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# DIFFERENT EFFECTS OF LONG-TERM HALOPERIDOL ADMINISTRATION ON $GABA_A$ AND BENZODIAZEPINE RECEPTORS IN VARIOUS PARTS OF THE BRAIN

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Long-term administration of neuroleptics has been shown to cause substantial structural changes in many neurotransmitter systems of the brain, including the GABA-ergic system and benzodiazepine receptors [1, 4, 6]. On long-term administration of the typical neuroleptic haloperidol, a marked decrease in the density of $GABA_A$- and benzodiazepine receptors has been observed in various structures of the forebrain and cerebellum [1, 2]. Besides these changes, if muscimol, an agonist of $GABA_A$ receptors is used, its sedative action is reversed [2]. Instead of inhibiting orienting-motor activity in mice muscimol had the opposite effect — it stimulated motor activity in animals treated beforehand with haloperidol. However, the paradoxical change in the action of muscimol cannot be attributed entirely to a decrease in the density of $GABA_A$ and benzodiazepine receptors in the forebrain. Direct injection of muscimol into the substantia nigra is known to induce stereotyped behavior analogous to that found after systemic administration of dopaminomimetics [3]. Microinjection of muscimol into the medial nucleus raphe leads to the development of hyperactivity in rats; simultaneous administration of chlordiazepoxide, a benzodiazepine agonist, moreover, potentiates the stimulating effect of muscimol considerably [8]. After long-term administration of neuroleptics neurons of the substantia nigra develop hypersensitivity to GABA agonists [4] and the density of GABA receptors in the substantia nigra is increased [6].

The data described in this paper are evidence that long-term administrationof haloperidol has an opposite effect on the density of $GABA_A$ and benzodiazepine receptors in the fore- and hindbrain. These changes are reflected at the molecular level as reversal of the behavioral effect of the $GABA_A$ agonist muscimol and the benzodiazepine agonist Ro15-1788.

## EXPERIMENTAL METHOD

Experiments were carried out on 160 male mice weighing 25-30 g and on 100 male Wistar rats weighing 250-270 g. Haloperidol in a dose of 0.25 mg/kg (from Gedeon Richter, Hungary) or physiological saline was injected intraperitoneally twice a day for 15 days. Behavioral tests and radioligand binding experiments were carried out 12 h after the last injection of the neuroleptic. The sedative action of muscimol (from Serva, West Germany), a $GABA_A$ receptor agonist, was determined in mice by means of a photoelectric actometer. The animals were placed in the actometer 15 min after intraperitoneal injection of muscimol in doses of 0.75 and 1.5 mg/kg, and their orienting-investigative activity was determined for 30 min. The effect of the benzodiazepine antagonist Ro15-1788 (from Hoffmann-La Roche, Switzerland) on the rats' behavior was assessed by the open field method. The animals were placed in the center of an open field (measuring 100 × 100 × 40 cm) 30 min after intraperitoneal injection of 5 mg/kg of Ro15-1788. The animals' behavior was recorded for 5 min. The following parameters were de-

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termined: motor activity, by a photoelectric method (using five independent channels), number of times of standing up on the hind limbs, and the number of head dippings. According to data in the literature [5], an increase in the number of head dippings is highly specific for Ro15-1788 in the open field test. By means of parallel behavioral tests binding of $^3$H-muscimol in the fore- and hindbrain of the rats was investigated in experiments *in vitro*. $^3$H-flunitrazepam binding experiments were carried out *in vivo* on mice. To obtain the forebrain structures, a frontal section was made on the line of the optic chiasma, and the structures remaining in front of the line of section were used in experiments *in vitro* and *in vivo*. To separate the hindbrain the frontal section was made on the posterior line of the diencephalon, after which the structures of the brain stem were separated from the cerebellum and cortical formations. Interaction between muscimol and $GABA_A$ receptors were studied by the method described by the writers previously [2]. The binding experiments were carried out after the tissue had been rinsed 8 times (30,000g, 25 min). The concentration of $^3$H-muscimol (specific activity 10.9 Ci/mmole, from Amersham Corporation, England) in the incubation medium was constant (10 nM), whereas that of the unlabeled ligand varied from 10 to 1000 nM. Incubation was carried out at 0°C for 15 min. In the experiments to study binding of $^3$H-flunitrazepam (specific activity 84 Ci/mmole, from Amersham Corporation) *in vivo* the isotope was injected subcutaneously in a dose of 0.3 μg/kg. The mice were decapitated 30 min after receiving the injection of $^3$H-flunitrazepam. The fore- and hindbrain material from the corresponding groups was pooled and homogenized in a Potter's homogenizer. To determine nonspecific binding, 10 μM of unlabeled flunitrazepam was added to some of the samples. The samples were incubated for 60 min at 0°C. The difference between the radioactivity levels of samples without ligand and with unlabeled ligands gave the specific binding of flunitrazepam in the experiments *in vivo*.

## EXPERIMENTAL RESULTS

After long-term administration of haloperidol a change was observed in the behavior effect both of muscimol (Tables 1 and 2) and of Ro15-1788 (Tables 3 and 4). The sedative action of 0.75 mg/kg of muscimol on behavior was replaced by a stimulating action, and tolerance developed to the strong inhibitory action of a larger dose (1.5 mg/kg) of muscimol. Ro15-1788 (5 mg/kg increased the motor activity of the control rats and the number of times they stood on their hind limbs, and there was a significant increase in the number of head dippings (Table 2). After treatment with haloperidol for 15 days the behavioral activity of the rats in the open field test was increased. Ro15-1788 (5 mg/kg had a sedative action on the orienting-investigative activity of these rats, i.e., reversal of the effect was observed. Parallel with reversal of the behavioral effects of muscimol and Ro15-1788 the number of binding sites both for $^3$H-muscimol (Table 1) and for $^3$H-flunitrazepam (Table 2) in the forebrain was reduced. In the hindbrain the opposite process took place: the number of binding sites for muscimol and flunitrazepam was greater than in the control.

The results are evidence that long-term administration of the typical neuroleptic haloperidol causes opposite changes in the density of $GABA_A$ and benzodiazepine receptors in the fore- and hindbrain. In the hindbrain, where bodies of monoaminergic neurons innervating various structures of the diencephalon and forebrain are located, haloperidol led to an increase in the number of GABA- and benzodiazepine receptors, whereas in the forebrain their density diminished. The reflection of these changes at the level of GABA- and benzodiazepine receptors is a change in the action of muscimol and of the benzodiazepine antagonist Ro15-1788;

TABLE 1. Effect of Muscimol on Orienting-Investigative Activity of Mice during 15 days (number of impulses in 30 min)

| Substance | Control | Experiment |
|---|---|---|
| Physiological saline | 415±28 | 352±24 |
| Muscimol mg/kg: | | |
| 0,75 | 284±26 | 514±42* |
| 1,5 | 120±14 | 284±29* |

Legend. Here and in Tables 2-4: control — administration of physiological saline for 15 days, experiment — of haloperidol for 15 days. *P < 0.05 compared with control; n = 3.

TABLE 2. Binding of $^3$H-Muscimol in Experiments

| Part of brain | Parameter | Control | Experiment |
|---|---|---|---|
| Forebrain | $K_d$, nM | 9,6±1,6 | 10,2±1,8 |
| | $B_{max}$, pmoles/mg protein | 0,90±0,08 | 0,54±0,05+ |
| Hindbrain | $K_d$, nM | 12,6±1,3 | 13,2±1,3 |
| | $B_{max}$, pmoles/mg protein | 0,38±0,03 | 0,50±0,04+ |

Legend. $K_d$) Dissociation constant, $B_{max}$) maximal number of binding sites according to Scatchard plot.

TABLE 3. Effect of Ro15-1788 on Behavior of Rats in Open Field Test

| Parameter | Substance | Control | Experiment |
|---|---|---|---|
| Motor activity, number of impulses during 5 min | Physiological saline | 29,5±5,2 | 42,0±5,1 |
| | Ro15-1788, 5 mg/kg | 38,5±4,7 | 26,0±3,8 |
| Number of times rats stood up on hind limbs | Physiological saline | 5,2±1,6 | 5,1±1,3 |
| | Ro15-1788 5 mg/kg | 8,5±1,7 | 4,8±1,4+ |
| Number of head dippings | Physiological saline | 6,7±0,7 | 11,8±1,5+ |
| | Ro15-1788 5 mg/kg | 11,5±1,4 | 6,0±0,7+ |

TABLE 4. Binding of $^3$H-Flunitrazepam in Experiment (in impulses/g brain tissue)

| Part of brain | Control | Experiment |
|---|---|---|
| Forebrain | 14 840±620 | 11 760±660+ |
| Hindbrain | 9 180±720 | 11 260±680+ |

both substances, moreover, had effects opposite to those observed in the control animals. It can accordingly be postulated that the $GABA_A$ and benzodiazepine receptors of the fore- and hindbrain have opposite functional roles. This hypothesis is supported by data obtained by other workers, who found that direct injection of muscimol into dopaminergic and serotoninergic structures in the hindbrain has a stimulating effect on the behavior of experimental animals [3, 8]; stimulation of benzodiazepine receptors by chlordiazepoxide, moreover, potentiates this action of muscimol. Long-term administration of haloperidol leads to a shift toward predominance of benzodiazepine and $GABA_A$ receptors of the "stimulating"type, which are mainly located in the hindbrain. This phenomenon also lies at the basis of reversal of the behavioral effects of muscimol and Ro15-1788.

Consequently, besides the existence of subtypes of $GABA_A$ and benzodiazepine receptors ($GABA_A$, $GABA_B$, benzodiazepine$_1$ and benzodiazepine$_2$), demonstrated in binding experiments [7, 9], $GABA_A$ and benzodiazepine receptors are also subdivided from the functional point of view. "Stimulating" receptors predominate in the hindbrain, whereas $GABA_A$ and benzodiazepine receptors with an inhibitory influence on behavior predominate in the forebrain. An unequal balance between these functional subtypes of $GABA_A$ and benzodiazepine receptors may give rise to individual differences in the response of man or animals to administration of tranquilizers of the benzodiazepine series.

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# EFFECT OF NICOTINIC AND MUSCARINIC CHOLINOMIMETICS AND CHOLINOLYTICS ON EPILEPTOGENESIS IN A PENICILLIN FOCUS IN THE DORSAL HIPPOCAMPUS

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KEY WORDS: epilepsy; penicillin focus; galanthamine; muscarinic and nicotinic cholinolytics.

Most investigators prefer the penicillin model of epilepsy, for epileptiform changes produced under these conditions correspond most closely to the clinical forms of epilepsy in man [12, 15]. This process is evidently based on inhibition of GABA release from nerve endings [10, 11] and a decrease in the number of GABA receptors [9].

Cholinergic mechanisms are known to play an important role in the formation of epileptiform activity. Mainly muscarinic cholinomimetics and muscarinic cholinolytics are used to study this problem [13, 14]. However, analysis of the experimental data obtained by Gerasimyan [4], who studied many clinically effective anticonvulsants (derivatives of succinimides), reveals that all these preparations have a marked nicotinic cholinolytic action.

In this investigation the role of muscarinic (M) and nicotinic (N) cholinergic mechanisms in the formation of epileptiform activity induced by application of penicillin was studied.

## EXPERIMENTAL METHOD

Chronic experiments were conducted on six rabbits weighing 3-3.5 kg, with electrodes implanted into the dorsal part of the hippocampus, the mesencephalic reticular formation, and the sensomotor cortex. An epileptogenic focus was created by introduction of 250 U of a solution of the sodium salt of benzylpenicillin in a volume of 1 µl through a chemical electrode [4] implanted into area CA1 and CA2 of the dorsal hippocampus. Eterofen (a Soviet anticholinergic drug) was used in a dose of 8 mg/kg, gangleron (1,2-dimethyl-3-diethylaminopropyl p-isobutoxybenzoate hydrochloride), in a dose of 3 mg/kg, metamizil (2-diethylamino-1-methylethyl ester of benzylic acid hydrochloride) in a dose of 0.5 mg/kg, and galanthamine in a dose of 1 mg/kg. The drugs were injected intravenously. In each experiment the animals remained under observation for 2.5 h. Experiments in which penicillin alone was injected into the hippocampus served as the control. The number of interictal discharges in the focus and the number of seizures were analyzed. These parameters were counted for 15 consecutive 10-minute periods. The number of spikes was counted during 1 min of recording and the number of seizures during 10 min of observation. Values obtained in the control experiments were taken as 100.

The drugs were injected 20 min before formation of the epileptogenic focus or 30 min after the appearance of epileptiform activity. In the second case mean values of the number of interictal epileptiform discharges and of seizures, counted during 30 min of recording, were

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