EFFECT OF DEPRIVATION OF THE PARADOXICAL PHASE OF SLEEP ON ROTATIONAL AND STEREOTYPED BEHAVIOR INDUCED BY SELECTIVE AGONISTS OF DOPAMINE RECEPTORS

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It is not yet sufficiently clear on the results of which mechanisms actions, such as deprivation of the paradoxical phase of sleep (DPPS) and/or administration of antidepressants, give rise to an antidepressive effect in man. In animals, chronic administration of antidepressants causes suppression of the paradoxical phase of sleep (PPS) [10], whereas long-term DPPS, by behavioral methods, is accompanied by intensive stress reactions [1]. The increased excitability of animals induced by these procedures is associated with changes in central dopaminergic (DA) processes, aimed at strengthening DA-regulation. It has been shown that in both cases the sensitivity of postsynaptic receptors to direct DA-agonists increases [7, 13], and the concentration of endogenous DA and tyrosine hydroxylase (TH) activity of the brain also are increased in DPPS [1, 5]. However, we do not know the aftereffects of prolonged DPPS in relation to subtypes of D_1- and D_2-postsynaptic DA-receptors, which have opposite links with adenylate cyclase activity and are responsible for different pharmacologic effects.

The aim of this investigation was to study the effects of selective D_1- and D_2-agonists of DA receptors on the induction of rotational (qualitative and quantitative behavioral parameter of DA-receptor functions) and of stereotyped behavior before and after long-term DPPS.

EXPERIMENTAL METHOD

Experiments were carried out on noninbred male rats weighing 150-180 g. As a first step the character of the rats' avoidance behavior in an acute stress situation, described previously [2] was assessed, and this also was done after DPPS in order to determine changes in the animals' reactivity. The number of attempts to avoid and the latent period (LP) of avoidance were recorded. Animals incapable of avoidance behavior were discarded. Under ether anesthesia, 1 μg of kainic acid ("Sigma") in 1 μl was injected into the selected rats twice into the left striatum, at coordinates A = +1, L = 2.3, H = 4.5 and A = 0, L = 3.8, and H = 4.5 by the method in [6]. To prevent the onset of convulsions diazepam (10 mg/kg, Relanium, from "Polfa") was injected. After a recovery period lasting 2 weeks the intensity of rotational behavior induced by apomorphine 0.5 mg/kg was estimated. The number of ipsilateral rotations was recorded every 10 min for 1 h. Animals with a stable rotation pattern (100-180 turns per hour) were chosen. The effect of each dose of the selective DA-agonists on induction of rotational and the corresponding stereotyped behavior was studied on 4-6 selected animals 1 week before and 1 day after a 5-day period of DPPS, using the method of small areas (14 cm^3/100 g) [11]. Stereotyped behavior was recorded during a 15-sec series every 2 min for 1 h. The prevalence of each form of stereotyping was expressed as a percentage of the total number of intervals of recorded behavior. In separate experiments, in order to reduce the stimulating effect of endogenous DA on DA-receptors of the intact striatum, the TH inhibitor a-methylparatyrosine (α-MPT, from "Sigma") was injected into two groups of rats 3 h before injection of the D_1- and D_2-agonists in a dose of 200 mg/kg intraperitoneally. In these cases behavioral manifestations were recorded for 90 min. Selective D_1-receptor SKF-38393 ("Lundbeck") and D_2-receptor Ly-171555 ("Lundbeck") agonists were dissolved in distilled water and injected intraperitoneally. In all cases the control animals were given an injection of an equal volume of the base. The results were subjected to statistical analysis by the Wilcoxon–Mann–Whitney tests.

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TABLE 1. Effect of a 5-Day Period of DPPS on Rotational Behavior Induced by Selective Agonists of Dopamine Receptors (M ± m)

Experimental conditions	Number of ipsilateral rotations during 1 h of recording
Control (distilled water)	9.8 ± 3.0
Before DPPS:	
quinpyrol, 0.5 mg/kg	117.0 ± 16.4
SKF-38393, 3.2 mg/kg	0**
SKF-38393, 5 mg/kg	0**
quinpyrol, 0.5 mg/kg + SKF-38393, 3.2 mg/kg	13.4 ± 4.3
Control (distilled water)	8.3 ± 2.5
Before DPPS:	
quinpyrol, 0.5 mg/kg	185.8 ± 18.3*
SKF-38393, 3.2 mg/kg	14.2 ± 4.1*
SKF-38393, 5 mg/kg	27.3 ± 5.0**
quinpyrol, 0.2 mg/kg + SKF-38393, 3.2 mg/kg	54.5 ± 9.6*

Note. *) Differences compared with effect of corresponding agonists in animals without DPPS are significant; **) compared with corresponding control. In both cases $p < 0.001$.

EXPERIMENTAL RESULTS

Stimulation of normosensitive postsynaptic D_2-receptors by Ly-171555 (quinpyrol) induced ipsilateral rotations, the number of which increased with an increase in the dose: 0.5, 2, and 5 mg/kg, corresponding to 117, 257.3, and 426.5 rotations per hour of recording. Rotations were accompanied by stereotyped reactions, mainly sniffing (prevalence 8% with a dose of 0.5 mg/kg). Prevalence of oral forms of stereotypy (chewing, licking, grooming) was low and largely indistinguishable from the control. Conversely SKF-38393, in doses of 1.5, 3.2, and 5 mg/kg, induced intensive oral stereotyped reactions, but not rotations. With a dose of 3.2 mg/kg, the prevalence for chewing was 36%, for licking 17%, for sniffing 31%, and for grooming 48%. With increased doses, apomorphine, a mixed agonist of DA-receptors, is known to reduce the locomotor component as a result of strengthening of oral stereotype. In the same way, combined injection of quinpyrol and SKF-38393 induced a considerable (by 85.5%) fall in the number of ipsilateral rotations (Table 1), as a result of strengthening or oral stereotypy with similar prevalence of forms as when SKF-38393 was given separately. This result agrees with data obtained previously [3].

DPPS led to worsening of avoidance behavior in an acute stress situation. The number of unsuccessful attempts at avoidance was increased (6.3 ± 2.7 before and 14.3 ± 7.1 after DPPS, $p < 0.05$). LP of avoidance was significantly increased (14.8 ± 6.0 and 45.5 ± 12.3, respectively, $p < 0.001$), which, as was pointed out above, was connected with strengthening of the animals' emotional behavioral reactivity, correlating with increased brain TH activity [1] and with an increased concentration of endogeneous DA [10]. Increased reactivity of the animals' distress after DPPS was connectd with strengthening of the effect of the agonists in relation to rotational behavior (Table 1). The effect of quinpyrol rose by 59%, whereas that of SKF-38393 rose by 229% (compared with the control with a dose of 5 mg/kg). The effect of their combined injection amounted to 307%. Under these circumstances prevalence of oral forms of stereotypy, with the exception of grooming (62%, $p < 0.01$), was significantly reduced both when SKF-38393 was given separately and when given together with quinpyrol (chewing 12%, licking 4%, sniffing 20%, $p < 0.001$). The results suggest that strengthening of ipsilateral rotations by the agonists after DPPS was mediated through unequal changes in sensitivity of the DA-receptor subtypes, not only of the nigrostriatal, but also of the mesolimbic system, predominant involvement of which takes place during chronic administration of antidepressants [8].

Considering that potentiation of the locomotor response to separate injection of quinpyrol appeared only after combined chronic administration of quinpyrol and SKF-38393 [4], chronic administration of L-DOPA simultaneously counteracted enlargement of the ^{3}H-spiroperidol binding sites, increased hypersensitivity of adenylate cyclase (bound with the D_1-receptor) to DA, and potentiated contralateral rotations to apomorphine in animals with a unilaterally denervated striatum by the neurotoxin 6-hydroxydopamine [9], it can be tentatively suggested that prolonged and intensive interaction of endogenous DA with both

TABLE 2. Effect of 5-Day DPPS on Dynamics of Ipsilateral Rotations Induced by the D_2 Agonist Quinpyrol in a Dose of 0.5 mg/kg

Experimental conditions	Number of rotations in 10-min recording periods					
	0–10	11–20	21–30	31–40	41–50	51–60
Before DPPS	9,1	30,0	25,3	15,0***	12,1***	25,5
After DPPS	15,5	39,3*	32,2*	34,6**	33,4**	30,8

Note. Differences significant compared with effect of agonist in animals without DPPS during corresponding time intervals. *$p < 0.05$, **$p < 0.001$, ***$p < 0.001$ (differences significant compared with values during 21-30-min interval in animals before DPPS).

TABLE 3. Effect of α-MPT on Rotational Behavior Induced by Selective Dopamine Agonists before and after 5-Day DPPS

Experimental conditions	Number of rotations in 10-min recording periods								
	0–10	11–20	21–30	31–40	41–50	51–60	61–70	71–80	81–90
Before DPPS									
Quinpyrol 0.5 mg/kg + SKF-38393, 5 mg/kg 30 min after quinpyrol	7,5	18,5	14,0	13,5	7,0	5,5	5,0	0	0
After DPPS	0*	1*	0*	13,0	16,0*	32,5*	22,5*	13,5*	19,0*

Note. Differences significant compared with effect agonists in animals without DPPS. *$p < 0.001$.

DA-receptor subtypes during DPPS leads to a selective increase in sensitivity of the D_1-subtypes.

After DPPS the pattern of rotational behavior under the influence of quinpyurol changed (Table 2). After a sharp rise in the number of rotations in the initial periods of recording (11-20 and 21-30 min) no significant decrease in the intensity of the rotations took place, as was observed in the control with normal receptor sensitivity. In time, as was shown previously on intact animals, this coincides with reduced release of endogenous DA after the same dose of quinpyrol [12], which is connected with activation of presynaptic D_2-receptors, inhibiting DA release [12]. Stability of rotations under the influence of quinpyrol after DPPS therefore reflects the state of subsensitivity of the D_2 receptors.

The DPPS behavioral procedure, accompanied by stress, leads to increased TH activity [1], coupled with increased affinity of TH for tyrosine and α-MPT. A single injection of α-MPT into animals subjected to DPPS completely prevented the development of the effect of quinpyrol (Table 3). Additional injection of the D_1-receptor agonist SKF-38393 30 min after quinpyrol restored rotational behavior, and this was accompanied by a low level of oral stereotyped reactions and, conversely, as a result of their induction in animals not subjected to DPPS, it inhibited rotational behavior. These results are evidence that deficiency of tonic activation of D_1-receptors by endogenous DA prevents manifestation of the effect of the D_2-receptor selective agonist quinpyrol. Postsynaptic D_2-receptors cannot therefore be supersensitive and they are functionally linked with D_1-receptors.

The results show that induction of rotational and stereotyped behavior by selective agonists of DA-receptors and its intensity depend on the degree and duration with which the complementary subtype of receptor is stimulated by endogenous DA. It can be postulated on the basis of these results that prolonged and intensive tonic activation of DA-receptors, caused both by behavioral DPPS and by chronic administration of antidepressants, can lead to nonidentical changes in sensitivity of D_1- and D_2-subtypes of DA-receptors.

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EFFECT OF DELTA-9-TETRAHYDROCANNABINOL ON RECEPTOR AND PHYSICOCHEMICAL PROPERTIES OF RAT BRAIN MEMBRANES

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Despite many publications on cannabimimetic agents, the mechanism of action of this group of compounds at the cell level has not been fully explained. The molecular mechanism of action of the cannabinoids has been linked either with their powerful nonspecific lipotropic action on the cell membrane or with specific interaction with their own receptor [8, 10-14]. It is claimed that the influence of cannabinoids on membrane enzymes is determined by their membranotropic properties [14]. Meanwhile the action of cannabinoids on receptors of the principal neurotransmitters of the CNS and drugs has not been adequately studied.

The aim of this investigation was to study the action of delta-9-tetrahydrocannabinol (delta-9-THC) on β-adrenergic, muscarinic acetylcholine, D_2-dopamine, 5-HT (serotonin), μ-opioid, and benzodiazepine receptors and on the physicochemical state of rat brain neuronal membranes.

EXPERIMENTAL METHOD

Male Sprague-Dawley rats weighing 150-200 g were used. Rat brain membrane preparations were obtained by methods described previously [3]. The following tritium-labeled ligands were used for finding with β-adrenergic muscarinic acetylcholine, D_2 dopamine, 5-HT (serotonin), benzodiazepine, and μ-opioid receptors: ^{3}H-dihydroalprenolol (^{3}H-DHA), ^{3}H-LSD, ^{3}H-quinuclidinyl benzylate (QNB), spiperone, flunitrazepam, DAGO (D-Ala^2,MePhe^4,Glyol^5-enkephalin), with specific radioactivity of 52, 24, 4, 38, 77, 78, and 60 Ci/mmole, respectively ("Amersham," England). Experiments with binding of labeled ligands with rat brain membranes were carried out in accordance with methods already familiar and described previously for DHA and QNB [2], LSD [5], spiperone [12], flunitrazepam [13], and DAGO [15]. The concentration of membrane-bound protein in all the binding experiments was 1-2 mg/ml.

Microviscosity of the lipid phase of the membranes was judged from the ratio of the peaks of pyrene fluorescence (F_e) at 480 nm (excimer) to the maximum F_m at 373 nm (monomer), with excitation wavelength of 335 nm, as described in [1]. The value of the F_e/F_m ratio is inversely proportional to microviscosity of the membranes. Quenching of tryptophan fluorescence (λ_e = 285 nm, λ_f = 340 nm) was calculated by the ratio $F_0/\Delta F$, where F_0 is the intensity of tryptophan fluorescence in the absence of the quenching agent and ΔF the change in tryptophan fluorescence after addition of the quencher. Fluorometric measurements were made on the "Hitachi-650-10" spectrofluorometer (Japan).

Freshly obtained membranes were treated with pro-oxidants (a mixture of 50 μM $FeCl_2$ and 250 μM ascorbic acid) for 10 min at 37°C and were used immediately for subsequent experiments.

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