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INTERACTION BETWEEN KYNURENIN AND DIAZEPAM

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Diazepam, a universal anticonvulsant, prevents seizures induced by injection of L-kynurenin sulfate, an endogenous metabolite of tryptophan, into the cerebral ventricles of mice only in a dose as high as 30 mg/kg (ED_{50}), whereas against metrazol, maximal electric shock, and strychnine, its ED_{50}, according to our data and those published in the literature, is 0.5-1.0, 3.0-4.0, and 9.0-10.0 mg/kg respectively. It has been suggested that the uniquely low activity of diazepam against kynurenin may perhaps be connected with the fact that the latter, with a similar structure to that of diazepam, either as a ligand reduces binding of diazepam with benzodiazepine receptors or, as a modulator with no effect on binding, depresses the physiological effect of this binding.

It was accordingly decided to study interaction between kynurenin and diazepam in tests other than those with kynurenin-induced seizures. This paper describes an investigation into this problem.

EXPERIMENTAL METHOD

Experiments were carried out on male SHR albino mice and C57BL/6 black mice weighing 16-18 g, from the "Rappolovo" nursery, Academy of Medical Sciences of the USSR, in September-December. An aqueous solution of L-kynurenin sulfate (subsequently described as kynurenin), from Sigma (USA), was injected into the cerebral ventricles of waking animals by means of a semiautomatic apparatus. Control mice received injections of 5 μl of physiological saline. The method was described in detail previously [13]. Diazepam (packed in ampuls, from Gedeon Richter, Hungary), metrazol, and caffeine (caffeine sodium-benzoate or base) in distilled water were injected intraperitoneally in a volume of 1% of body weight. Diazepam was injected 30 min before metrazol or caffeine, and 20 min before determination of orienting motor activity; kynurenin was injected 1 min before caffeine or metrazol and 10 min before determination of orienting motor activity. The convulsant effect of metrazol and caffeine was assessed on the basis of five criteria: the latent period of clonicotonic seizures, the number of animals with clonic seizures in the group, the number of animals with tonic extension in the group, mortality, length of survival. Equally effective doses (ED_{90}, intraperitoneally) were used: the metrazol 80 mg/kg, of caffeine sodium-benzoate 170 mg/kg, and of caffeine base 250 mg/kg. Orienting motor activity was recorded in a single mouse for 2 min in a metal chamber measuring 20 × 15 × 10 cm, at the number of times the mouse crossed lines dividing the floor into four rectangles (locomotion), and as the number of times the mouse stood up on its hind limbs. A conflicting situation test [8, 9], which has proved to be adequate and reliable for the study of benzodiazepine and other tranquilizers, was carried out in a chamber consisting of light (27 × 28 × 27 cm) and dark (27 × 16.5 × 27 cm) parts. Locomotion and standing movement of a single mouse were recorded for 2 min in both compartments, and the number of crossings from one compartment into the other through a gate 10 cm wide and 6 cm high was recorded. The mice were placed in a corner of the light compartment. Diazepam and kynurenin were injected simultaneously 30 min before the test.

The alternative data were compared by means of tables [1] and the graduated data by Student's t test. Each group of mice contained 10-12 animals.

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TABLE 1. Weakening of Anticaffeine Effect of Diazepam by Kynurenin (results of three experiments)

No. of group	Preparations injected before caffeine		Latent period of "caffeine" seizures (M ± m)
	Intraperitonally	into cerebral ventricles	
1	Distilled water	physiological saline	3,2±0,2
2	The same	kynurenin 10 μg	3,1±0,1
3	Diazepam 5 mg/kg	physiological saline	5,7±0,4°
4	The same	kynurenin 10 μg	4,3±0,2**
1	Distilled water	physiological saline	3,2±0,1
2	The same	kynurenin 10 μg	3,2±0,2
3	Diazepam 5 mg/kg	physiological saline	7,0±0,6°
4	The same	kynurenin 10 μg	5,2±0,4*
1	Distilled water	physiological saline	5,3±0,3
2	The same	kynurenin 10 μg	5,1±0,2
3	Diazepam 5 mg/kg	physiological saline	9,8±0,3°
4	The same	kynurenin 10 μg	5,7±0,4***

Legend. °P < 0.01 compared with control (group 1), asterisks — significance of difference compared with group 3: *P < 0.05, **P < 0.02, ***P < 0.01. Caffeine sodium-benzoate in dose of 750 mg/kg.

EXPERIMENTAL RESULTS

The anticaffeine effect of diazepam, namely lengthening of the latent period of "caffeine" seizures (the only parameter of antagonism between these drugs when diazepam was used in doses of 1-20 mg/kg) was almost completely abolished by kynurenin in all experiments. In the control, kynurenin had no effect on "caffeine" seizures. Diazepam reduced the number of animals with clonic seizures but only in doses of 25 and 30 mg/kg. "Caffeine" seizures, like "kynurenin seizures," were much more resistant to the anticonvulsant action of diazepam than "metrazol," "strychnine," seizures, and so on. ED_{50} for diazepam against caffeine (both caffeine sodium-benzoate and base) and of kynurenin was 27 and 30 mg/kg respectively, whereas against other convulsants it was much smaller (see above). Correlation between the effects of kynurenin and caffeine may have profound significance, since both substances are related to the biochemical consequences of stress. The kynurenin level rises during stress in the brain [12] and in the body as a whole [13], and this may play an important role in the development of the late consequences of chronic emotional stress [4]. Caffeine potentiates pathophysiological and hormonal manifestations of stress in mice [11]. Excessive caffeine consumption and a predilection for it increased the frequency and severity of anxiety states in psychiatric patients [10]. Antagonism of kynurenin with the anticaffeine effect of diazepam probably reflects interaction between the anxiogenic effect of kynurenin and the anxiolytic effect of diazepam. Caffeine displaces [^{3}H]diazepam *in vitro* from benzodiazepine receptors [16] but does not displace [^{3}H]flunitrazepam *in vivo* [17]. The action of caffeine on benzodiazepine receptors is considered to be indirect, through the intermediaty of purinergim mechanisms [17].

The antimetrazol effect of diazepam was unchanged by kynurein (0.1-10 μg). In doses of 0.1-5 μg kynurenin did not potentiate the defensive action of subthreshold (0.1 mg/kg) and threshold (0.25 mg/kg) doses of diazepam. The hypothetical endogenous ligand of benzodiazepine receptors — inosine, hypoxanthine, and nicotinamide, in doses of 1000 and 2000 mg/kg (lower doses are ineffective) — counteracted the convulsant effect of metrazol and caffeine injected 30 min later, about equally and weakly merely lengthening the latent period of the seizures by 2-4 times depending on the dose. A dissimilarity in the action of the hypothetical ligands and of diazepam is again exhibited in this ase. Diazepam was much more effective against metrazol than against caffeine: according to our data the corresponding values of ED_{50} were 0.5 and 27 mg/kg. Previously diazepam was shown to have little effect against

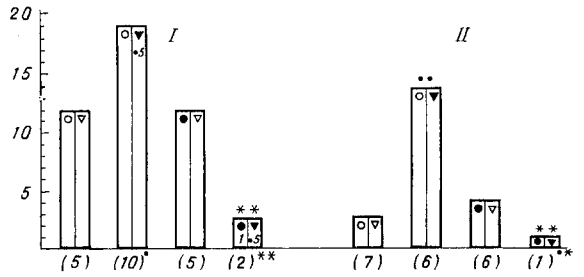

Fig. 1

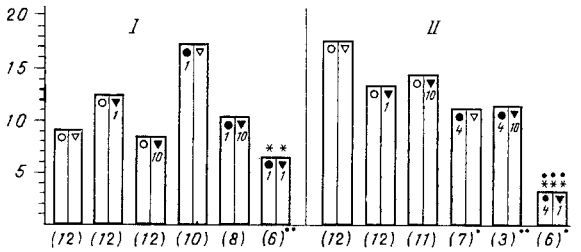

Fig. 2

Fig. 1. Prevention of excitatory effect of kynurenin by diazepam. Height of columns denotes number of lines crossed by mouse in 2 min (locomotion). Circles denote intraperitoneal injection of distilled water (empty) and diazepam (filled). Triangles denote injection of physiological saline (empty) and kynurenin (filled) into cerebral ventricles. Numbers inside columns give doses of diazepam (in mg/kg) and kynurenin (in μg); in parentheses below columns is number of mice (in groups of 12 animals) placed in chamber. Significance of differences: dots — compared with control: distilled water (intraperitoneally) + physiological saline (into ventricles); asterisks — compared with group 2: distilled water (intraperitoneally) + kynurenin (into ventricles). One dot (or asterisk) $P < 0.05$, two dots (or asterisks) $P < 0.02$, three dots (or asterisks) $P < 0.01$. Results of two experiments (I and II).

Fig. 2. Potentiation of sedative effect of diazepam by kynurenin. I) Experiment on SHR mice; II) experiment on C57BL/6 mice. Remainder of legend as to Fig. 1.

"kynurenin" seizures whereas the ligands were highly effective [15]. Such considerable differences between the pharmacologic effects of diazepam and the hypothetical ligands make it questionable whether weak binding of purines and nicotinamide with benzodiazepine receptors plays a significant physiological role and whether the pharmacologic effects of the hypothetical ligands, such as their anticonvulsant effect, are due to their action on benzodiazepine receptors.

In some experiments shortening of the latent period of metrazol seizures was observed after preliminary injection of small doses of diazepam (0.05 and 0.1 mg/kg). Since kynurenin does not influence the convulsant effect of metrazol [14] and does not change the antimetrazol effect of diazepam, but weakens the anticaffeine effect of diazepam, it is logical to suggest that the anticonvulsant action of diazepam against caffeine and against metrazol has different mechanisms. Only the similarity in the effects of these two convulsants and in their antagonism with diazepam was emphasized previously [16, 17].

The excitatory effect of kynurenin was significantly and reproducibly prevented or even reversed by diazepam in a dose of 1 mg/kg (Fig. 1). Excitation of locomotion was observed after injection of kynurenin in doses of 0.5 and 2.5 μg. The convulsant effect of 50 μg kynurenin was prevented by diazepam in a dose of 30-35 mg/kg [7], i.e., in an almost identical ratio between doses.

The sedative effect of diazepam was potentiated by kynurenin (Fig. 2). The effective dose of kynurenin, namely 1 μg, was 10 times less than the dose reducing the anticaffeine effect of diazepam. In doses of 0.1 and 10 μg kynurenin did not affect the sedative action of diazepam (Fig. 2). The opposite effect of smaller (1 μg) and larger (10 μg) doses of kynurenin on the action of diazepam (synergism and antagonism respectively) is in harmony with the theory of competitive relations [2].

The anticonflict effect of diazepam — an increase in the number of passages between the light and dark compartments (Fig. 3) — was unchanged by kynurenin, although kynurenin itself had the opposite action — it reduced the number of passages. No increase in locomotion of the mice was observed under the influence of diazepam in a conflicting situation chamber of similar design [8]. Diazepam (0.5 mg/kg) prevented excitation induced by kynurenin (1 μg) in the light compartment (Fig. 3). The possibility cannot be ruled out that optimal conditions, more especially the time of injection of drugs, for demonstration of their interaction were not found.

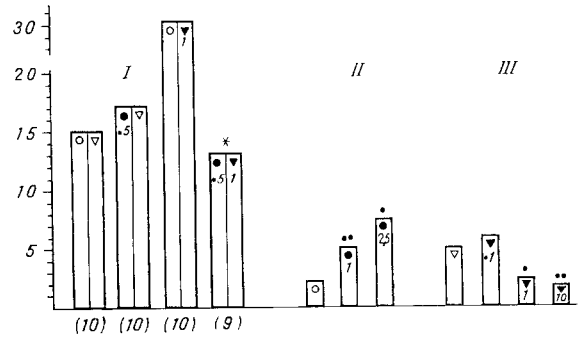

Fig. 3. Opposite effect of diazepam and kynurenin on behavior of mice in conflicting situation chamber. Vertical axis — number of lines crossed by mouse in light compartment in 2 min (experiment I) or number of passages through gate in 2 min (experiments II and III). Remainder of legend as to Fig. 1.

Kynurenin thus modified the anticaffeine and sedative effects of diazepam and did not change its antimetrazol and anticonflict effects. This is indirect evidence against the view that kynurenin may displace diazepam from the same benzodiazepine receptors. In the conflicting situation test kynurenin had an action opposite to that of diazepam. The most likely explanation of the results of this investigation is as follows: some effects of kynurenin, such as antagonism or synergism with diazepam, may be linked with benzodiazepine receptors, where, through antagonism with purines [5], with GABA [6], and with serotonin and its precursors [3], it may modulate the function of these receptors, whereas its other effects, such as potentiation of the effects of the conflicting situation, are unconnected with benzodiazepine receptors.

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