BIBLID: ISSN 0253-9756 中国药理学报 *Acta Pharmacologica Sinica* 1992 Jul; 13 (4) · 319-322

Effect of intracerebral injection of pirenzepine on electroencephalography and convulsions in conscious rabbits[1]

JIANG Yuan-Xu, PENG Jian-Zhong
(*Department of Pharmacology, Ningxia Medical College, Yinchuan 750004, China*)

ABSTRACT Intracerebroventricular (icv) injection of pirenzepine (Pir) 2 mg · kg^{-1} caused EEG spike discharges, which first appeared in amygdala or hippocampus and then in midbrain reticular formation and cortex. After that rabbits developed clonic convulsions. Injection (300 μg) into amygdala or hippocampus also produced EEG spike discharges Hemicholinium 3 (HC-3) 50-150 μg · kg^{-1} icv inhibited the EEG spike and seizure discharges in varying degress produced by Pir. It is suggested that Pir has a central stimulatory action which is related to cholinergic mechanism.

KEY WORDS pirenzepine; hemicholinium 3; electroencephalography; convulsions

Pirenzepine (Pir) is a selective muscarinic M_1 receptor antagonist[1], with a strong inhibitory action on gastric acid secretion and has been used for treatment of gastric and / or duodenal ulcers[2]. Central effects of Pir in intact animals are conflicting, some presenting central inhibitory effects[3] and others showing no effect on CNS[4]. In this paper, to overcome the blood-brain barrier, we injected Pir intracerebrally to study the Pir effects on the electroencephalographic (EEG) activities and behavior in conscious rabbits.

MATERIALS AND METHODS

Rabbits of either sex ($n=96$) weighing $2.2 \pm s$ 0.4 kg were used.

The stainless steel electrodes were pretreated with routine insulating method in cortex and subcortex (amygdala, hippocampus and midbrain reticular formation) were used for icv of Pir. The cerebral electrical activities and behavior were recorded[5].

The rabbits cannula electrodes (0.8 mm OD) were implanted into amygdala or hippocampus. A stainless steel stylet was left in the cannula electrode. On d 6-7, 2-5 μl Pir was injected at 1 μl · min^{-1}. The injecting depth (0.1 mm below the implanted cannula) was marked by a plastic pipe on the microsyringe needle. Before and after the injection of Pir, EEG and behavior were recorded. The recording electrodes were the stainless steel cannula. At the end of each experiment, the subcortical electrode positions were verified histologically by Prussian-blue stain.

To investigate the influences of HC-3 on Pir effects, the rabbits were pretreated with icv of HC-3, 90 min prior to icv Pir 2 mg · kg^{-1}. The EEG activities and behavior were recorded for 60 min. In this study, the EEG spike discharges and clonic convulsive seizures were used as the criteria of the central stimulating action.

Pirenzepine (Pir, Chongqing Institute of Pharmaceutical Industry) and hemicholinium 3 (HC-3, Sigma) were dissolved in normal saline.

RESULTS

Effect of icv Pir Under quiet conditions, before Pir injection, the EEG in frontal cortex revealed mixed waves of high voltage (300-500 μV) slow wave (1-6 Hz) and low voltage (25-100 μV) rapid rhythm (16-22 Hz) and spindle waves appeared now and then. Irregular low voltage (10-100 μV) waves emerged in amygdala. High voltage (500-900 μV) θ rhythm were presented in hippocampus. EEG in midbrain reticular formation was low voltage (100-250 μV) of mixed θ and α waves.

After icv Pir 2 mg · kg^{-1}, EEG single spike appeared in 3-13 min and then multiple

Received 1990 Mar 15 Accepted 1992 Apr 3

[1] Part of this work was presented at the 4th Chinese Neuropharmacological Science Conference (1989 Oct 30, Wuhan) and posted at the 11th International Congress of Pharmacology (1990 Jul 6, Amsterdam).

spikes and clonic convulsions in all of 4 rabbits. These lasted > 160 min (Fig 1). The EEG spike discharges appeared first in amygdala in 10 / 16 rabbits, and in hippocampus in 5 / 17 rabbits (Tab 1). Prior to the clonic convulsions, the rabbits displayed excitation with restlessness, nystagmus, and tachypnea. At 3–60 min after icv Pir 1.5 mg · kg^{-1}, EEG spike discharges and clonic convulsions appeared in 6 / 9 rabbits. When the dosage of Pir was reduced to 0.5 mg · kg^{-1}, no marked alteration of EEG pattern and behavior occurred in 4 rabbits.

Tab 1. Rate of EEG spike discharges and clonic convulsions in conscious rabbits induced by icv pirenzepine. C = Cortex, A = Amygdala, H = Hippocampus, RF = Reticular formation.

Pir / mg · kg^{-1}	EEG spike discharges C	A	H	RF	Clonic convulsions
0.5	0 / 4	0 / 4	0 / 4	0 / 4	0 / 4
1.0	3 / 9	4 / 8	5 / 9	4 / 9	6 / 9
1.5	1 / 4	3 / 4	2 / 4	2 / 4	3 / 4
2.0	4 / 4	4 / 4	4 / 4	4 / 4	4 / 4

Four rabbits in the control group were injected icv saline of equal volume and the EEG and behavior did not show any changes.

Effect of Pir injected into amygdala or hippocampus When Pir (300 μg) was injected into amygdala or hippocampus, EEG spike discharges appeared in 4 / 5 rabbits, but convulsions did not occur. At 15–54 min after intraamygdala injection, the spike discharges first appeared in amygdala in 4 / 5 rabbits. Meantime, spike discharges came forth in hippocampus and reticular formation only in 1

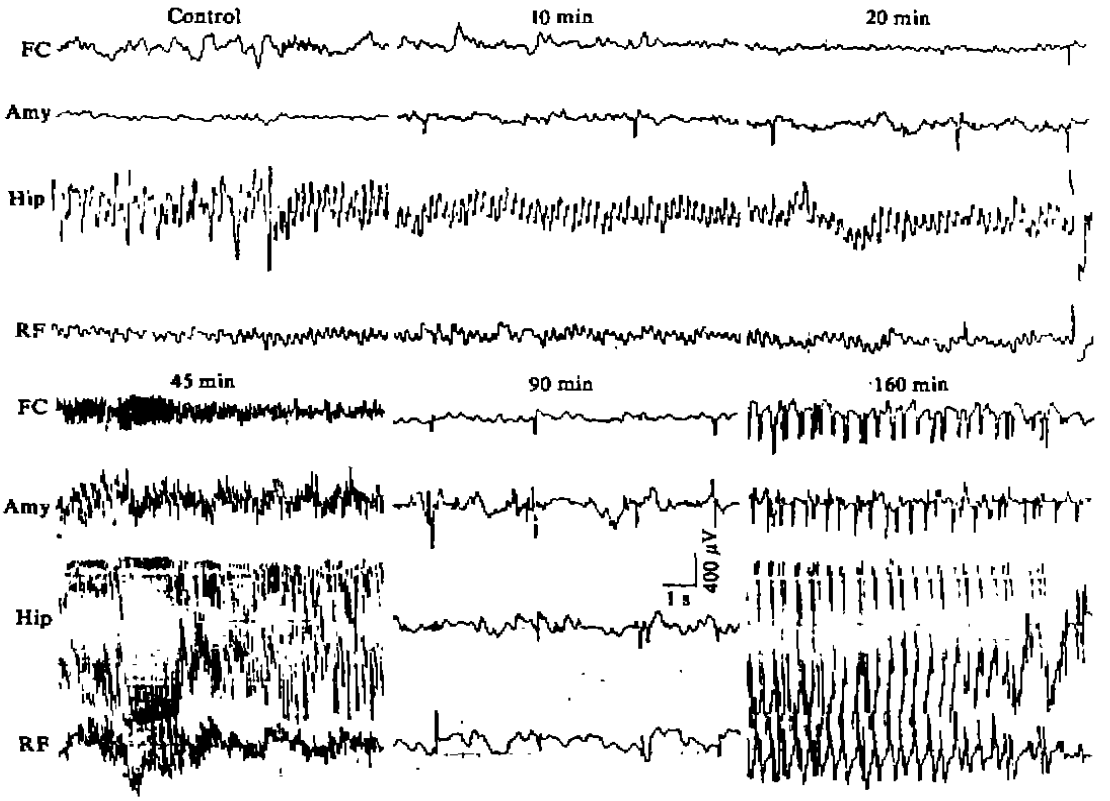

Fig 1. EEG activities of right brain in a conscious rabbit after injection of pirenzepine 2 mg · kg^{-1} into right lateral ventricle. frontal cortex (FC), amygdala (Amy), hippocampus (Hip), and reticular formation (RF).

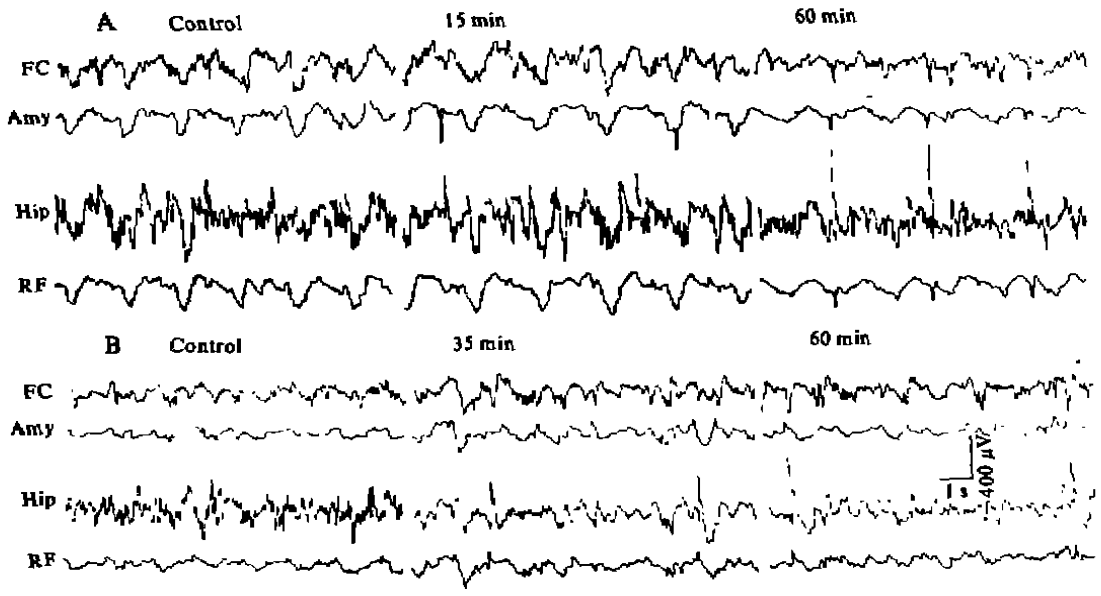

Fig 2. EEG activities after intraamygdala and intrahippocampal injections of Pir 300 μg in conscious rabbits. A) Amygdala spikes began after 15 min. Hippocampus and reticular formation low-voltage spikes appeared 35 min later and lasted > 60 min. B) Hippocampus spikes began after 35 min. The other electrode leads were not markedly affected. Electrode placements were same as in Fig 1.

rabbit (Fig 2-A). At 9-50 min after intrahippocampal injection, the spike discharges were recorded in hippocampus in 4 / 5 rabbits (Fig 2-B). But the spike discharge appeared in the cortex and reticular formation only in 1 rabbit each.

Upon intraamygdala Pir 150 μg, the spike discharges occurred in amygdala, hippocampus, reticular formation, and cortex in 1 / 5 rabbits. No significant alterations of EEG and behavior were noted after intrahippocampal Pir 150 μg.

Intraamygdala and intrahippocampal injections of equal volume of saline to 8 rabbits did not vary the EEG and behavior.

HC-3 antagonism of Pir effects HC-3 (25-150 $\mu g \cdot kg^{-1}$) was tested in 14 rabbits. At 25-40 min after icv HC-3, the EEG activities were characterized by mixed high voltage (600-900 μV, 1-2 Hz) and low voltage (100-200 μV, 4-6 Hz) waves in the cortex and subcortex, and intermittent spindle waves in the cortex. The reaction continued for at least 4 h. Most of the rabbits were quiet, but 2 were excited with restlessness, nystagmus, and the beard trembling after icv HC-3 150 $\mu g \cdot kg^{-1}$.

HC-3 50-150 $\mu g \cdot kg^{-1}$ antagonized the effects of Pir to a certain extent. In 3 / 5 rabbits after icv HC-3 150 $\mu g \cdot kg^{-1}$, spike discharge and convulsion did not occur within 150 min. Other 1 rabbit showed a partial antagonism with high amplitude sharp waves in EEG and nontypical clonic seizures. Only 1 rabbit exhibited no antagonism. HC-3 50 $\mu g \cdot kg^{-1}$ icv completely antagonized the central stimulatory effect of Pir in 2 / 4 rabbits and displayed partial antagonism in the other 2. HC-3 25 $\mu g \cdot kg^{-1}$ did not antagonize the effect of Pir in 4 rabbits.

DISCUSSION

Our results indicated that after icv of Pir to conscious rabbits, central stimulation was induced. The areas involved included at least the amygdala, hippocampus, midbrain reticular formation, and cortex. Earlier reports showed that icv of Pir produced central inhibitory effects in rabbits[3], yet in another, no such effects to the CNS were seen after ip Pir in rats and mice[4]. These contradictory findings may be related to the different routes of administration and different indices of investigation.

The EEG spike discharges appeared first in amygdala or hippocampus after icv of Pir. Intraamygdala or intrahippocampal Pir 300 μg also induced spike discharges. These indicated that the origins of the spike discharges were the amygdala and / or hippocampus. It has been known that both amygdala and hippocampus are the places having high affinity for Pir by radioligand binding study and autoradiography technique[6,7].

Since HC-3, an inhibitor of acetylcholine synthesis[8], could obviously be antagonistic to the central excitatory action of Pir, it was suggested that the Pir-induced central stimulation was related to cholinergic mechanisms. Although large doses of HC-3 were used in some rabbits, there were no complete antagonism to the central stimulation of Pir. It is possible that the central excitatory effect of Pir may also involve some non-cholinergic mechanism.

ACKNOWLEDGMENTS We are grateful to Professor LU Man-Wen for his advice and support, and to the Chong-qing Institute of Pharmaceutical Industry for the generous supply of Pir.

REFERENCES

1 Hammer R, Berrie CP, Birdsall NJM, Burgen ASV, Hulme EC. Pirenzepine distinguishes between different subclasses of muscarinic receptors. *Nature* 1980; **283**: 90-2.

2 Brunner H, Veritō M, Polterauer P, Grabner G. Effect of pirenzepine, a new gastric acid-inhibiting agent, on exocrine pancreatic secretion in man. *Arzneimittelforsch* 1977; **27**: 684-7.

3 Bian CF, Xing SH, Shao LN, Qin W. Central inhibitory effects of pirenzepine. *Acta Pharmacol Sin* 1987; **8**: 396-9.

4 Rogóz Z, Skuza G, Sowinska H. Central action of pirenzepine. *Pol J Pharmacol Pharm* 1981; **33**: 615-26.

5 Peng JZ, Jin LR. Sites of seizure discharges after intracerebroventricular injection of anisodamine and the antagonism by diazepam in rabbits. *Acta Pharmacol Sin* 1989; **10**: 484-7.

6 Waser PG, Rimvall K. Subtypes of muscarinic receptor in cultured explants of hippocampus of the rat. *Neuropharmacology* 1987; **26**: 1027-9.

7 Spencer DG Jr, Horvath E, Traber J. Direct autoradiographic determination of M_1 and M_2 muscarinic acetylcholine receptor distribution in the rat brain: relation to cholinergic nuclei and projections. *Brain Res* 1986; **380**: 59-68.

8 Dren AT, Domino EF. Effects of hemicholinium (HC-3) on EEG activation and brain acetylcholine in the dog. *J Pharmacol Exp Ther* 1968; **161**: 141-54.

319-322

脑内注射哌仑西平对清醒兔脑电描记和惊厥的影响

R971

蒋袁絮, 彭建中
(宁夏医学院药理学教研室, 银川 750004, 中国)

提要 清醒兔 icv 哌仑西平 Pir 2 mg · kg^{-1}, 首先在杏仁核或海马出现单个棘波放电, 尔后在中脑网状结构和大脑皮层出现棘波放电及全身阵挛性惊厥; 杏仁核或海马局部注射 Pir 300 μg 均可引起棘波放电. icv 密胆碱 50-150 μg · kg^{-1} 明显阻抑 Pir 所致的棘波放电和惊厥发作, 提示 icv Pir 具有中枢兴奋作用, 棘波放电的原发作用部位为杏仁核或海马

关键词 哌仑西平; 密胆碱; 脑电描记术; 惊厥