LOWERING THE SENSITIVITY OF CHOLECYSTOKININ RECEPTORS IN THE BRAIN BY CHRONIC HALOPERIDOL ADMINISTRATION

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According to existing data, long-term administration of neuroleptics has a significant effect on cholecystokinin-8-ergic (CCK-8-ergic) brain processes. Administration of haloperidol or reserpine for 2 weeks has been shown to increase the density of CCK-8-receptors in the mouse forebrain [2]. Administration of various neuroleptics (clozapine, chlorpromazine, and haloperidol) increases the CCK-8 concentration in subcortical limbic structures and the caudate nucleus [5]. The writers' previous investigations showed that chronic administration of haloperidol reverses the inhibitory effect of coerulein, a CCK-8 agonist, on ^{3}H-spiroperidol binding in experiments *in vivo* [7]. Coerulein stimulated binding of ^{3}H-spiroperidol with dopamine$_2$- and serotonin$_2$-receptors after administration of haloperidol for 15 days [7]. The opinion is held that the lowering of the CCK-8 concentration in some forebrain structures may lie at the basis of resistance of mental patients to neuroleptics [3]. In the present investigation the effect of chronic administration of the typical neuroleptic, haloperidol, on binding of ^{3}H-cholecystokinin in the mouse forebrain and on the behavioral effects of coerulein, an agonist of CCK-8-receptors, was studied.

EXPERIMENTAL METHOD

Experiments were carried out on male albino mice weighing 25-30 g. Physiological saline or haloperidol (0.25 mg/kg, from Gedeon Richter, Hungary was injected twice a day for 15 days. The behavioral experiments and radioligand binding experiments were carried out 48-72 h after the last injection of haloperidol. In the experiments to study binding of ^{3}H-CCK-8 (86 Ci/mmole, Amersham International, England) in the mouse forebrain, a modified method in [4] was used. Brain tissue was homogenized in 10 volumes of cold Tris-HCl-buffer solution (pH 7.4) at 20°C in a Potter C homogenizer. The homogenized tissue was centrifuged at 48,000g for 15 min, after which the pellet was rehomogenized in 10 volumes of Tris-HCl buffer solution and the homogenate was centrifuged at 48,000g for 15 min. The final residue was homogenized in 100 volumes of incubation buffer consisting of 10 mM HEPES, 130 mm NaCl, 5 mM KCl, 5 mM $MgCl_2$, and 1 mM EDTA (the pH was adjusted to 7.4 with potassium hydroxide). ^{3}H-CCK-8 was added to the incubation mixture in various concentrations from 50 pM to 3 nM. Nonspecific

TABLE 1. Effect of Chronic Haloperidol Administration on ^{3}H-Cholecystokinin Binding in Mouse Forebrain

Substance injected	High-affinity binding sites		Low-affinity binding sites	
	K_d	Bs_{max}	K_d	Bs_{max}
Physiological saline	0,35±0,05	7,3±0,8	2,66±0,25	31,5±2,5
Haloperidol	0,45±0,05	11,7±1,0*	1,14±0,12**	21,4±2,0*

Note. K_d) Dissociation constant (in nM); Bs_{max}) maximal density of binding sites (in fmoles/mg protein). Here and in Tables 2 and 3: *P < 0.05, **P < 0.01.

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TABLE 2. Effect of Chronic Haloperidol Administration on Sedative and Antiaggressive Action of Coerulein

Substance injected	Chronic administration of physiological saline		Chronic administration of haloperidol	
	Orienting-investigative activity of mice: number of impulses during			
	15 min	30 min	15 min	30 min
Physiological saline	187±17	325±39	160±12	276±17
Coerulein (10 μg/kg)	115±19	198±23	142±23	252±27
	Aggressiveness of mice in response to painful electrical stimulation: number of aggressive contacts in 2 min			
Physiological saline	13,5±0,95		8,6±0,93	
Coerulein (40 μg/kg)	5,8±0,82		21,8±2,99	

TABLE 3. Changes in Anticonvulsant Action of Coerulein after Chronic Haloperidol Administration

Parameters of picrotoxin convulsions	Picrotoxin (10 mg/kg)+ physiological saline	Picrotoxin (10 mg/kg)+ coerulein (125 μg/kg)
Chronic administration of physiological saline		
Latent period of clonic convulsions, sec	441±25	785±72**
Latent period of tonic convulsions, min	16±1,5	24,9±2,0**
Length of survival, min	17,4±1,7	26,1±1,6**
Chronic administration of haloperidol		
Latent period of clonic convulsions, sec	427±32	776±65**
Latent period of tonic convulsions, min	15,8±1,4	20,5±1,5*
Length of survival, min	18,1±1,5	21,5±1,5

binding was determined by addition of 1 μM coerulein (from Farmitalia, Italy). The samples were incubated at 24°C for 90 min. After incubation the samples were centrifuged at 1200 g for 2.5 min. The supernatant was decanted and the residue carefully washed several times with cold incubation buffer. The radioactivity of the samples (four parallel determinations) was measured by Bray's scintillator on an LS-6800 β-particle counter (Beckman, USA). The experiments were repeated three or four times. The results were subjected to analysis by the Scatchard plot method.

Parallel with the radioligand binding experiments, changes in the behavioral effects of the CCK-8-receptor agonist coerulein were studied. The effect of coerulein (10 μg/kg, subcutaneously) on the orienting-investigative activity of the mice was recorded with a photoelectric actometer. Immediately after injection of coerulein or physiological saline the animals were placed in the individual cages of the actometer and their motor activity determined for 30 min. The method of painful electrical stimulation was used to study the action of coerulein (40 μg/kg) on the aggressive behavior of mice after 2 weeks of haloperidol injections. Two animals were placed in the chamber for painful electrical stimulation 20 min after injection of coerulein, and in the course of the next 2 min they received 48 electric shocks with a strength of 40 V. The parameter of intensity of aggressive behavior was the number of aggressive contacts between the animals. The anticonvulsant action of coerulein (125 μg/kg, subcutaneously) was investigated on a model of picrotoxin convulsions. CCK-8 and its analogs, in small doses, were found to antagonize picrotoxin convulsions, whether injected by the intraventricular or systemic routes [6]. Coerulein was given 10 min before injection of 10 mg/kg of picrotoxin. The three principal parameters of picrotoxin convulsions were determined: latent periods of clonic convulsions, latent periods of tonic convulsions, and length of survival of the mice. All the data obtained in the behavioral experiments were subjected to statistical analysis by Student's t test.

EXPERIMENTAL RESULTS

Administration of haloperidol for 15 days (0.25 mg/kg twice a day) in these experimental increased the density of high-affinity binding sites of CCK-8 (Table 1). The number of low-affinity binding sites was reduced, but their affinity for CCK-8 was appreciably increased. Parallel with the changes at the CCK-8 binding sites, weakening or reversal of the behavioral effects of the CCK-8 agonist, coerulein, was observed. Coerulein (10 μg/kg), in mice receiving haloperidol beforehand for 15 days, largely lost its ability to inhibit the orienting-investigative reaction, whereas the antiaggressive action of coerulein was reversed (Table 2). Coerulein in a dose of 40 μg/kg considerably increased the number of aggressive contacts between mice (Table 2). Prolonged preliminary administration of haloperidol significantly weakened the antagonistic effect of coerulein (125 μg/kg) on picrotoxin convulsions (Table 3).

The results of this investigation thus demonstrate that, despite an increase in the number of high-affinity and a growth in the affinity of the low-affinity CCK-8 binding sites,

prolonged administration of the typical neuroleptic haloperidol caused weakening or reversal of the behavioral effects of coerulein. The results of the writers' previous investigations [1, 7], which showed that long-term administration of haloperidol restores the inhibitory effect of coerulein on ^{3}H-spiroperidol binding in experiments *in vivo*, are also evidence of reduced sensitivity of the CCK-8 receptors. Meanwhile prolonged administration of haloperidol significantly reduces the number of high-affinity dopamine$_2$- and serotonin$_2$-receptors [1, 7]. Reduction of the density of these monoaminergic receptors evidently also determines the hyposensitivity of the CCK-8 receptors. Considering the close morphological and functional connection between CCK-8, dopamine, and serotonin, and also the considerable adaptive changes of CCK-8 receptors during long-term administration of the typical neuroleptic, haloperidol, it can be tentatively suggested that CCK-8 plays an important role in the realization of both antipsychotic [3] and side effects of neuropeptics.

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ACTION OF ANTIHYPOXIC AGENTS ON ISCHEMIC MYOCARDIAL DAMAGE

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KEY WORDS: sodium hydroxybutyrate; pyracetam; pyridoxinyl-glyoxylate; creatine phosphokinase; myocardial infarction.

The possibility of limiting the size of a myocardial infarct (MI) by the use of drugs has been discussed widely in recent years. Opposite points of view have been expressed even as regards such traditional "anti-infarct" drugs as the β-adrenoblockers [8, 9, 13].

The aim of this investigation was to study the effect of antihypoxic agents — pyridoxinyl-glyoxylate (glyo-6), sodium hydroxybutyrate, and the α-pyrrolidone derivative pyracetam — on the development of ischemic damage and on the final size of MI resulting from occlusion of the coronary artery.

EXPERIMENTAL METHOD

To assess the effect of the drugs on the development of ischemic damage in the heart following coronary arterial occlusion experiments were carried out on cats weighing 3-4 kg, anesthetized with pentobarbital sodium (40 mg/kg, intravenously), and artificially ventilated. The anterior descending branch of the left coronary artery of the animals was ligated in its middle third. Heparin was injected intravenously in a dose of 1000 U/kg. Blood samples were taken from the coronary sinus before coronary occlusion and 20 and 60 min thereafter. There were four series of experiments. The drugs were injected immediately after coronary arterial occlusion, intravenously: sodium hydroxybutyrate 200 mg/kg, pyracetam 400 mg/kg, and glyo-6 100 mg/kg body weight. In the control series the equivalent volume of physiological saline was injected into the animals. Plasma creatine phosphokinase (CPK) activity was determined by the method in [10]. Linear regression coefficients were determined by Theil's nonparametric test. Significance of differences between angles of slope of the regression lines was determined by Hollander's one-way nonparametric test.

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