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Functional Supersensitivity of the Hippocampal Dopaminergic System After Prolonged Treatment With Haloperidol

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BIJAK, M. AND A. ŚMIAŁOWSKI. *Functional supersensitivity of the hippocampal dopaminergic system after prolonged treatment with haloperidol.* PHARMACOL BIOCHEM BEHAV 32(1) 95–99, 1989.—The effect of acute and prolonged (21 days) treatment with haloperidol (1 or 5 mg/kg SC) on the dopamine-, apomorphine- and LY 171555-induced changes in the firing rate of CA1 layer neurons was studied in a hippocampal slice preparation. Dopamine and apomorphine administration evoked either an excitatory or inhibitory reaction, while the selective D2 receptor agonist LY 171555 increased the firing rate of hippocampal neurons. The excitatory effects of dopamine and LY 171555 were blocked by sulpiride and haloperidol. Prolonged administration of haloperidol potentiated the excitatory reaction of dopamine and apomorphine; however, even a single dose of the neuroleptic enhanced the dopamine-induced effect. The reaction evoked by LY 171555 was not significantly affected by either acute or chronic treatment with haloperidol. The present findings indicate that long-term administration of haloperidol results in an increased sensitivity of hippocampal neurons to the mixed dopamine agonists, dopamine and apomorphine, but not to selective stimulation of the D2 receptor.

Haloperidol Repeated treatment Supersensitivity Hippocampus LY 171555 Dopamine Spontaneous firing rate

ALTHOUGH the role of dopamine as a transmitter substance in the hippocampal formation has been under discussion for a long time, the latest experimental evidence not only confirmed the specific action of this amine on hippocampal neurons, but also suggested that the hippocampal dopaminergic system may be a target of an antipsychotic action of neuroleptics (2,10).

Since the first studies which showed that intrahippocampally administered apomorphine evokes a behavioral arousal and EEG changes in the rabbit (25), our knowledge of the dopaminergic system in the hippocampus has greatly proliferated, notwithstanding the fact that a low level of dopamine and its receptors in this structure hindered the investigation. It has been shown that a dopaminergic innervation of the hippocampus originates in the ventral tegmental area and partly in the substantia nigra (23). More detailed data were obtained in biochemical studies which demonstrated the presence of D1 and D2 dopamine receptors in the hippocampus (5, 9, 12) and the influence of neuroleptics on the dopamine turnover in this brain area (3,22). As in the dopamine-rich brain structures, chronic administration of haloperidol in the hippocampus evokes an increase in spiperone binding (1). However, there are no data concerning the functional significance of this rise in the number of D2 dopamine receptors.

Our earlier studies demonstrated that dopamine strongly affects the firing rate of hippocampal neurons in vitro. Having used relatively specific dopaminergic agonists and antagonists, we demonstrated that in all likelihood the activation of D1 dopamine receptors leads to inhibition of spontaneous discharges of CA1 layer cells, while activation of D2 dopamine receptors evokes an excitatory reaction (26). This finding permits a functional differentiation of the effects related with D1 and D2 receptors in the hippocampus.

We attempted to study the influence of acute and prolonged treatment with haloperidol on the sensitivity of hippocampal neurons to exogenously applied agonists of the dopamine receptor.

METHOD

Male Wistar rats, weighing approximately 250 g at the time of decapitation, were used. The rats were kept under natural light conditions, with free access to food and water. The animals were divided into three groups: 1) nontreated controls; 2) animals receiving 2 ml/kg SC of saline for 20 days and a single dose of haloperidol at 21st day (1 or 5 mg/kg SC); 3) the chronic haloperidol group receiving haloperidol (1 or 5 mg/kg SC) once daily for 21 days. The rats were examined in the morning, 2, 3 or 7 days after the final dose of haloperidol.

All the rats were decapitated under chlorophorm narcose and the hippocampal formation was rapidly dissected. Transverse hippocampal slices (400 μm) were cut by hand using a glass guide and gently transferred, for more than one hr, into a Petri dish containing an oxygenated medium consisting of (mM): NaCl 124, KCl 5, KH_2PO_4 1.25, $MgSO_4$ 1.3, $CaCl_2$ 2.4, $NaHCO_3$ 26, and glucose 10, at 32°C, pH 7.4. In the recording chamber a single slice was placed on a fluid-gas

TABLE 1

THE EFFECT OF HALOPERIDOL ADMINISTRATION ON THE RESPONSIVENESS OF HIPPOCAMPAL NEURONS TO DOPAMINE AND LY 171555

Pretreatment†	N	(n)	Type of Response (%N) Excitation	Inhibition	No Effect
			Dopamine (2 μM)		
Control	13	(7)	77	—	23
Haloperidol 1 mg/kg 1×	10	(5)	70	30	—
Haloperidol 1 mg/kg 21×	10	(5)	80	20	—
Haloperidol 1 mg/kg 21× + sulpiride (10 μM)*	6	(4)	17	66	17
Haloperidol 5 mg/kg 1×	12	(4)	42	25	33
Haloperidol 5 mg/kg 21×	13	(4)	62	31	7
			Dopamine (2 mM)		
Control	22	(12)	64	32	4
Control + haloperidol (0.1 μM)*	13	(6)	39	46	15
Haloperidol 1 mg/kg 1×	12	(5)	42	58	—
Haloperidol 1 mg/kg 21×	12	(6)	75	25	—
Haloperidol 1 mg/kg 21× + haloperidol (1 μM)*	12	(6)	33	67	—
			LY 171555 (10 μM)		
Control	16	(6)	69	—	31
Haloperidol 1 mg/kg 1×	10	(5)	70	10	20
Haloperidol 1 mg/kg 21×	16	(6)	50	6	44
Haloperidol 1 mg/kg 21× + sulpiride (10 μM)*	6	(4)	67	—	33
Haloperidol 5 mg/kg 1×	12	(4)	75	8	17
Haloperidol 5 mg/kg 21×	12	(4)	58	—	42

*Superfused through the slice.
†Testing was carried out 3 days after the last dose of haloperidol.
N—number of slices; n—number of animals.

interface and was perfused at a rate of 1 ml/min; the medium was maintained at 34°C.

The spontaneous activity of unidentified cells in the CA1 layer of the hippocampus was recorded extracellularly using a tungsten microelectrode (Clark Electromedical Instr., 12 MΩ). The recorded signals were amplified, filtered (200 Hz to 2 kHz), displayed on an oscilloscope and photographed. The band-pass-filtered signal was fed to an amplitude discriminator, a signal integrator and a frequency meter. Pulses were integrated over 10-second intervals and displayed on a chart recorder. Additionally, the frequency of spontaneous activity was measured at 1-min intervals.

The tested substances were dissolved in the medium and added to the perfusion line in an amount of 0.1 ml in case of dopamine (2 μM) and LY 171555 (10 μM), or 0.3 ml in the case of dopamine (2 mM) and apomorphine (1 mM). When used, haloperidol and sulpiride were continuously superfused through the experimental chamber.

The tested substances were added to the perfusion line after ca. 10 min of a stable level of basal spontaneous activity. The effect was measured for 15–20 min after administration of the drug, and expressed as a percent change of the mean control spontaneous discharge rate. The experiment was classified as excitation when the frequency of firing exceeded 120% of the control mean, or an inhibition when the firing rate decreased below 80% of the control mean. Mean values of the reaction in experimental groups were compared statistically using the ANOVA and the two-tailed Student's *t*-tests.

Drugs

Dopamine HCl (Intern. Enz. Ltd. Windsor), apomorphine HCl (Sigma), LY 171555 (quinpirole HCl, Lilly Labs.), sulpiride (Serva) and haloperidol (Polfa) were used.

RESULTS

Dopamine administration to the rat hippocampal slice preparation in a dose of 2 μM resulted mainly in an increase in the spontaneous firing rate of CA1 layer neurons. However, a higher dose of the substance (2 mM) also evoked a decrease in the frequency of neuronal discharges in part of the slices (Fig. 1A, Table 1). The excitatory reaction to a low dose of dopamine was significantly potentiated by both acute and prolonged treatment with haloperidol, but the potency of the enhancing effect did not depend on the applied

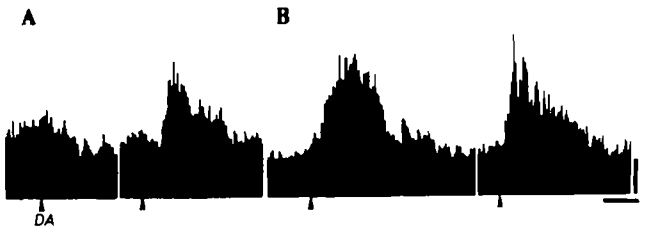

FIG. 1. A typical example of the dopamine effect (2 mM) on the spontaneous firing rate of hippocampal CA1 layer neurons in slices from the control (A) and haloperidol-treated animals (B; 1 mg/kg SC); vertical bar: 20 impulses/10 sec; horizontal bar: 5 min.

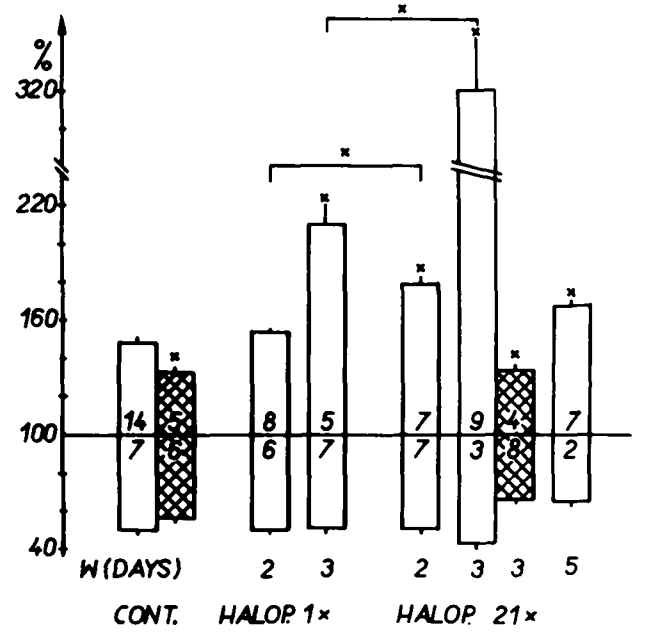

FIG. 3. The time-course of changes in the responsiveness of hippocampal neurons to dopamine (2 mM) after acute (1×) and long-term (21×) haloperidol administration (1 mg/kg SC). The results are expressed as a percentage change (mean±S.E.M.) in the baseline firing rate (the number of experiments for each group is given inside the bars). Cross-hatched bars: effect of bath applied haloperidol (1 μM) on the reaction of hippocampal neurons to dopamine. W—withdrawal. *$p<0.001$.

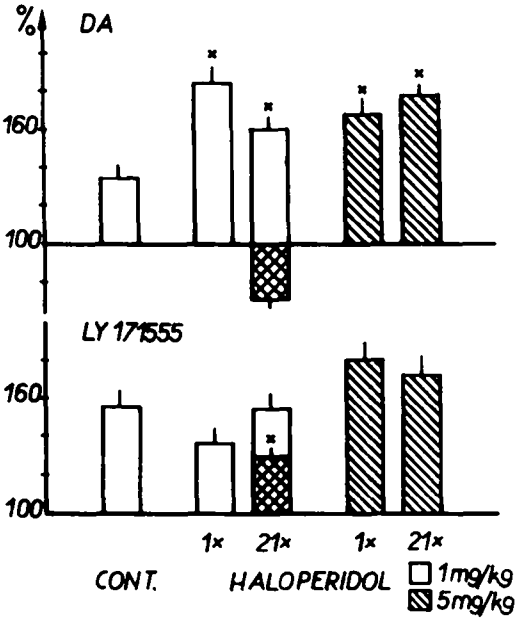

FIG. 2. The effect of acute (1×) and prolonged (21×) application of haloperidol on the excitatory reaction to dopamine (DA, 2 μM) and LY 171555 (10 μM), tested 3 days after the last dose of haloperidol. The results are expressed as a percentage change (mean±S.E.M.) in the baseline firing rate. Bar below the baseline firing rate shows inhibitory reaction. *$p<0.05$, cross-hatched bars: effects of bath applied sulpiride (10 μM).

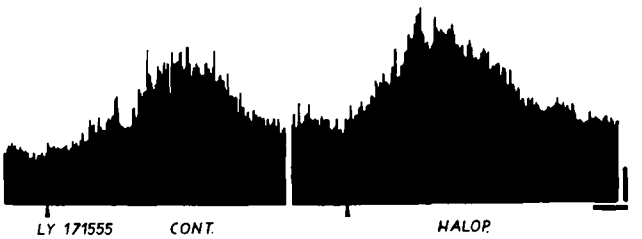

FIG. 4. A ratemeter record of the effect of LY 171555 on the spontaneous firing rate of hippocampal CA1 layer neurons in slices from control and haloperidol- (5 mg/kg SC) treated animals; vertical bar: 10 impulses/10 sec; horizontal bar: 5 min.

dose of the neuroleptic (1 or 5 mg/kg; Fig. 2). This reaction to dopamine was blocked by sulpiride (10 μM) (Fig. 2).

Administration of a higher dose of dopamine (2 mM) evoked both an excitatory and inhibitory reaction, but chronic administration of the neuroleptic augmented the dopamine-evoked excitation only (Fig. 3). The potentiating effect of haloperidol was at a maximum 3 days after the last dose, and it was readily diminished when slices were incubated with haloperidol (1 μM) (Fig. 3). That reaction was still efficient on the 7th day of the drug-free period. Interestingly, even a single injection of the neuroleptic potently enhanced the excitatory reaction to dopamine, that effect being observed at three but not two days after the last haloperidol treatment (Fig. 3).

The application of apomorphine (1 mM), another mixed D1/D2 dopamine receptor agonist, evoked an excitatory (an approximately 50% increase in a basal firing rate; N=7) or inhibitory (a mean 50% decrease in the basal firing; N=6) reaction in an equal number of hippocampal slices. Two days after chronic haloperidol administration (1 mg/kg) most of the slices reacted to apomorphine with enhancement in the frequency of spontaneous discharges (70%, N=10) the potency of that effect was significantly higher than in the control (on an average of 210% of basal firing).

The selective D2 receptor agonist LY171555 evoked an excitatory reaction in CA1 layer neurons (Table 1, Fig. 4). Neither prolonged nor acute administration of haloperidol in doses of 1 or 5 mg/kg changed the number of neurons excited by LY 171555 (Table 1), but the potency of excitation was affected in some neurons (Fig. 4). The elevation in the firing rate evoked by LY 171555 was attenuated by sulpiride (10 μM, Fig. 2).

DISCUSSION

A vast body of experimental evidence revealed that a long-term treatment with neuroleptics leads to supersensitivity of the dopaminergic system in the brain [for review see (20)]. Although it has been suggested that the mesolimbic system is a target for an antipsychotic action of neuroleptics while the nigro-striatal one is an anatomical substratum of extrapyramidal side effects, most of the studies on the consequences of prolonged blockade of dopaminergic receptors concentrated on dopamine-rich nigro-striatal brain areas. Several data support the hypothesis that the hippocampal dopaminergic system may be an important site of the neuroleptic action (2,10). Binding studies with neuroleptics which show a low potency in inducing extrapyramidal disturbances demonstrated their selectivity for hippocampal dopamine receptors (4). Moreover, it has been shown that haloperidol administration more readily evokes an increase in the dopamine D2 receptor density in the hippocampus than in the striatum (1).

Our study demonstrates that the increase in the number of hippocampal D2 receptors, observed by Bischoff (1) after a prolonged treatment with the neuroleptic, results in a functional supersensitivity of hippocampal neurons to dopamine and apomorphine. Although both dopamine and apomorphine are mixed dopamine D1/D2 receptor agonists and can evoke either an inhibitory or an excitatory effect on the spontaneous firing of CA1 layer cells, only an excitatory reaction to these substances was augmented by haloperidol administration. Our earlier data (26), as well as the present results obtained with LY 171555, sulpiride and haloperidol, strongly suggest that the excitatory reaction to dopamine and apomorphine results from stimulation of the dopamine D2 receptor. The reported enhancing action of prolonged haloperidol administration on the excitatory effect of dopamine and apomorphine further confirms the latter assumption, as the increase in the number and function of D2 but not D1 receptor has been the most consistent alteration found after prolonged treatment with haloperidol (1, 15, 20, 21).

Surprisingly, we did not observe a similar potentiating effect of haloperidol administration on the excitatory reaction evoked by the selective D2 receptor agonist LY 171555 (the applied dose of LY 171555 was not the most effective one; data not shown). The requirement of stimulation of both subtypes of dopamine receptors for a full expression of the effect of a selective activation of one of them, observed in many experimental models, may account for our negative results obtained with LY 171555 (6,29).

In our study both acute and prolonged application of haloperidol evoked supersensitivity of hippocampal neurons to dopamine. However, the potency of that sensitization was higher after long-term treatment with haloperidol. Differentiation between the effects of single and chronic treatment with the neuroleptic was possible only when a higher dose of dopamine was applied, a low concentration being probably not adequate to stimulate all the dopamine receptors available.

A single dose of haloperidol was shown to effectively block dopamine receptors for 2–4 hours after its application (7), and the level of the neuroleptic in the brain declined with a half time of ca. 4 hr (19). Interestingly, even a single dose of different neuroleptics evoked behavioral signs of supersensitivity to dopaminergic agonists 2 and 3 days after the application (8). Our results, which show the sensitizing effect of an acute dose of haloperidol, are in line with the data that demonstrate that dopamine receptors in the brain can undergo rapid adaptational changes (13, 18, 27).

Several authors demonstrated that there is little dependence of the dosage upon the effects evoked by the chronic neuroleptics (23). Similarly our experiments show no dependence of the dosage upon the haloperidol action on the responsiveness of hippocampal neurons to dopamine and LY 171555. Nevertheless, the concentrations of haloperidol used in this present study were rather high, therefore it is likely that, like in other experiments (17,24), the effects might be dose-related within a lower dose range of neuroleptic.

The observed sensitizing effect of haloperidol may in part be not specific to the dopaminergic system, but rather lie in an increased excitability of neurones, evoked by indirect effects of haloperidol. While acting on other than dopamine receptors (7) or presynaptic D2 receptors in the hippocampus (16,28) and affecting the noradrenergic activity of the locus coeruleus and the cholinergic activity in the septum (11,14), haloperidol may change the sensitivity of hippocampal neurons to neurotransmitters. However, the excitatory effect of noradrenaline application to hippocampal slices was not potentiated by haloperidol treatment ($N=7$; data not shown).

The results of the present study give further support to the existence and functional significance of the dopaminergic system in the hippocampus. They demonstrate that, like other typical dopaminergic areas the hippocampal dopaminergic system undergoes adaptive changes in the sensitivity after blockade of dopamine receptors. Moreover, the obtained data confirm our earlier assumption that the D2 receptor activation has a stimulatory effect on the spontaneous activity of CA1 layer neurons.

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