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Dopamine D_2 Receptor Blocking Effect of Imipramine in the Rat Hippocampus

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ŚMIAŁOWSKI, A. *Dopamine D_2 receptor blocking effect of imipramine in the rat hippocampus*. PHARMACOL BIOCHEM BEHAV **39**(1) 105–108, 1991.—The effect of dopamine receptor agonists on the spontaneous bioelectrical activity of CA1 layer neurons in the hippocampal slice preparation from the rat brain was studied. Two groups of rats were used: control and imipramine-pretreated ones (twice a day, for two weeks, 10 mg/kg PO). Dopamine and the selective D_2 receptor agonist quinpirole induced an excitatory reaction; a similar effect was evoked by amphetamine, an indirect dopamine agonist. The effects of the three compounds were diminished by sulpiride. Perfusion of control slices with imipramine decreased the excitatory effect of dopamine, amphetamine, and quinpirole. The effect of dopamine agonists was also inhibited 2 h after repeated pretreatment with imipramine. The excitatory effect of the dopamine agonist was enhanced 48 h after the last dose of imipramine, the latter effect was blocked by a 60-min perfusion of slices with imipramine or sulpiride in the experimental chamber. The obtained data show that acute imipramine induces blockade of dopamine D_2 receptors in the hippocampus. This effect is probably responsible for development of supersensitivity of dopamine D_2 mechanisms after prolonged treatment with imipramine in this brain region.

Hippocampus D_2 dopamine receptors Imipramine Antidepressants Supersensitivity

THE first information referring to the existence of some influence of antidepressant drugs on the brain dopamine system was obtained some years ago. During the early investigation into the influence of tricyclic antidepressants on the apomorphine-induced hypermotility in rats, Grabowska et al. (4) found that imipramine or amitriptyline injected before apomorphine significantly weakened the apomorphine-induced hypermotility. A further study found that a single dose of desipramine, amitriptyline or citalopram delayed the onset of the apomorphine-induced stereotyped sniffing, licking and gnawing; only citalopram diminished accumulation of apomorphine in the limbic forebrain and striatum of the rat (15). According to other authors a single dose of imipramine diminished all the apomorphine-induced behavioral activities in the open field test (8), and prevented the food- and apomorphine-induced place preference in rats (12). A single dose of desipramine injected 2 h before the test decreased the number of tritiated spiperone binding sites in the limbic forebrain but not in the striatum, whereas no change in [^{3}H]-SCH 23390 binding was observed (7). In the same paper a single dose of desipramine diminished the enhanced exploration induced by quinpirole injection (0.5 mg/kg SC).

Different results were obtained when the influence of antidepressant drugs was studied after repeated injection. Following this schedule, enhancement of the stimulating effect of dopamine receptor agonists was found when the rats were tested two days after the last dose of antidepressant (8, 10, 12, 18, 19, 21). The results obtained after the shorter withdrawal period (2 h) were equivocal: in some tests enhancement of the apomorphine effect was observed (8, 9, 16, 20), whereas in others the effect of dopamine agonist was inhibited (17). Since the results presented above are somewhat controversial, the interaction of imipramine with dopamine receptor in the hippocampus was studied.

METHOD

Male Albino Wistar rats, initially weighing 150 g, were used. The rats were housed in groups of eight and had free access to food and water under controlled light-dark conditions (light on between 7–19 h). Imipramine dissolved in water was administered orally in a dose of 10 mg/kg every 12 h for 14 days. The animals were decapitated 2 or 48 hours following discontinuation of imipramine administration.

Transverse slices of the dorsal hippocampus (400 μm thick) were prepared and rapidly transferred to an incubation chamber. After at least 1 h of preincubation, a single slice was placed in the experimental chamber on a nylon net and was continuously superfused at a rate of 1 ml/min with an oxygenated Krebs-Ringer solution consisting of (in mM): NaCl 124, KCl 5, $CaCl_2$ 2.4, $MgSO_4$ 1.3, KH_2PO_4 1.25, $NaHCO_3$ 26, glucose 10. The temperature was maintained at 34°C, pH at 7.4.

Spontaneous discharge rates of neurons located in the CA1 cell layer of the hippocampal slice were recorded with a tungsten microelectrode (Clark Electromedical Instr., 12 MΩ). Neurons were selected on the basis of the characteristics of their spontaneous activity. Units displaying a low firing rate (mean 6.96 Hz) and multiple irregular action potential of long duration were chosen. These neurons are related to complex-spike cells, which may represent pyramidal neurons (1,11). Action potentials were filtered using a spike filter, and separated from background signals with a window discriminator. The frequency of spontaneous dis-

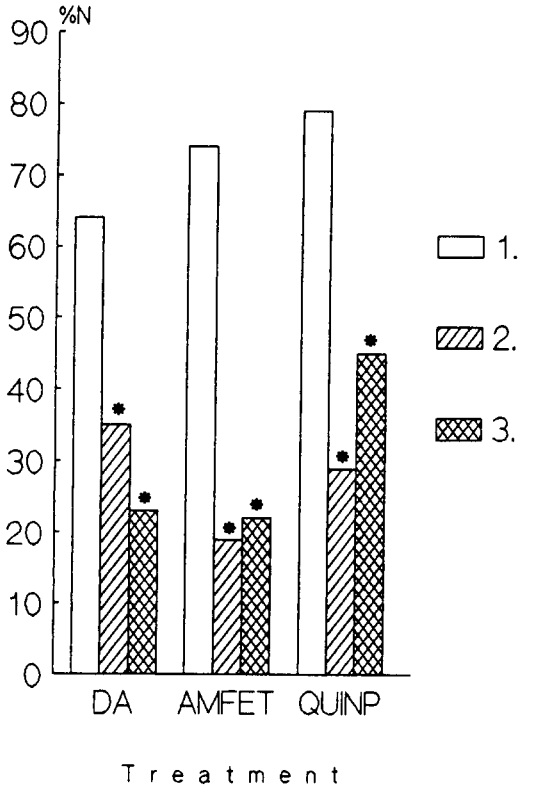

FIG. 1. The effect of imipramine (2) and sulpiride (3) on the excitatory reaction to dopamine agonists (1): dopamine (DA), amphetamine (AMFET) and quinpirol (QUINP). Each bar represents the number of units revealing excitatory reaction presented as a percentage changes of total number of units tested (%N). *$p<0.05$, Chi^2 test.

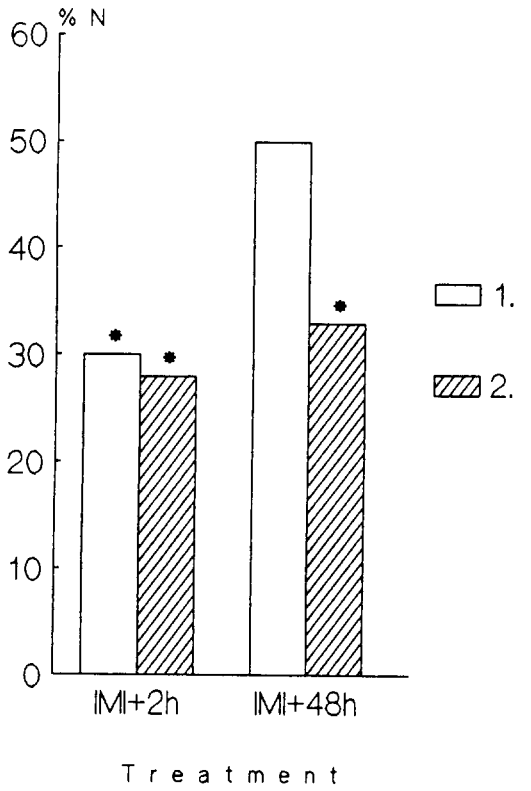

FIG. 2. The effect of prolonged treatment (14 days) on the excitatory reaction to dopamine tested 2 (IMI + 2 h) or 48 h (IMI + 48) after the last dose of imipramine tested without (bar 1) and during imipramine perfusion (bar 2). Data are presented as a percentage changes of total number of units tested (%N). *$p<0.05$, Chi^2 test in comparison with control group (see Fig. 1).

charges was calculated as the number of spikes per minute, and was then simultaneously integrated at 10-s intervals and recorded on a chart pen-recorder. The tested compounds were dissolved in an incubation medium and added to the perfusion line just before its access to the experimental chamber in a volume of 0.1 ml after at least 10 min of a stable basal firing rate. When used, imipramine or sulpiride were continuously superfused through the chamber (throughout approx. 60 min before adminstration of dopamine agonists). The reaction to the tested compounds was expressed as percentage of the mean firing rate before drug administration. The mean percent increases in the neuronal firing rate after administration of the tested compounds were calculated and compared by a one-way analysis of variance and a two-tailed Student *t*-test. The significance of group differences in the number of units reacting with excitation, inhibition or showing no reaction to the substance was evaluated using the Chi-square test. Number of units tested was 12–34 per dose.

The drugs used were d-amphetamine (Merck), dopamine HCl, imipramine (Polfa), quinpirole HCl (Lilly, IN), sulpiride (Sigma).

RESULTS

Excitatory Effect

Dopamine. Dopamine applied in a dose of 20 μM (Fig. 1) induced mainly the excitatory effect (60% of units). A 60-min perfusion of hippocampal slices with sulpiride (30 μM) or imipramine (30 μM) diminished the number of units excited by dopamine, but had no effect on the potency of the excitatory reaction (150% vs. 156% respectively).

Repeated treatment with imipramine diminished the number of units excited at 2 h after the last dose (Fig. 2), but potentiated the number of the excitatory reaction two days after the last dose. Application of imipramine to the perfusing medium diminished significantly the potency of the dopamine effect in imipramine-pretreated rats tested 48 h later (179% vs. 145%) and diminished the number of units excited 2 and 48 h after repeated imipramine.

Amphetamine. Amphetamine applied in a dose of 90 μM induced mainly the excitatory reaction in 74% of the tested units (Fig. 1). That effect was potently diminished by perfusion of the slices with sulpiride or imipramine. Repeated imipramine enhanced the number of units excited by amphetamine (Table 1), as well as the potency of the excitatory reaction (202% vs. 160%) at 48 but not 2 h after the last dose. Application of imipramine to the perfusing medium significantly reduced the number of the excited units in the group of rats previously pretreated with imipramine, tested 48 h after the last injection.

Quinpirole. Quinpirole in a dose of 0.1 μM induced enhancement of the firing rate of CA 1 hippocampal neurons in 85% of the units. That effect was diminished by pretreatment of slices with sulpiride and imipramine (Table 1). Repeated imipramine diminished the number of slices excited by quinpirole at 2 h after the last dose, but increased it after 48 h. Enhancement of the potency of the tested quinpirole effect was observed 48 h after re-

TABLE 1

THE INFLUENCE OF REPEATED TREATMENT OF IMIPRAMINE ON THE EXCITATORY EFFECT OF HIPPOCAMPAL SLICES TO AMPHETAMINE AND QUINPIROLE

Treatment	Withdrawal, h	N	Excitatory effect (%n)
	Amphetamine (90 μM)		
1. Control	—	23	74
2. Repeated imipramine	2	20	35*
3. Repeated imipramine	48	12	83*
4. Repeated imipramine + imipramine perfusion	48	16	31*
Significance between group 3–4: $p<0.05$, Chi^2 test.			
	Quinpirole (0.1 μM)		
1. Control	—	13	85
2. Repeated imipramine	2	36	22†
3. Repeated imipramine	48	18	82
4. Repeated imipramine + sulpiride perfusion	48	28	43*
Significance between groups 2–3 and 3–4: $p<0.001$, Chi^2 test.			

*$p<0.05$; †$p<0.01$ vs. control group, Chi^2 test.

peated imipramine (192% vs. 138%); perfusion of sulpiride in that experimental group significantly diminished both the number and the potency of the excitatory reaction.

Inhibitory Effect

Dopamine. In the control group dopamine inhibited 7% of the tested units only. This value was higher after perfusion of slices with imipramine, and ranged from 32 to 50%. The potency of the inhibitory reaction was significantly enhanced (24%) only in units pretreated with repeatedly imipramine, tested 48 h after the last dose.

Amphetamine. Amphetamine induced the inhibitory effect in 26% of the control units. The number of the inhibited units was higher after perfusion of slices with imipramine or sulpiride (64–81%). Changes in the potency of the inhibitory effect did not reach the level of statistical significance in any amphetamine-treated groups.

Quinpirole. In the control group quinpirole inhibited 15% of the units only. Repeated administration with imipramine and sulpiride perfusion slightly enhanced the number of the inhibited units. The potency of the inhibitory effect of quinpirole was significantly diminished (27%) 2 h after repeated imipramine. In the remaining groups changes in the potency of the inhibitory effect did not reach the level of statistical significance.

DISCUSSION

The results presented above show that dopamine, quinpirole and amphetamine induce enhancement of the firing rate of hippocampal neurons. Since the effect of dopamine, amphetamine and quinpirole was blocked by sulpiride, the dopamine D_2 receptor is responsible for the observed reaction.

Perfusion of control slices with sulpiride or imipramine decreased the number of slices showing on excitatory reaction to dopamine agonists tested. A similar effect was observed 2 h after repeated treatment with imipramine when a high level of imipramine was found in the brain tissue (3). This result shows the blocking effect of dopamine D_2 receptor by acute imipramine application and confirms the finding of Grabowska et al. (4) that imipramine decreases the locomotor effect of apomorphine.

A different effect appeared after 48 h, i.e., when the concentration of imipramine was below the detection level (3); at that time the sensitivity of dopamine receptors to the excitatory effect of dopamine agonists and the number of the excited units were higher. The reaction recorded 48 h after imipramine was also diminished by pretreatment of slices with sulpiride or imipramine. The above mentioned results show that both sulpiride and imipramine are capable of blocking the effect of dopamine D_2 agonists in the rat hippocampus.

The observed effect of repeated treatment with imipramine is generally similar to that found earlier after a prolonged blockade of the dopamine D_2 receptor in the rat hippocampus by haloperidol (2). A prolonged treatment with haloperidol (3 weeks) enhanced the potency of the dopamine excitatory effect. That enhancement of the dopamine effect was diminished by sulpiride. When we compare the above data it appears that prolonged imipramine induces supersensitivity to dopamine D_2 agonists in the hippocampus by means of an interaction with a dopamine D_2-sensitive mechanism. The presented results are confirmed by an increase in the affinity of quinpirole for ^{3}H-spiperone binding sites after repeated imipramine found in membranes prepared from the limbic system, but not from the striatum (6).

Presented data, together with other results, point to the influence of imipramine and some other antidepressant drugs, as well as electroconvulsive treatment on the dopamine D_2 system in the limbic brain (5, 13, 21). Since a reduced functional dopamine system activity was described in some subgroups of depressive patients (5, 14, 22, 23) the imipramine-induced enhancement of sensitivity of dopamine D_2 system is capable of improving the function of this disturbed mechanism upon the normal level.

In conclusion, repeated imipramine induces enhancement of the sensitivity of dopamine D_2 mechanisms in the rat hippocampus. This phenomenon is probably induced by the blocking effect of dopamine D_2 hippocampal receptors by acute imipramine. Although in our study acute imipramine-like sulpiride blocks the effect of the dopamine D_2 agonist, according to the results of other authors it probably influences some other than a classic neuroleptics part of the brain dopamine mechanisms.

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