Pharmacology Biochemistry & Behavior, Vol. 35, pp. 999–1001. © Pergamon Press plc, 1990. Printed in the U.S.A. 0091-3057/90 $3.00 + .00

BRIEF COMMUNICATION

Inhibitory Influence of Excitatory Amino Acid Antagonists on Penicillin-Induced Epileptiform Bursting in Rat Hippocampal Slices

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Received 24 March 1989

SAGRATELLA, S., C. FRANK AND A. SCOTTI DE CAROLIS. *Inhibitory influence of excitatory amino acid antagonists on penicillin-induced epileptiform bursting in rat hippocampal slices.* PHARMACOL BIOCHEM BEHAV **35**(4) 999–1001, 1990.—The inhibitory influence of excitatory amino acid (E.A.A.) antagonists such as kynurenic acid, 2-amino-5-phosphonopentanoic acid (AP5), cis-2,3-piperidine dicarboxylic acid (cis-2,3 PDA) and (+)-5-methyl-10,11,-dihydro-5H-dibenzo(a,d)cyclo-hepten-5,10-imine maleate (MK 801), has been studied on the epileptiform activity elicited in rat hippocampal slices, bathed in penicillin (1 mM). The rank of the inhibitory potency was: MK 801 > kynurenic acid > cis 2,3 PDA > AP5. Moreover, only MK 801 was able to block the last population spike of the penicillin-induced epileptiform bursting in 100% of the experiments. The data indicate that the antiepileptic activity of E.A.A. antagonists on the penicillin epileptiform bursting in CA1 pyramidal cells is low and limited, indicating that the hippocampal area is not the primary site of the anticonvulsant activity of E.A.A. antagonists.

E.A.A. antagonists Penicillin Epileptiform activity Hippocampal slices Rat

EXCITATORY amino acid (E.A.A.) antagonists have been shown to act as anticonvulsants in several experimental tests on chemically, electrically induced and reflex seizures (5). The competitive antagonists for a type of E.A.A. receptors the N-methyl-D-aspartate (NMDA) receptors, DL-2-amino-5-phosphonopentanoic acid (DL-AP5) and 2-amino-7-phosphonoheptanoic acid (APH), were able to reduce kindled amygdala seizures in rats (6) as well as audiogenic seizures in mice (2) and photically induced myoclonus in baboons (5). Moreover, cis-2,3-piperidine dicarboxylic acid (cis-2,3 PDA), a nonselective antagonist of all the types of E.A.A. receptors (10), has been found to be effective against sound-induced seizures in mice, photically induced seizures in baboons and high pressure-induced seizures in rats (5).

This paper studies the influence of some E.A.A. antagonists on the epileptiform activity induced by penicillin in rat hippocampal slices. In particular, the effects of DL-AP5, the competitive antagonist of NMDA receptors, and of cis-2,3 PDA, a broad spectrum antagonist of E.A.A., were examined. In addition, the effects of the noncompetitive antagonist of NMDA receptors, the (+)-5-(methyl-10,11-dihydro-5h-dibenzo(a,d)cyclohepten-5,10-imine maleate) MK 801 (11), and of kynurenic acid, another broad spectrum E.A.A. antagonist with some selectivity for NMDA receptors (3), were studied.

METHOD

Male Wistar rats (200–250 g) were killed by decapitation, the skull was opened and the hippocampus rapidly removed. Hippocampal slices (450 μm thick) were cut with a tissue chopper and placed in a recording chamber, where they were constantly perfused with an artificial cerebrospinal fluid (A.C.S.F.) (122 mM NaCl, 0.4 mM KH_2PO_4, 3 mM KCl, 1.2 mM $MgSO_4$, 25 mM $NaHCO_3$, 10 mM glucose, 1.3 mM CaCl) saturated with 95% O_2 and 5% CO_2. Field potentials were recorded in the CA1 cell layer after electrical stimulation of the Schaffer collaterals (1–5 V, 70 μsec, 0.1 Hz).

The influence of the tested drugs was assessed for a period of 60 min after drug perfusion on the number of additional epilepti-

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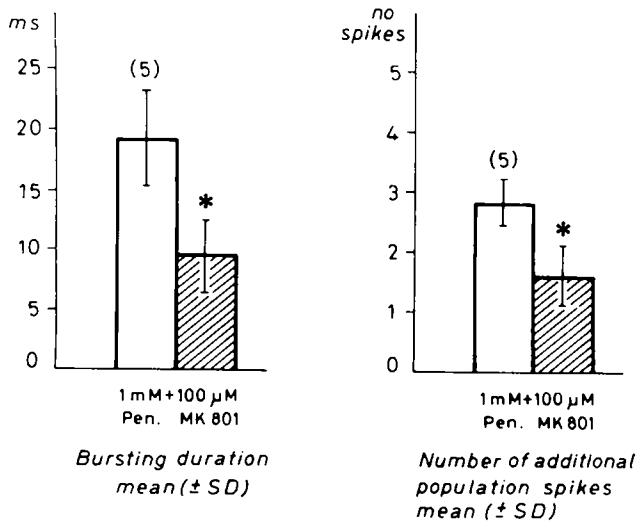

FIG. 1. Inhibitory effects of MK 801 on penicillin-induced epileptiform bursting. The histograms show the inhibitory influence of MK 801 (60 min after the addition of the drug to the perfusion solution containing the epileptogenic agent) on the parameters of the stimulated epileptiform bursting. In parentheses is the number of experiments. *Significantly different from before MK 801, $p<0.01$.

form population spikes (NAEPS) and on the duration of the epileptiform bursting (EBD) due to 1 mM penicillin. As literature (8) has reported that the E.A.A. antagonist AP5 would preferentially abolish the last additional epileptiform population spike (LAEPS) of the CA1 hippocampal epileptiform bursting, the minimal concentration of the drugs able to block LAEPS has been determined in the present study.

RESULTS AND DISCUSSION

Slice perfusion with penicillin (1 mM) solution elicited within 30–40 min the appearance of an epileptiform bursting characterized by 1) the occurrence of several (2–5) additional population spikes, and 2) a duration of 15–40 msec (Figs. 1 and 2). Low concentrations (up to 20 μM) of MK 801 did not significantly affect the penicillin-induced epileptiform bursting within 60 min of drug perfusion (NAEPS 3.5 ± 0.54 spikes × burst before drug, 3.6 ± 0.48 spikes × burst after drug; EBD 19 ± 4.1 msec before drug, 20 ± 3.2 msec after drug).

Within 60 min of drug perfusion a 50–75 μM solution of MK 801 was able to block the LAEPS in 50% of the experiments (N = 4), and, on average, it decreased the EBD and the NAEPS by 20.5 ± 8% and 16 ± 5%, respectively. One hundred μM of MK 801 blocked the LAEPS within 60 min of drug perfusion in 100% of the experiments (N = 5), and, on average, decreased the EBD and the NAEPS by 49 ± 12% and 43 ± 3.68%, respectively (Fig. 1).

Up to the highest tested concentration (400 μM) DL AP5 was able to block the last epileptiform additional population spike in 25% of the experiments (N = 8), and, on average, it did not significantly affect the EBD or the NAEPS within 60 min of drug perfusion (NAEPS 2.5 ± 0.4 spikes × burst before drug, 2.25 ± 0.7 spikes × burst after drug; EBD 22.7 ± 3 msec before drug, 21.7 ± 3 msec after drug).

Within 60 min of drug perfusion, a 50 μM solution of kynurenic acid blocked the LAEPS in 50% of the experiments (N = 4), and, on average, it reduced the EBD and the NAEPS by 29 ± 6% and 20.8 ± 7%, respectively (Fig.2). Higher concentrations of kynurenic acid (up to 200 μM) blocked the last epileptiform additional population spike in 66.6% of the experiments (N = 9), and, on average, it decreased the EBD and the NAEPS by 21.1 ± 5% and 22 ± 7%, respectively.

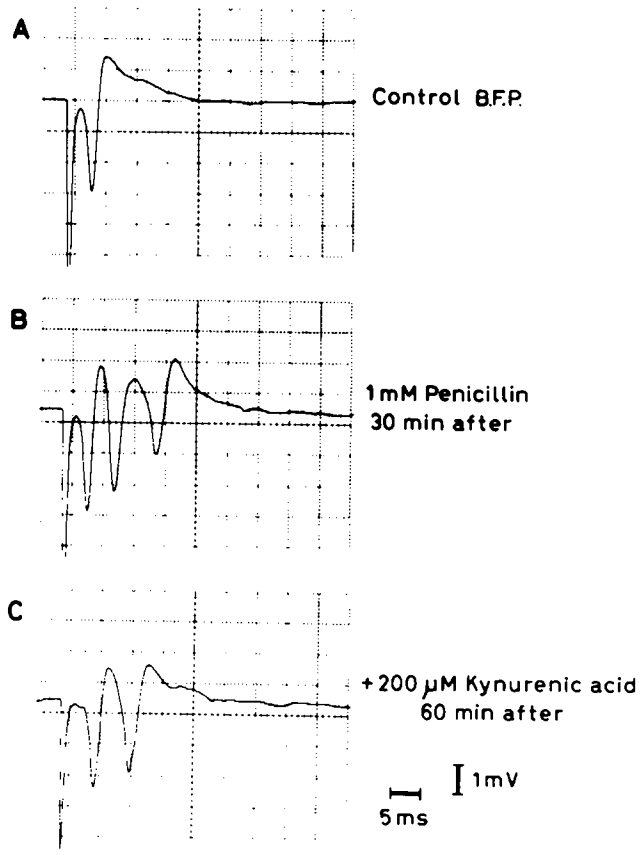

FIG. 2. Inhibitory effects of kynurenic acid on penicillin-induced epileptiform bursting. (A) Control basal field potential of CA1 hippocampal area. (B) Effect of penicillin on field potential. (C) Inhibitory influence of kynurenic acid (60 min after the addition of the drug to the penicillin solution).

Up to a concentration of 100 μM, cis-2,3 PDA did not significantly affect the penicillin-induced epileptiform bursting within 60 min of drug perfusion (NAEPS 3.28 ± 0.4 spikes × burst before drug, 3.25 ± 0.6 spikes × burst after drug; EBD 16.33 ± 4 msec before drug, 17.05 ± 6 msec after drug). A 200–400 μM solution of cis-2,3 PDA was able to block the LAEPS in 66.6% of the experiments (N = 6) and, on average, it reduced the EBD and the NAEPS by 23.6 ± 5% and 27.5 ± 4%, respectively.

In the present paper the inhibitory influence of some E.A.A. antagonists has been tested on the epileptiform activity elicited in vitro by the GABA antagonist penicillin in rat hippocampal slices. The rank of the inhibitory potency was: MK 801 > kynurenic acid > CIS-2,3 PDA > DL AP5. Kynurenic acid and the noncompetitive antagonist of NMDA receptors, MK 801, turned out to be the most potent, while the competitive antagonist of NMDA receptors, DL AP5, the least potent. These data are in line with other reports (9) in which kynurenic acid has been found 2–3 times more potent than D-AP5 against picrotoxin-induced epileptiform activity in rat hippocampal slices.

The higher antiepileptic activity of kynurenic acid did not seem to be correlated with its antagonistic activity in regard to "non-

NMDA'' receptors (3), because the broad spectrum antagonist of E.A.A. receptors, cis-2,3 PDA, presented limited antiepileptic effects at very high concentrations. The complete blockage of LAEPS in penicillin-induced epileptiform bursting was obtained in 100% of the experiments only with MK 801. This is probably related to the high antagonistic potency of MK 801 against NMDA receptors (11) and to the data provided by Coan *et al.* (1) who found MK 801 20 times more potent than D-AP5 against the epileptiform bursting elicited in rat hippocampal slices bathed in a ''Mg^{++}-free'' solution.

Our previous work (7) showed that the noncompetitive antagonists of NMDA receptors ketamine and (+) cyclazocine inhibited the epileptiform bursting elicited in rat hippocampal slices bathed in penicillin and ''Mg^{++}-free'' solutions. In particular, the noncompetitive antagonists of NMDA receptors were found ten times more potent against the epileptiform activity produced by ''Mg^{++}-free'' solution than against the penicillin-induced epileptiform bursting. The same finding was reported by Stone (9), who described that the antiepileptic activity of the competitive antagonist of NMDA receptors D-AP5 was five times more potent against the epileptiform activity elicited in the absence of Mg^{++} ions than that elicited by the GABA antagonist picrotoxin in rat hippocampal slices. The low antiepileptic potency of DL-AP5 demonstrated in the present study against the penicillin-induced epileptiform bursting is in agreement with these data and confirms the specific influence of DL-AP5 towards NMDA receptor-channel complexes (5). The lower inhibitory potency of E.A.A. antagonists is probably limited to the hippocampal area, because in cortical slices D-AP5 for a 100 μM concentration was equally effective on ''Mg^{++}-free'' and bicuculline epileptiform activities (4).

In conclusion, these data indicate that the hippocampal area is not the primary site of the anticonvulsant activity of E.A.A. antagonists.

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