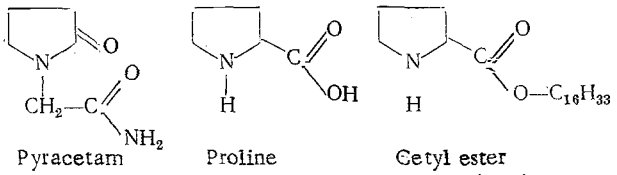
ANTAGONISM BETWEEN PYRACETAM AND PROLINE IN THEIR EFFECT ON MEMORY

R. U. Ostrovskaya, S. S. Trofimov,
N. M. Tsybina, T. A. Gudasheva,
A. P. Skoldinov, and V. V. Zakusov*

UDC 615.214.3:547.745].015.23:615.31:
547.747].015.4:612.821

KEY WORDS: cetyl ester of proline; pyracetam; amnesia.

Despite extensive clinical use of substances with nootropic activity the mechanism of their action is still largely unexplained. This applies above all to pyracetam, whose ability to accelerate recovery of memory functions when disturbed by harmful influences, and to increase resistance to hypoxia [1, 11] has not yet been sufficiently explained. There is now no doubt about the important role of mediator amino acids in realization of the effect of various neurotropic substances. In an attempt to analyze the mechanism of action of pyracetam, our attention was drawn to the amino acid proline, the only mediator amino acid which possesses some degree of structural similarity with the cyclic part of the pyracetam molecule.



The ability of proline to impair reproduction of conditioned-reflex responses has been described [7]. Since this effect was obtained on chickens, characterized by immaturity of the blood-brain barrier (BBB), this model, if the amino acid is injected intracerebrally, cannot be regarded as adequate in order to describe the effect of proline on memory processes in animals with a mature brain. The writers showed previously in the case of GABA that an amino acid which does not pass through the BBB can penetrate into the brain if injected systemically in the form of the lipophilic cetyl ester [2, 4]. This was subsequently confirmed by other workers [8, 10]. In view of the facts described above, it was decided to synthesize and study the neurotropic activity of CEP and its interaction with pyracetam, as reflected in parameters of the nootropic effect, and also with sodium hydroxybutyrate, as a compound which, within a certain dose range, exhibits a nootropic effect [3], but which does not possess a cyclic structure.

EXPERIMENTAL METHOD

To characterize its neurotropic activity CEP was tested in experiments on mice by studying the following parameters: general action, acute toxicity, effect on motor activity recorded by means of an "Opto-Varimex" multichannel actometer, movement coordination (revolving rod), and the maximal electric shock test, by studying its action on the convulsant effect of metrazol (110 mg/kg, subcutaneously) and bicuculline (2.5 mg/kg, subcutaneously). The effect of CEP on the animals' resistance to hypoxia and on their memory functions also was studied. Normobaric hypoxic hypoxia was created by keeping the mice in an airtight chamber in which the original oxygen concentration in the air was 8 vol. %.

Effects on memory processes were studied on a modified model of the passive avoidance conditioned reflex (PACR) in rats [6]. The time spent by the animal in the experimental two-compartment chamber was 150 sec, and at its end irremovable painful electrical stimula-

*Academician of the Academy of Medical Sciences of the USSR.

Institute of Pharmacology, Academy of Medical Sciences of the USSR, Moscow. Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 99, No. 3, pp. 311-314, March, 1985. Original article submitted April 19, 1984.

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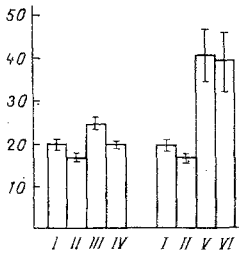

Fig. 1. Selectivity of antagonism of CEP and pyracetam on a model of normobaric hypoxic hypoxia. Vertically, length of survival of mice in airtight chamber (in min). I) Isotonic NaCl solution 75 min before animals placed in airtight chamber; II) CEP (30 mg/kg) 75 min before hypoxia; III) pyracetam (300 mg/kg) 60 min before hypoxia; IV) CEP (30 mg/kg) 75 min and pyracetan (300 mg/kg) 60 min before hypoxia; V) sodium hydroxybutyrate (300 mg/kg) 60 min before hypoxia; VI) CEP (30 mg/kg) 75 min and sodium hydroxybutyrate (300 mg/kg) 60 min before hypoxia.

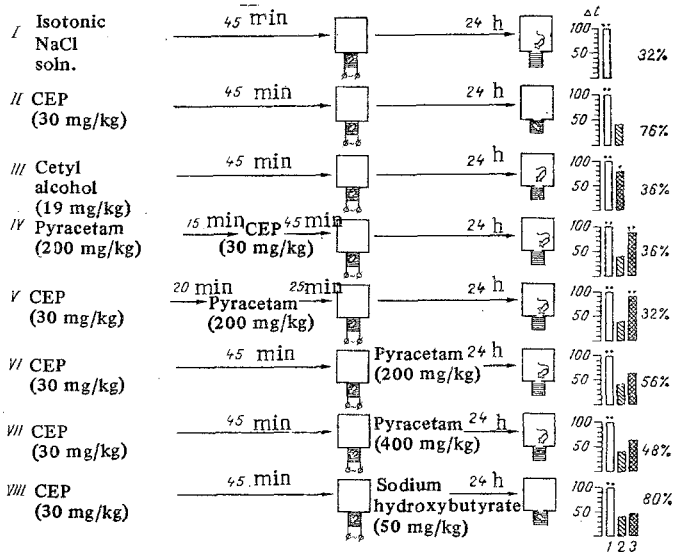

Fig. 2. Scheme of experiment and activity of compounds in PACR test. Order of administration of substances and formation of PACR and testing its preservation after 24 h shown on left side of figure. Histograms: ordinate, Δt (in sec). On right: number of animals (in %) going into darkened compartment of experimental chamber during testing preservation of PACR. 1) Injection of isotonic NaCl solution (series I); 2) injection of CEP (series II); 3) injection of other compounds (series III-VIII). *P < 0.05, **P < 0.01 compared with series II (Wilcoxon–Mann–Whitney U test). [No asterisks are present in the original Russian figure.]

tion was applied to rats in the darkened compartment through the floor (six stimuli with a strength of 2 mA and each 1 sec in duration, separated by intervals of 2 sec. The degree of preservation of PACR was estimated after 24 h: the number of animals which had not gone into the "dangerous" darkened compartment was counted and the difference between the lengths of the animal's stay in the darkened compartment was determined before learning and on testing 24 h later (Δt). Altogether 200 noninbred male rats weighing 170-220 g were used. The animals as a whole were divided into eight groups with 25 mice in each group. All substances were injected intraperitoneally.

EXPERIMENTAL RESULTS

In doses of 10-30 mg/kg CEP evoked no visible changes in the animals' general condition, but in a dose of 30 mg/kg motor activity was very slightly reduced (according to the results of actometry, by up to 20% compared with the control). Movement coordination was undisturbed and the response to external stimuli was preserved. LD_{50} was 310 mg/kg. CEP had a moderately strong protective action against maximal electric shock; ED_{50} for the appearance of tonic extension was 27 mg/kg. Against the convulsant action of metrazol and bicuculline, CEP was inactive. It very slightly reduced the length of survival of mice in the airtight chamber and reduced the severity of the antihypoxic effect of pyracetam (Fig. 1). At the same time, it did not weaken the protective action of sodium hydroxybutyrate in hypoxia.

A single reinforcement of the parameters described above by painful electric shock was sufficient to form a PACR (Fig. 2, series I). In a dose of 30 mg/kg (series II) CEP exhibited a marked amnesic effect: Unlike the animals of series I, which spent the time during testing preservation of PACR mainly in the illuminated compartment, most animals which received this compound went into the darkened compartment, and spent almost as long in it as before receiving painful stimulation, which was manifested as a decrease in Δt. Cetyl alcohol in a dose of 19 mg/kg (series III), equimolar with 30 mg/kg of CEP, did not induce an amnesic effect. This indicates that the amnesia induced by CEP is connected with the action of the amino acid itself and not with that of the lipophilic radical. Pyracetam, if injected before training (series IV and V) abolished, and if given after training (series VI and VII), it considerably reduced the intensity of the amnesic effect of CEP. Sodium hydroxybutyrate (series VIII), on the other hand, had no activity on this model of amnesia.

It follows from the results, first, that CEP, when injected extracerebrally (intraperitoneally) induces amnesia. In the intensity of this effect it is not inferior to amnesia due to electric shock (ES): Δt in experiments with CEP was 39.2 sec, compared with 42.7 sec in experiments with ES [1]. The suggested model of amnesia has definite advantages over other models. By contrast with ES, which induces profound, although transient, changes in functional state of the brain [9], CEP does not change the animal's general condition. It causes a disturbance of memory in a dose equal to one-tenth of toxic. In this respect "proline" amnesia has advantages over amnesia due to actinomycin D and other inhibitors of protein synthesis, whose amnesic effect develops only if toxic doses are used [5]. The advantage of the suggested method of obtaining amnesia with the aid of CEP over amnesia obtained in experiments on chickens by intracerebral injection of the amino acid itself, is not only that it is possible to work with adult animals with a properly formed BBB, but also that by systemic administration of the lipophilic derivative of proline, it is possible to study the generalized action of this amino acid on the brain, unlike in the model with intracerebral injection of proline, when it acts mainly on the hippocampus [7].

On the basis of the facts described above, and also since this amino acid is a metabolite of brain tissue (its concentration in the brain is 0.12 μmole/g [13]), proline can be regarded conjecturally as a natural amnesic factor. The effect of this factor is not weakened by sodium hydroxybutyrate (50 mg/kg), a compound which in this same dose exhibits antiamnesic properties when memory is disturbed by ES [3]. At the same time, in the present experiments antagonism was found between pyracetam and proline. It is most clearly exhibited on a model of PACR, although a definite tendency toward antagonism was observed in experiments with hypoxia.

The presence of definite similarity between proline and the cyclic part of the pyracetam molecule may perhaps be the cause of the direct competition between these compounds at the receptor level. Glutamate may also be involved in the mechanism of their interaction: We know that proline exhibits antagonism with glutamate in its action on postsynaptic receptors [15] and it prevents presynaptic liberation of the latter [12]. Glutamate activates phosphorylation of specific cerebral cortical proteins [14], whose important role in memory processes has now been conclusively demonstrated. Antagonism with glutamate is evidently one cause of the amnesic action of proline. Pyracetam, by weakening the effect of proline, may lead to strengthening of the action of endogenous glutamate on memory processes. Although these suggestions on the mechanisms of interaction of pyracetam with proline require further experimental verification, the fact that amnesia is obtained by systemic injection of the lipophilic derivative of proline and that pyracetam is selectively effective on this model may open up new aspects in the study of the mechanism of action of compounds possessing nootropic activity, and the biochemical basis of learning and memory.

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BINDING OF ^{3}H-SPIPERONE IN THE MOUSE BRAIN AFTER INTRAPERITONEAL INJECTION

A. M. Zharkovskii, T. A. Zharkovskaya and K. S. Chereshka

UDC 615.214.2.033.81.073

KEY WORDS: ^{3}H-spiperone; dopamine receptors; neuroleptics; tolerance.

Methods of radioligand binding with neuronal membranes *in vitro* have become widely used in the study of the action of psychotropic drugs on various brain receptors [4]. In the last few years methods of binding *in vivo* also have been successfully developed, and they possess a number of important advantages [2, 3, 5]. In particular, by this method it is possible to assess the character of binding of a radioactive ligand in the living organism, while maintaining the ionic composition, the presence of mediators, and pH [2]. To study the action of neuroleptics on dopamine and serotonin receptors, ^{3}H-spiperone, which has high affinity for these receptors [1-3, 6], is used as the radioligand.

In experiments *in vivo* minimal doses of ^{3}H-spiperone are injected into the caudal vein of mice or rats, and a short time (30 min-4 h) later, the level of radioactivity in the brain tissues is measured. Intravenous injection into mice is technically quite difficult, for it is not possible to guarantee that all the ligand enters the blood stream, and this results in loss of both animals and substance. Intravenous injection in rats is not difficult, but in this case a large quantity of ligand is required, and this makes the experiments too expensive. In the investigation described below binding of ^{3}H-spiperone was studied after intraperitoneal injection into mice.

EXPERIMENTAL METHOD

Experiments were carried out on male mice weighing 20 ± 1 g. Different quantities of ^{3}H-spiperone (16.7 Ci/mmole, from Amersham Corporation, England) were injected intraperitoneally into the animals. For comparison, ^{3}H-spiperone was injected into the caudal vein of animals of another group. The mice were decapitated 1.5 h after injection of the radioligand and the brain was removed in the cold and divided into parts: frontal cortex, basal

Department of Pharmacology and Laboratory of Psychopharmacology, Research Institute of General and Molecular Pathology, Tartu University. (Presented by Academician of the Academy of Medical Sciences of the USSR V. V. Zakusov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 99, No. 3, pp. 314-315, March, 1985. Original article submitted June 15, 1984.

