ANTAGONISM OF CERULEIN, A CCK-8 RECEPTOR AGONIST TO THE BEHAVIORAL EFFECTS OF KETAMINE IN MICE AND RATS

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Phencyclidine and other aryl cyclohexylamines in subamnesic doses have a psychomimetic action on man [1]. The use of phencyclidine or its weaker analog ketamine is accompanied by amnesia in man [2, 6]. In rats and mice phencyclidine and ketamine induce enhanced motor activity and stereotyped behavior: behaviorial effects reminiscent in many respects of the action of amphetamine and other dopaminomimetics in these animals [9]. Activation of motor activity after administration of phencyclidine or ketamine is accompanied by ataxia [14]. It has been shown that administration of phencyclidine to mice completely inhibits the formation of a defensive reflex by the passive avoidance method [11]. The view is held that the stereotyped behavior induced by phencyclidine is due to its interaction with serotonin$_2$-receptors [9], whereas the amnesic action of phencyclidine is realized through opioid receptors [11]. The octapeptide cholecystokinin (CCK-8) and its close analog cerulein have an antidopaminergic action, and antagonise the excitatory action of amphetamine on the behavior of rats and mice [15]. Data have also been obtained to show that CCK-8 induces a marked antiamnesic effect in rats [7].

The aim of the present investigation was to study the effect of cerulein, an agonist of CCK-8 receptors, on the behaviorial effects of ketamine in rats and mice, paying special attention to changes in opiodergic and dopaminergic systems.

EXPERIMENTAL METHOD

All behavioral experiments were conducted on male mice weighing 25-30 g and on male rats weighing 220-270 g. In experiments on mice the effect of cerulein on the principal behavioral effects of ketamine were studied: enhanced motor activity, stereotyped behavior, and ataxia. Ketamine (Gedeon Richter, Hungary), in a dose of 15-30 mg/kg, was injected subcutaneously into the mice 5 min before subcutaneous injection of cerulein in a dose of 75-375 μg/kg (Farmitalia-Carlo Erba, Italy). Haloperidol (0.1-1.5 mg/kg, intraperitoneally, from Gedeon Richter, Hungary, an antagonist of dopamine$_2$-receptors, was given 30 min before the injection of ketamine. The intensity of stereotyped behavior was studied by the method in [4] at the 10th minute after injection of ketamine. The intensity of ataxia also was estimated at the 10th minute after injection, on a conventionally prepared scale [3]. From the 10th to the 15th minute after injection of ketamine its effects on the orienting and investigative activity of mice in an open field was studied. The open field (30 × 30 × 15 cm) was divided by lines into 16 sectors (with a nest in the center of each sector). For 5 min the number of sectors visited by the mice, the number of rearings on the hind limbs, and the number of nests investigated were determined. The effect of cerulein on the amnesic action of ketamine was studied in rats by the passive avoidance method in a shuttle box. On the 1st day of the investigation the animals were adapted to the experimental situation. On the 2nd day the animals were trained. After the rat moved into the dark compartment the door between the two parts of the shuttle box was closed and the animal received four electric shocks (40 V) through the floor of the box. The interval between the electric shocks was 45 sec. Only those animals which moved from the light compartment of the box into the dark compartment in the course of 20 sec were chosen for the experiment. Immediately after training the animals were given an injection of ketamine (7.5-30 mg/kg, subcutaneously), of ceru-

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TABLE 1. Effect of Cerulein and Haloperidol on Behavioral Effects of Ketamine in Mice

Substance	Dose	Stereotyped behavior, points	Ataxia points	Orienting-investigative activity in the course of 5 min		
				No. of crossings between sectors	No. of nests investigated	No. of rearings
Physiological saline		0±0	0±0	40±3,2	9±1,2	7±1,6
Ketamine	15 mg/kg	1,75±0,15	1,20±0,20	60±5,8	8±1,8	0±0
The same	30 mg/kg	1,92±0,12	1,83±0,25	85±6,6	8±0,9	0±0
Ketamine + cerulein	30 mg/kg+75 μg/kg	1,75±0,20	1,75±0,15	48±4,2*	6±0,8	0±0
Ketamine + cerulein	30 mg/kg+150 μg/kg	1,20±0,18*	1,50±0,20	35±4,0**	6±0,9	0±0
Ketamine + cerulein	30 mg/kg+225 μg/kg	0,83±0,12*	1,40±0,20	24±5,2**	4±0,5*	0±0
Ketamine + cerulein	30 mg/kg+375 μg/kg	0,33±0,15**	1,32±0,15	11±1,6***	3±0,6**	0±0
Ketamine + haloperidol	30+0,1 mg/kg	1,50±0,20	1,42±0,20	58±5,2	6±0,9	0±0
Ketamine + haloperidol	30+0,5 mg/kg	1,20±0,16*	1,17±0,25	21±4,2**	4±0,5*	0±0
Ketamine + haloperidol	30+1,5 mg/kg	0,33±0,15**	1,83±0,20	4±0,2***	0±0***	0±0

Legend. Asterisks indicate significance of differences between parameters observed and those obtained with ketamine alone, by Mann–Whitney U test: *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

TABLE 2. Effect of Cerulein and Naloxone on Amnesic Action of Ketamine in Rats

Substance	Dose	Latent period of passage into dark compartment, sec		Total time of stay in dark compartment, sec
		before training	after training	
Physiological saline		11±1,2	105±20	58±10
Ketamine	7,5 mg/kg	14±2,0	96±25	59±15
Ketamine	15 "	8±1,4	66±20	95±17
Ketamine	30 "	13±1,2	33±12	135±26
Ketamine + cerulein	15 mg/kg + 10 μg/kg	11±1,8	180±0**	0±0**
Ketamine + cerulein	30 mg/kg+10 μg/kg	9±2,0	158±15*	3±3**
Cerulein	10 μg/kg	12±2,0	120±15	47±12
Ketamine + naloxone	30+5 mg/kg	10±2,2	180±0**	0±0**
Naloxone	5 "	14±1,8	110±25	45±15

Legend. Asterisks indicate significance of difference (Student's t test) between parameters obtained and those following injection of ketamine: *$p < 0.05$, **$p < 0.01$.

lein (10 μg/kg, subcutaneously), or naloxone (5 mg/kg subcutaneously, from Endo Laboratories, USA), or a combination of ketamine with cerulein or naloxone. The latent period of passage of the animal from the light into the dark compartment of the shuttle box and also the duration of the rat's stay in the dark compartment were determined 24 h after training. The behavior of each animal was kept under observation for 3 min. Parallel with the behavioral experiments, we studied the effect of ketamine on binding of ^{3}H-spiroperidol in the frontal cortex of rats in the presence of 5 μM sulpiride (Ravizza, Italy), a selective antagonist of dopamine$_2$-receptors, and in the caudate nucleus in the presence of 1 μM pirenperone (Janssen Pharmaceutica, Belgium), an antagonist of serotonin$_2$-receptors. Binding of ^{3}H-spiroperidol (16 Ci/mmole, Amersham International, England) was investigated by the method in [5]. The effect of ketamine on binding of ^{3}H-etorphine (36 Ci/mmole, Amersham International) was studied in the rats' forebrain by the method in [12].

EXPERIMENTAL RESULTS

Subcutaneous injection of ketamine in doses of 15 and 30 mg/kg caused definite intensification of motor activity: motor excitation in mice in the open field, intensive stereotyped sniffing, and ataxia (Table 1). Because of ataxia, rearing by mice receiving ketamine was not observed. Cerulein in a dose of 75 μg/kg antagonized the motor excitation caused by ketamine (30 mg/kg), and only in a dose of 360 μg/kg did it completely suppress ketamine stereotypy. Under these circumstances cerulein had only a weak effect on ketamine-induced ataxia (Table 1). Haloperidol in a dose of 0.5 mg/kg significantly weakened motor excitation and stereotyped behavior caused by ketamine (30 mg/kg). In rats ketamine induced amnesia

(Table 2). In doses of 15 and 30 mg/kg the drug significantly disturbed training of the rats. In a dose of 10 μg/kg cerulein had no effect on training of the rats in the shuttle box, but it completely abolished the amnesic action of ketamine. Naloxone, an antagonist of opioid receptors, with a dose of 5 mg/kg had a similar action (Table 2). In experiments to study radioligand binding, ketamine did not affect binding of ^{3}H-spiroperidol (0.25 mM) in the caudate nucleus (dopamine$_2$-receptors) and in the frontal cortex (serotonin$_2$-receptors), even in a concentration of 100 μM. Ketamine caused semi-inhibition of binding of ^{3}H-etorphine (0.25 nM) in a concentration of 30 μM. With a further increase in the concentration of ketamine, its effect on binding of ^{3}H-etorphine was unchanged.

The results are thus evidence that the CCK-8 receptor agonist cerulein can antagonize definite behavioral effects of ketamine, a stimulator of phencyclidine receptors. It has been suggested that the motor excitation induced by phencyclidine is realized in rats and mice through serotonin$_2$-receptors [9]. However, according to the results of the present investigation, ketamine had no such action. Ketamine did not interact with serotonin$_2$-receptors and did not induce any behavioral effects characteristic of serotoninomimetics (head shaking, "wet dog" shaking, and so on). Evidently the stereotyped behavior and motor excitation observed after injection of ketamine are due, just as in the case of amphetamine, to increased dopamine released from presynaptic terminals in the caudate nucleus and mesolimbic structures. Cerulein, as we know, has an antidopaminergic action [13, 15]. The antagonism of cerulein with the stereotyped behavior and motor excitation evoked by ketamine also is probably connected with the antidopaminergic action of cerulein. This hypothesis is supported by the fact that haloperidol, predominantly an antagonist of dopamine$_2$-receptors, has an inhibitory influence similar to that of cerulein on the behavioral effects of ketamine in mice. According to data obtained by Contreras et al. [3], the ataxia observed after injection of ketamine and phencyclidine is realized by different mechanisms from the stereotyped behavior. This state of affairs is evidently responsible for the weak effect of cerulein on ketamine-induced ataxia. In the amnesic action of ketamine, interaction of the drug with opioid receptors plays a leading role, just as in the case of phencyclidine [11]. The opioid antagonist naloxone is an effective antagonist of the amnesic action of ketamine. It has been shown that cerulein, in a dose of 10 μg/kg and less blocks morphine-induced analgesia in rats [8]. It can be tentatively suggested that functional antagonism with opioid receptors lies at the base of the antagonism of cerulein with the amnesic action of ketamine.

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