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EFFECT OF DEPRIVATION OF THE PARADOXICAL PHASE OF SLEEP ON ACTIVITY OF OPIATE RECEPTORS ISOLATED FROM RAT BRAIN SYNAPTIC MEMBRANES

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Paradoxical sleep deprivation (PSD) in mammals is a convenient model with which to study the effect of extremal factors on metabolic and mediator processes in the brain [1]. It has been found that during PSD not only is the sensitivity of the rat brain to dopamine reduced [8], but the total concentration of dopamine receptors and their affinity for ligands also are reduced [14]. Similar changes in the number of binding sites and the affinity of β-adrenoreceptors during PSD have been observed by Mogilnicka [11]. PSD in rats also is reflected in the state of their opiatergic system: the concentration of Met-enkephalin is increased whereas that of Leu-enkephalin is reduced in the hypothalamic region and hippocampus [3], although the β-endorphin concentration is unaffected [3, 12]. Workers in our laboratory also have shown that 24-hourly PSD in rats causes a change in the state of the opiate receptors located in synaptic membranes, and leads to a decrease in the concentration of binding sites of the antagonist, namely ^{3}H-naloxone [5]. In this case a tendency was observed for affinity of the receptors for this ligand to be increased. Meanwhile a change in the molecular organization of the synaptic membranes was found after PSD: a decrease in their lipoperoxide content, an increase in their degree of hydrophobicity, and a shift of the cationic—anionic balance [6]. In this connection the following problem arises: are the changes in functional properties of the receptor apparatus the result of changes in the molecular organization of the synaptic membranes, or are they due to a change in the properties of the opiate receptors themselves and, in particular, of opiate-binding proteins.

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In order to shed light on this problem we undertook a comparative study of the properties of opiate receptors isolated from brain synaptic membranes of control rats and rats subjected to PSD. Another of our aims was to determine whether the modified properties of the opiate receptors are preserved during their isolation under harsh conditions from synaptic membranes and their subsequent purification.

EXPERIMENTAL METHOD

Experiments were carried out on male Sprague—Dawley rats weighing 200-220 g. PSD was carried out for 24 h by the use of small platforms [9]. Isolation of synaptic membranes from the rats' brain and their solubilization with 0.5% digitonin solution and purification from excess of the detergent were carried out by the method described previously [2].

For affinity purification of the isolated proteins we used a specially prepared affinity sorbent, containing the Leu-enkephalin analog Dalargin (Tyr-D-Ala-Gly-Phe-Leu-Arg) synthesized at the All-Union Cardiologic Scientific Center, Academy of Medical Sciences of the USSR (Moscow). Chromatography was carried out by the method described by us previously [4]. The column was washed with 50 volumes of buffer A (10 mM $MgCl_2$, 1 mM $CaCl_2$, 1 mM EDTA-Na, 1 mM phenylmethylsulfonyl fluoride (PMSF), 0.01% bacitracin, and 0.05 M Tris-HCl, pH 7.5), then with 0.2 M NaCl. Proteins specifically bound with the sorbent were eluted either with 0.5 M NaCl, and designated Dalargin-binding proteins — DBP_{NaCl}, or with a 10 μM solution of DAGO (Tyr-D-Ala-Gly-N-Me-Phe-Gly-ol) and designated DBP_{DAGO}, in buffer containing 0.01% bacitracin, 1 mM PMSF, 0.05 M Tris-HCl. The proteins were recorded by means of a Uvicord-S spectrophotometer ("LKB," Sweden) at 280 nm.

Binding of ^{3}H-naloxone (55 Ci/mmole, from "Amersham," England) with synaptic membranes and with solubilized proteins was investigated by the method described previously [2], and in the case of isolated proteins, by Bidlak's method [7] in 0.05 M Tris-HCl buffer at 37°C in the course of 20 min. The protein content in the sample (100 μl) was: for synaptic membranes and solubilized proteins — 100 μg, for DBP_{NaCl} 10 μg, for DBP_{DAGO} 0.01 μg. Nonspecific binding was determined in the presence of 1 μM unlabeled naloxone. The free ligand was separated from bound by filtration to GF/C glass fiber filters (Whatman, England) on a "Minifold" instrument (Dynateck, USA). The filters were washed with buffer and transferred to flasks containing dioxan scintillation fluid. The radioactivity of the samples was determined on a Mark III counter. The kinetic characteristics of the binding sites were calculated by the "Pharmacologic Calculation System — Version 4.0" program on a personal computer [10].

The homogeneity of proteins isolated from brain synaptic membranes of control rats and of rats subjected to PSD was investigated by HPLC on a "Gilson" chromatograph (France) with detection of proteins at 210 nm. The samples (10-20 μg) were applied to a TSK 3000 SW (30 cm) + TSK 3000 SW (60 cm) column, equilibrated with 0.05 M Tris-HCl, pH 7.5 Elution was carried out at the rate of 1 ml/min, using the same buffer.

EXPERIMENTAL RESULTS

Specific binding of ^{3}H-naloxone with opiate receptors was studied at different stages of their isolation from rat brain synaptic membranes and further purification. The results, shown in Fig. 1, indicate that on solubilization of synaptic membranes the naloxone-binding activity (calculated per milligram protein) of the solubilized proteins was reduced by 60-70%. This confirms data according to which the use of the nonpolar detergent digitonin reduces the opiate-binding activity of the solubilized proteins but does not cause its total loss, as when polar detergents are used [13]. On further purification of the proteins by affinity chromatography their naloxone-binding activity increased regularly (Fig. 1), when both the nonspecific eluent (NaCl solution; sevenfold) and the specific eluent (DAGO: 6000-fold) was used. A precise rule also was discovered: after PSD the naloxone-binding activity fell: not only of opiate receptors located in synaptic membranes (by 35%), but also of isolated receptors (by 25-28%), obtained from membranes, irrespective of the method of elution and the degree of purification (Fig. 1). This fact not only confirms previous data showing a decrease in the concentration of active binding sites of opiates in synaptic membranes after PSD [5], but also is evidence of the involvement of opiate receptors in structural and functional reorganization of the synaptic membranes under exposure to extremal factors, such as 24-hourly PSD. We accordingly undertook a more detailed study of the effect of PSD on the kinetic characteristics of purified opiate receptors.

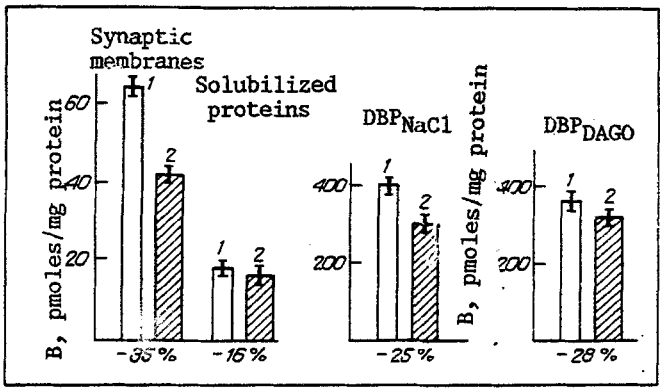

Fig. 1. Effect of paradoxical sleep deprivation on specific binding of ^{3}H-naloxone (7 nM) with different degrees of purification of opiate receptors. Columns: 1) control, 2) after PSD for 24 h. B) Specific binding.

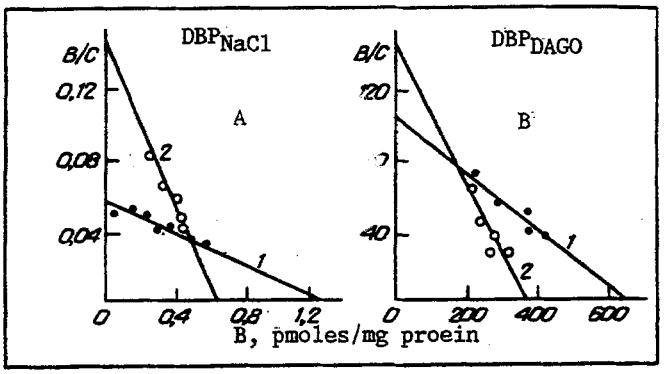

Fig. 2. Results of analysis of specific binding of ^{3}H-naloxone with purified opiate receptors, shown by Scatchard plot. A) DBP$_{NaCl}$, B) DBP$_{DAGO}$. 1) Control, 2) after PSD for 24 h. B) Number of binding sites (in pmoles/mg protein). C) ^{3}H-naloxone concentration.

Investigation of binding of different concentrations of ^{3}H-naloxone with isolated receptors of control animals showed that binding of the antagonist both with DBP$_{NaCl}$ and with DBP$_{DAGO}$ is specific and saturatable. The results were analyzed by Scatchard plot (Fig. 2a, b). The linear character of the Scatchard plots is evidence of homogeneity of the binding sites of these proteins with the antagonist and also of binding of ^{3}H-naloxone with binding sites of only one type. The kinetic characteristics of interaction between ^{3}H-naloxone and isolated receptors indicate the high-affinity nature of the binding (Table 1) and point to significantly higher activity of receptors eluted with DAGO solution (DBP$_{DAGO}$) than of DBP$_{NaCl}$. In the case of DBP$_{DAGO}$ affinity for naloxone was trebled whereas the density of active binding sites (B_{max}) was 600 times greater than that of DBP$_{NaCl}$. These results are evidence not only of different degrees of purification of opiate-binding proteins, but also that the use of DAGO, a specific ligand of opiate receptors of mu-type, to elute proteins during affinity chromatography enables a highly purified fraction of opiate receptors, possessing a higher concentration of active binding sites for ^{3}H-naloxone and higher affinity for this radioligand, to be isolated.

PSD led to a decrease in the density of naloxone binding sites and an increase in affinity for naloxone in both DBP$_{NaCl}$ and DBP$_{DAGO}$ (Table 1). The fact that the Scatchard plot remained linear in character (Fig. 2) indicates that in this case, just as in the case of opiate receptors of the control animals, ^{3}H-naloxone interacts with only one type of binding site.

The results thus showed the same trend of changes in functional properties of opiate receptors located in synaptic membranes and isolated from them, induced by PSD. These changes are stable and persist under harsh conditions of their isolation and purification, despite exposure to high accelerations, detergents, and sorbents. It can be concluded from these results that during exposure to such extremal conditions as PSD, profound structural changes take place in the synaptic membranes of the brain at the molecular level, affecting not only the lipid components of the membrane, but also its inte-

TABLE 1. Binding of ^{3}H-Naloxone with Purified Opiate Receptors (M ± m)

Experimental conditions	K_d, nM	B_{max}, pmoles/mg protein
	DBP_{NaCl}	
Control	21,2±5,1	1,22±0,10
PSD for 24 h	4,6±0,2*	0,64±0,04*
	DBP_{DAGO}	
Control	6,6±0,9	690±40
PSD for 24 h	2,8±0,05*	376±26*

Legend. *$p < 0.05$ compared with control.

gral receptor proteins. Evidence in support of this hypothesis is given by the marked increase in affinity for naloxone in receptors isolated after PSD, whereas for receptor synaptosomes only a tendency for affinity to increase was noted [5].

Since the degree of affinity of receptors for ligands is regulated through the formation and dissociation of a complex with GTP-binding proteins, it can be tentatively suggested that a prolonged sleep disturbance leads to changes in the structural organization of the receptor complex due to weakening of bonds either between protein subunits of the receptor complex or between the recognizing part of the receptors and GTP-binding proteins.

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