SPECIES SPECIFIC DIFFERENCES IN BEHAVIORAL EFFECTS OF CERULEIN, A CHOLECYSTOKININ OCTAPEPTIDE RECEPTOR AGONIST, IN ALBINO MICE AND RATS

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The cholecystokinin octapeptide (CCK-8) and its close analog cerulein possess a broad spectrum of pharmacologic action [15]. They inhibit spontaneous motor activity, they counteract amphetamine-induced motor excitation, block stereotyped behavior induced by dopaminomimetics, and have an anticonvulsant action, and so on [3, 8, 14, 15]. However, investigators have not always succeeded in reproducing in their own experiments the results obtained by others. For instance, in some investigations cerulein and CCK-8 inhibited the behavioral effects of apomorphine [12, 15], whereas in others the opposite action was observed, namely potentiation of the effects of this dopaminomimetic [2, 13].

The aim of the present investigation was to discover the cause of these contradictory data. Accordingly it was decided to study species-specific differences in the action of cerulein, an agonist of CCK-8 receptors. In comparative experiments on male mice and rats the long-term antiamphetamine action of cerulein [7] and antagonism between cerulein and the endogenous convulsant quinolinic acid (QUA) were studied [1, 11].

EXPERIMENTAL METHOD

Noninbred male albino mice weighing 18-24 g and noninbred male rats weighing 180-220 g were obtained from the Rappolovo Nursery, Academy of Medical Sciences of the USSR (Leningrad Region) in the spring and summer.

Antagonism with amphetamine-induced motor excitation was studied by the following scheme: on the first day of the experiment rats or mice of one group were given an intraperitoneal injection of physiological saline, animals of a second group received a subcutaneous injection of cerulein (rats 40 μg/kg, mice 50 and 100 μg/kg body weight), a third group received haloperidol (0.25 mg/kg, intraperitoneally), and animals of a fourth group received both haloperidol and cerulein. The experiments with amphetamine were repeated three times: after 24 h and on the 7th and 14th days after a single injection of haloperidol (Gideon Richter, Hungary) and cerulein (Farmitalia Carlo Erba, Italy). The excitatory action of amphetamine (3 mg/kg) on motor activity of the mice was determined with the aid of a photoelectric actometer. The animals were placed in the actometer 15 min after intraperitoneal injection of amphetamine and the animals' motor activity was estimated for 30 min. The effect of amphetamine (2 mg/kg) on motor activity of the rats was determined 45 min after intraperitoneal injection, over a period of 5 min in the open field test (recorded by the photoelectric method, with the aid of five independent channels). In a separate series of experiments the action of a combination of proglumide (50 mg/kg, Rotta Research Laboratories, Italy), a CCK-8 receptor antagonist, with haloperidol and cerulein on the long-term antiamphetamine action of cerulein was determined in rats.

The influence of cerulein on the effects of the different convulsants in mice was determined as follows: QUA (5 μg), L-kainic acid (0.2 μg), L-kynurenin sulfate (50 μg), and N-methyl-D-aspartate (0.1 μg; all from Sigma, USA) were injected into the lateral cerebral ventricle by means of a semiautomatic apparatus by the method described previously [5] in convulsant doses (ED_{100}), in a constant volume of 2 μl. Proglumide was injected into the mice intraperitoneally 5 min before the intraventricular injection of cerulein or 10 min before the convulsant. Cerulein (1-50 ng, intraventricularly, and 100-500 μg/kg, subcutane-

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TABLE 1. Effect of a Single Preliminary Subcutaneous Injection of Cerulein (50-100 μg/kg) and Intraperitoneal Injection of Haloperidol (0.25 mg/kg) on Motor Excitation Induced by Amphetamine (3 mg/kg) in Mice (M ± m)

Substance	Number of impulses during 30 min	
	1st day	7th day
Physiological saline + physiological saline	314±36	356±34
Physiological saline + amphetamine (3 mg/kg)	589±69	496±57
Cerulein (50 mg/kg) + amphetamine (3 mg/kg)	540±61	615±62
Cerulein (100 mg/kg) + amphatemine (3 mg/kg)	854±98*	563±70
Haloperidol (0.25 mg/kg) + amphetamine (3 mg/kg)	717±58	578±69
Haloperidol (0.25 mg/kg) + cerulein (50 mg/kg) + amphetamine (3 mg/kg)	815±80*	799±80*
Haloperidol (0.25 mg/kg) + cerulein (100 mg/kg) + amphetamine (3 mg/kg)	814±76*	747±76*

Legend. *p < 0.05 (Mann-Whitney U test) compared with group of mice receiving physiological saline + amphetamine (3 mg/kg).

ously) was injected 5 min before the convulsants. The animals remained under observation for 10 min after injection of the convulsant.

In the experiments on rats convulsions induced by QUA were studied and cerulein was injected subcutaneously in a dose of 200 μg/kg or into the cerebral ventricles in doses of 2-20 ng 5 min before QUA (30 and 60 μg into the cerebral ventricles). The cannula was inserted into the left lateral ventricle of the rats under pentobarbital anesthesia (40-50 mg/kg). Full details of the method of insertion were described previously [6]. The animals were used in the experiments 4-5 days after the operation. Solutions of the drugs were injected by means of a Hamilton's syringe and a polyethylene tube. The behavior of the rats was kept under observation for 90 min after injection of the convulsant. Four parameters were determined in all groups: the latent period of clonic convulsions, the frequency of clonic and tonic extensions, and mortality in the group.

EXPERIMENTAL RESULTS

A single combined injection of cerulein and haloperidol and, to a lesser degree, injection of cerulein alone, had a prolonged inhibitory effect on the excitatory action of 2 mg/kg of amphetamine (Fig. 1). Their antiamphetamine action was clearly manifested 24 h after combined injection of cerulein and haloperiodol. The opinion is held that the prolonged inhibitory effect of a combined injection of haloperidol and cerulein on amphetamine excitation of motor activity is realized through β-endorphin in nucleus accumbens [7]. Our own data indicate that the effect of cerulein on β-endorphinergic processes is mediated through CCK-8 receptors. This hypothesis is supported by the fact that the antiamphetamine action develops even after injection of cerulein alone. However, proglumide (50 mg/kg), a known antagonist of CCK-8, did not abolish the effect of a combined injection of haloperidol and cerulein. This suggests that proglumide does not interact with CCK-8 receptors. The role of haloperidol is to increase the sensitivity of opioid receptors to β-endorphin in mesolimbic structures [10]. In experiments on mice cerulein and haloperidol had no such antiamphetamine action. Preliminary injection of cerulein (50 and 100 μg/kg) and combined injection of cerulein and haloperidol actually potentiated the effect of amphetamine, i.e., hypersensitivity to the excitatory action of amphetamine was observed in the mice (Table 1).

Injection of cerulein (1 ng) into the cerebral ventricles prevented QUA-induced convulsions in mice. Proglumide (50 mg/kg) completely abolished the protective effect of cerulein. In a smaller dose (25 mg/kg) proglumide potentiated the convulsant effect of a

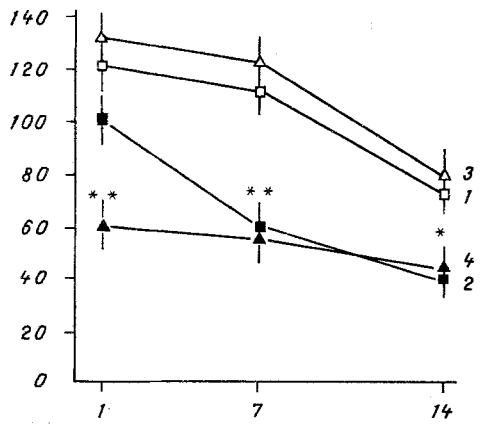

Fig. 1. Effect of a single preliminary subcutaneous injection of cerulein (40 μg/kg) and intraperitoneal injection of haloperidol (0.25 mg/kg) on amphetamine (2 mg/kg) induced motor excitation in rats. Abscissa, time after single injection of cerulein or haloperidol (in days); ordinate, number of impulses during 5 min. 1) Control (action of amphetamine in rats receiving preliminary injection of physiological saline); 2) cerulein; 3) haloperidol; 4) haloperidol + cerulein. *$p < 0.05$, **$p < 0.01$ compared with control animals receiving physiological saline (Mann–Whitney U test).

subthreshold dose of QUA (2.5 μg) – it increased the number of animals with convulsions from 0 to 5 in a group of six mice. Incidentally, the anticonvulsant action of cerulein in this model was evidently quite selective in character. Cerulein prevented convulsions induced only by QUA and N-methyl-D-aspartate. This confirms the view that these substances act on the same common N-methyl-D-aspartate receptor [11]. Cerulein was inactive against cainic acid and kynurenin. On subcutaneous injection into mice, cerulein over a wide range of doses (100-500 μg/kg) had only a weak effect on QUA-convulsions, simply lengthening the latent period of their onset. Preliminary subcutaneous (200 μg/kg) or intraventricular injection (2-20 ng) of cerulein did not prevent the development of QUA convulsions in rats or changed either the number of seizure attacks or the latent periods of clonic and tonic convulsions. The length of survival of the animals was actually shorter in the experimental group than in the control (52 and 92 min, respectively).

The results are thus evidence of considerable differences in the action of cerulein on rats and mice. The unequal effect of cerulein on the excitatory action of amphetamine is evidently due to differences in interaction between CCK-8 and dopaminergic systems in rats and mice. Whereas in the experiments on mice cerulein, injected subcutaneously in a dose of 75 μg/kg or more, abolished the increased motor activity due to amphetamine [13], in rats a dose of 40 μg/kg of cerulein (injected subcutaneously) did not change the effect of amphetamine [7]. On the basis of these data it can be postulated that cerulein, administered systemically to mice, has a direct inhibitory influence on dopaminergic processes in the limbic structures, and this leads to increased sensitivity of mice to the excitatory action of amphetamine after a single dose of cerulein. In rats, interaction between dopamine and CCK-8 is more complex, and may perhaps be mediated through increased release of β-endorphin in the nucleus accumbens [7], which ultimately leads to a prolonged decrease in sensitivity of rats to the excitatory action of amphetamine. In experiments on mice, cerulein, injected intraventricularly (but not systemically) proved to be a powerful and seleetive antagonist of endogenous convulsants, namely QUA and N-methyl-D-aspartate. It must be pointed out that in the grass frog (*Rana temporaria*), whose skin has been shown to contain large quantities of cerulein, preliminary administration of 1-5 ng of cerulein also prevented QUA-induced convulsions, whereas proglumide (50-100 mg/kg) abolished this effect. In experiments on rats cerulein had no such action. In addition, when injected intraventricularly into rats, the animals developed QUA-induced convulsions much more slowly than mice. The probable explanation of these differences in rats and mice is the unequal spatial arrangement of the lateral ventricles and hippocampus in the brain [4, 6, 9], leading to unequal penetration of the test substances into the hippocampus when injected into the cerebral ventricles.

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