6. M. Herrera-Marschitz and U. Ungerstedt, Eur. J. Pharmacol., 98, 165 (1984).
7. J. Maj, Z. Rogoz, G. Skuza, and H. Sowinska, Neuropharmacology, 85, 133 (1985).
8. J. Maj and K. Wedzony, J. Pharm. Pharmacol., 37, 362 (1985).
9. M. Parenti, C. Flauto, E. Parati, et al., Neuropharmacology, 25, 331 (1986).
10. P. Polc, J. Schneeberger, and W. Haffely, Neuropharmacology, 18, 259 (1979).
11. M. Radulovacki and R. Zak, Brain Res., 217, 420 (1981).
12. T. Sharp, T. Zetterström, A.-K. Collin and U. Ungerstedt, Eur. J. Pharmacol., 142, 187 (1987).
13. S. Tujik, Psychopharmacology, 72, 257 (1981).

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

A. V. Eremenko, I. S. Uvarova, and I. N. Kurochkin

UDC 612.82.014.467.06:615.217.22].08

KEY WORDS: delta-9-tetrahydrocannabinol; rat brain receptors; membrane microviscosity

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.

Research Center for Molecular Diagnosis, Ministry of Health of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR A. V. Val'dman.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 110, No. 11, pp. 497-499, November, 1990. Original article submitted January 29, 1990.

 0007-4888/90/0011-1514$12.50

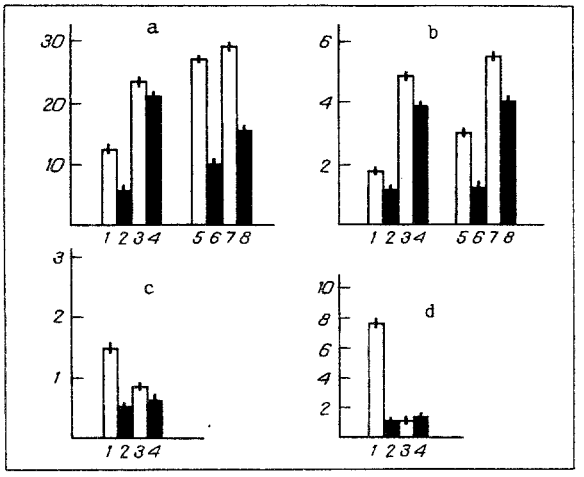

Fig. 1

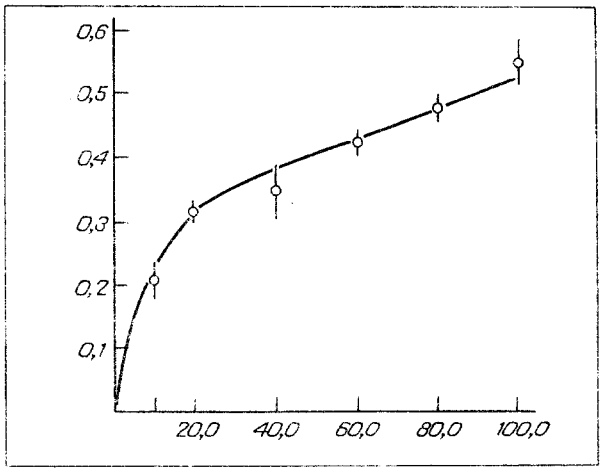

Fig. 2

Fig. 1. Effect of delta-9-THC and pro-oxidants on binding of labeled ligands by rat brain membranes. Abscissa, binding of ^{3}H-spiperone (a) and of ^{3}H-LSD (b, c), and ^{3}H-DAGO (d); ordinate, cpm ($\cdot 10^3$). 1, 2, 5, 6) control values of binding for corresponding ligands. a, b: 3, 4, 7, 8) binding in the presence of 10 μM delta-9-THC; 1-4) lyophilized; 5-8) freshly isolated membranes; c, d: 3, 4) after treatment with pro-oxidants. Unshaded columns) total binding, black columns) nonspecific binding of labeled ligands.

Fig. 2. Quenching of tryptophan fluorescence by rat brain membranes under the influence of delta-9-THC. Abscissa, concentration of delta-9-THC (in μM); ordinate, $F_0/\Delta F$.

TABLE 1. Effect of Delta-9-THC and LPO on Microviscosity of Rat Brain Membranes, Estimated from Excimerization of Pyrene (M ± m)

Experimental conditions	F_e/F_m	%
Control	0,390±0,020	100,0±5,1
Delta-9-THC, 40 μm	0,330±0,010*	84,6±3,0
Delta-9-THC, 80 μm	0,310±0,020*	79,5±6,5
Incubation for 10 min at 37°C without $FeCl_2$ and ascorbate	0,340±0,020	100,0±5,9
$FeCl_2$ and ascorbate	0,264±0,004*	77,7±1,5

Note. *$p \leq 0.05$.

All experiments were done in 50 mM Tris-HCl buffer, pH 7.4, at 37°C.

EXPERIMENTAL RESULTS

The study of the effect of delta-9-THC on receptors of the lyophilized rat brain membrane preparation showed that, in a concentration of 10 μM, delta-9-THC lowers the level of specific binding of labeled LSD and spiperone, accompanied by an increase in total and nonspecific binding (Fig. 1a, b). It reduces the specific binding of labeled flunitrazepam very slightly, but has no effect on binding of labeled QNB, DAGO, and DHA (results not shown). Incidentally, reduction of specific binding of the D_2-antagonist ^{3}H-spiperone with rat brain membranes also was found in [7], but no increase was observed in nonspecific binding, such as was observed in our own experiments.

It can be postulated that the effect of delta-9-THC on binding of labeled spiperone and LSD is effected through the lipid phase of the membranes, for this substance and its derivatives are strongly lipophilic compounds [9, 14]. We therefore estimated the effect of delta-9-THC on the physicochemical state of rat brain membranes. Data showing the effect of delta-9-THC on fluorescence of pyrene, inserted into the membrane, are given in Table 1. They show that under the influence of delta-9-THC there was a decrease in the ratio F_e/F_m of about 20-25% (the greatest observable decrease in concentrations was 40 and 80 μM), indicating an

increase in microviscosity of the lipid phase of the test membranes. The effect of delta-9-THC on membrane microviscosity which we obtained are in agreement with previous data obtained by the use of another hydrophobic fluorescent probe – 1,6-diphenyl-1,3,5-hexatriene [9]. Moreover, delta-9-THC is a quencher of fluorescence of membrane tryptophan (Fig. 2), indicating conformational changes in membrane proteins under the influence of this compound.

An increase in the viscosity of the lipid phase of biological membranes can be obtained, as we know, by initiating lipid peroxidation (LPO). Treatment of membranes with pro-oxidants, just as in the case with delta-9-THC, leads to an increase in microviscosity of the rat brain membranes (Table 1). However, assessment of receptor activity revealed that preliminary incubation of the membranes with pro-oxidants led to complete loss of ability of serotonin (^{3}H-LSD) and opioid (^{3}H-DAGO) receptors to bind the ligand (Fig. 1c, d). Incidentally, this effect of the pro-oxidants, given the equal strength and direction of the effect on membrane microviscosity, differs radically from the effect of delta-9-THC on these receptors. The action of delta-9-THC on the receptor systems therefore evidently has a more complex mechanism and cannot be explained simply by the membranotropic properties of this compound.

Analysis of data in the literature on the effect of delta-9-THC on the monoamine system (increased synthesis of dopamine and serotonin, inhibition of reuptake of these neurotransmitters) [4, 6, 11], and the results of the present investigation together suggest some selectivity of action of delta-9-THC on dopamine and serotonin receptor systems.

The authors are grateful to Candidate of Biological Sciences N. A. Avdulov for advice in the preparation of this paper.

LITERATURE CITED

1. N. A. Avdulov, A. V. Eremenko, and A. V. Val'dman, Byull. Éksp. Biol. Med., No. 2, 173 (1988).
2. S. G. Evdokimenko, O. A. Serebryakova, S. V. Zaitsev, et al., Nauch. Dokl. Vysshaya Shkolo, Biol. Nauki, No. 5, 24 (1989).
3. M. G. Sergeeva, O. A. Sklyankina, I. N. Kurochkin, et al., Neirokhimiya, 5, 11 (1986).
4. S. P. Banerjee, S. H. Snyder, and R. Mecholam, J. Pharmacol. Exp. Ther., 194, 74 (1975).
5. J. P. Bennet, Jr., and S. H. Snyder, Mol. Pharmacol., 12, 373 (1976).
6. A. S. Bloom and W. L. Dewey, Psychopharmacology, 57, 243 (1978).
7. A. S. Bloom and C. J. Hillard, Marijuana '84, Oxford (1985), pp. 217-231.
8. C. J. Hillard and A. S. Bloom, The Canabinoids: Chemical, Pharmacological and Therapeutic Aspects, New York (1984), pp. 591-602.
9. C. J. Hillard, R. A. Harris, and A. S. Bloom, J. Pharmacol. Exp. Ther., 232, 579 (1985).
10. J. S. Hye, H. H. Seltzman, C. C. Pitt, et al., J. Pharmacol. Exp. Ther., 234, 784 (1985).
11. K. M. Johnson, Serotonin in Biological Psychiatry, New York (1982), p. 330.
12. S. E. Leff and I. Creese, Brain Receptor Methodologies, Part B, New York (1984), pp. 249-277.
13. P. J. Marangos and J. Patel, Brain Receptor Methodologies, Part B, New York (1984), pp. 177-188.
14. B. R. Martin, Pharmacol. Rev., 38, 45 (1986).
15. R. S. Zukin, Brain Receptor Methodologies, Part B, New York (1984), pp. 77-100.