non-NMDA receptors seem to be less implicated in 4-aminopyridine epileptogenic action than the NMDA receptors.

It is interesting that, both behaviorally and by EEG, the seizures induced by i.c.v. 4-aminopyridine were more clearly antagonized by the three NMDA receptor antagonists tested than those produced by i.cx. 4-aminopyridine. This suggests that both 4-aminopyridine and the antagonists diffuse extensively when injected i.c.v. and therefore reach a relatively large number of synapses in several regions of the brain, whereas their diffusion is restricted when injected i.cx. Consistently with this interpretation, we have previously found that the NMDA receptor antagonists protect against wet-dog shakes induced by the intrahippocampal administration of 4-aminopyridine, when administered i.c.v. or i.p. (in the case of MK-801), but not when coinjected with the drug in the CA1 hippocampal area (Fragoso-Veloz and Tapia, 1992). In view of this, it is puzzling that the i.c.v. co-injection of 4-aminopyridine and the antagonists (or the i.p. administration of MK-801) induced wet-dog shakes behavior. Although we cannot offer an explanation for this finding, it may be relevant that a low dose of MK-801 (0.1 mg/kg) has been shown to increase the number of wet-dog shakes induced by systemic kainic acid during the first 40 min (Fariello et al., 1989). In addition, in hippocampal slices the NMDA receptor antagonists enhance the epileptiform neuronal burst activity induced by kainic acid or mast-cell degranulating peptide (Collingridge et al., 1983; Neuman et al., 1988).

4-Aminopyridine has been used in the treatment of several neurological disorders, such as miasthenia gravis, Lambert-Eaton myasthenic syndrome, multiple sclerosis, Alzheimer disease and spinal cord injury (Murray and Newsom-Davis, 1981; Wesseling and Agoston, 1984; Bever, 1994; Hayes, 1994). The benefit of this treatment is probably due to the restoration of current conduction by 4-aminopyridine in demyelinated axons, as a consequence of the blockade of K^+ channels, or to the increased release of acetylcholine at neuromuscular junctions, but one of the risks of 4-aminopyridine treatment is that a slight overdose might lead to convulsions (Bever, 1994; Bever et al., 1994; Hayes, 1994). Our findings indicate that this dangerous side effect is due to increased glutamatergic neurotransmission at central synapses, and suggest that pharmacological blockade of NMDA receptors, combined with the treatment with 4-aminopyridine, might reduce the risk of convulsions and other excitatory actions of the drug and therefore facilitate its therapeutic use.

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