Effects of Chronic Morphine Intake on Binding of NAD and GABA-Benzodiazepine Agonists to Synaptic Membranes

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Nine weeks of compulsory morphine drinking decreased the specific binding of ^{3}H-muscimol to GABA receptors and ^{14}C-NAD to rat brain synaptic membranes and increased the synaptosomal uptake of ^{14}C-GABA. These effects of morphine on the GABA-benzodiazepine receptor complex were reversed by excessive doses of vitamin B_3.

Key Words: *vitamin B3; NAD; morphine; GABA-benzodiazepine receptor complex*

Vitamin B_3 deficiency leads to severe dysfunction of the central nervous system. On the other hand, some nervous and mental diseases, such as schizophrenia, epilepsy, and alcohol dependence are characterized by impaired metabolism of this vitamin.

Two forms of vitamin B_3 (nicotinic acid and nicotinamide) and related drugs are widely used for the treatment of different mental diseases.

The discovery of central benzodiazepine (BD) receptors gave a new approach to understanding of the mechanisms of neurotropic effects of vitamin B_3 and its bioactive form NAD. Nicotinamide, the pharmacological profile of which is similar to BD [1], was identified as a possible endogenous ligand of BD receptors [9].

Previously, we have shown that nicotinamide through NAD interacts with the GABA-BD receptor complex and potentiates GABAergic neurotransmission [3]. Depending on the pathological conditions, this interaction occurs either with or without porticipation of the GABAergic component of the complex [2].

It is known that vitamin B_3 increases brain content of serotonin and acts as a tranquilizer [11]. It also potentiates the effects of a variety of anesthetic and hypnotic drugs [6]. Proceeding from these data, we studied the effects of vitamin B_3 on the GABA-BD receptor complex functioning under conditions of chronic morphine intake which is known to affect the GABAergic inhibitory system.

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MATERIALS AND METHODS

Experiments were carried out on male rats (body weight 80-120 g) showing alcohol preference in the free choice test. Reactions to alcohol were tested for a week under conditions of free choice between 15% ethanol and water. The rats whose alcohol intake was no less than 15 ml/kg per day were selected for further experiments. They were divided into two groups: control and experimental. The experimental group received morphine solution in increasing (from 10 to 100 mg/liter) concentrations as the sole source of drinking water for 9 weeks, while the control group was given normal saline.

During the last week before decapitation nicotinamide in a dose of 250 mg/kg was administered twice a day.

Synaptic membranes were isolated according to [5] with the protease inhibitor PMSF (1 mM) added to all buffer solutions.

Benzodiazepine receptors were assayed by specific binding of ^{3}H-flunitrazepam (specific activity 86 Ci/mmol, Amersham) [10]. Specific binding of ^{14}C-NAD to synaptic membranes was measured in a medium (0.2 ml) containing 5 mM Tris-HCl (pH 7.4), 120 mM NaCl, 1 mM $MgCl_2$, 5 mM KCl, 1 mM $CaCl_2$,

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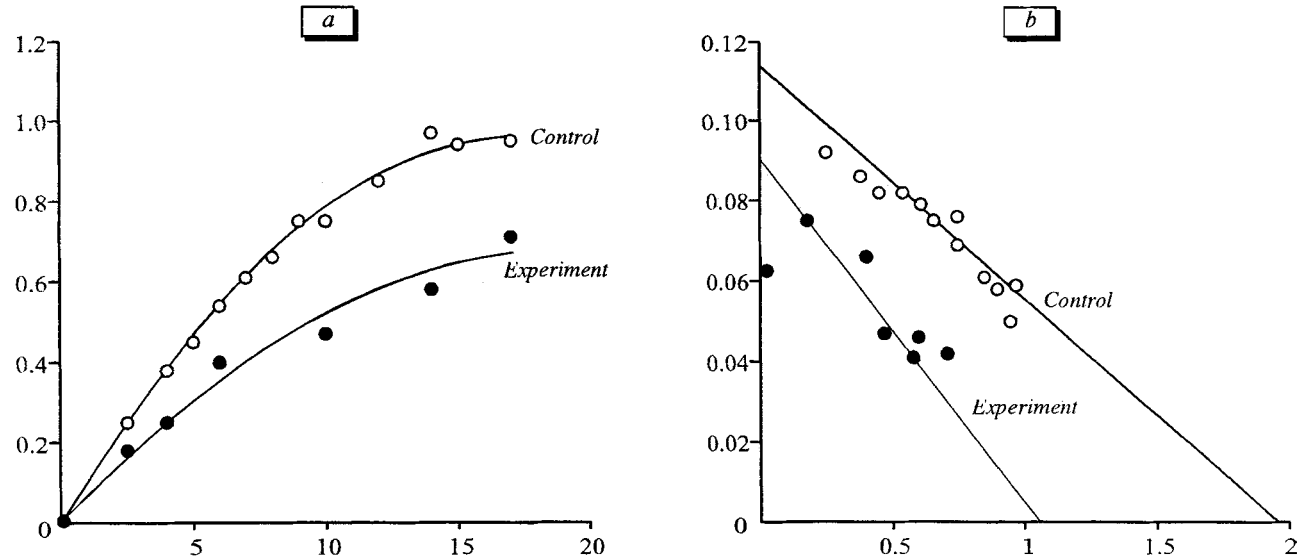

Fig. 1. Effects of morphine on specific binding of ^{3}H-muscimol to synaptic membranes (*a*) and the same data in Scatchard coordinates (*b*). *a*) Abscissa: free ligand, nM; ordinate: bound ligand, pmol/100 μg protein. *b*) Abscissa: bound ligand, pmol/100 μg protein; ordinate: B/F.

0.5 mM Na_2HPO_4, 10 mM glucose, and 20 nM ^{14}C-NAD (specific activity 285 mCi/mmol, Amersham). After 20-min incubation, binding assays were filtered through Whatman GF/C filters and washed three times with cold 5 mM Tris-HCl buffer. Nonspecific binding was measured in the presence of an excessive concentration of unlabeled NAD and was subtracted to compute specific binding.

Specific binding to GABA receptor was measured by the specific GABA agonist ^{3}H-muscimol (specific activity 70 Ci/mmol, Amersham) [7]. The membrane suspension (100 μg protein) was incubated for 40 min at 0°C in a 0.2 ml Tris-citrate buffer (50 mM, pH 7.4) containing 1 nM of the ligand.

Synaptosomal GABA uptake was measured with 4-amino-n-U-^{14}C-GABA (specific activity 232 mCi/mol, Amersham). Synaptosomal preparations (100 μg protein) were incubated at 37°C with various concentrations of ^{14}C-GABA in 0.2 ml medium containing 5 mM Tris-HCl (pH 7.4), 120 mM NaCl, 1 mM $MgCl_2$, 5 mM KCl, 1 mM $CaCl_2$, 0. 5 mM Na_2HPO_4, 10 mM glucose, 1 mM hydroxylamine, and 1 mM PMSF. After 3-min incubation, the samples were rapidly filtered through Whatman CG/C filters. The filters were washed with 5 mM Tris-HCl and counted for radioactivity. The GABA uptake was expressed in picomoles of ^{14}C-GABA incorporated for a 3-min period. The protein content was measured by the method of Lowry [8].

RESULTS

Chronic compulsory morphine intake decreased specific binding to GABA receptors. Using ^{3}H-muscimol as a pharmacologically active GABA agonist, we found that the dissociation constant (Kd) for its binding decreased from 16.5 nM in the control group to 11.4 nM (Fig. 1). This decrease was due to lowering of the mean binding capacity from 1.9 to 1.05 pmol/100 μg protein. Under the same conditions synaptosomal ^{14}C-GABA uptake significantly increased (Fig. 2).

Decreased specific binding of ^{3}H-muscimol and increased ^{14}C-GABA uptake suggest suppression of GABAergic transmission as a result of chronic morphine intake.

Taking into consideration the functional association between the GABA- and BD-recognition sites in the receptor complex, we studied the functional activity of BD receptors in the control and morphine-treated groups. Specific binding of ^{3}H-flunitrazepam in morphine-treated rats proved to be significantly lower than in the control animals (Table 1).

Conformational changes in the GABA-BD receptor complex associated with changes in its functional

TABLE 1. Specific Binding of ^{3}H-Muscimol and ^{3}H-Flunitrazepam to Synaptic Membranes (pmol/100 μg protein, *M±m*)

Ligand	Control	Morphine	Morphine+Nicotinamide
^{3}H-Muscimol	0.430±0.029	0.351±0.017	0.411±0.013
^{3}H-Flunitrazepam	0.151±0.011	0.120±0.009	0.126±0.021

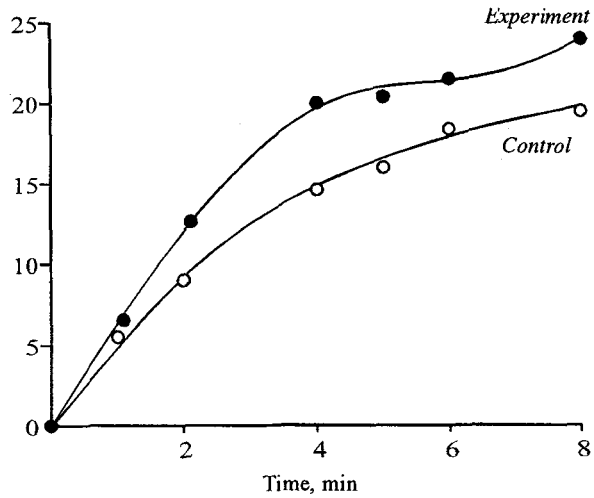

Fig. 2. Kinetics of synaptosomal ^{14}C-GABA uptake after chronic morphine intake. Ordinate: ^{14}C-GABA uptake, pmol/100 μg protein.

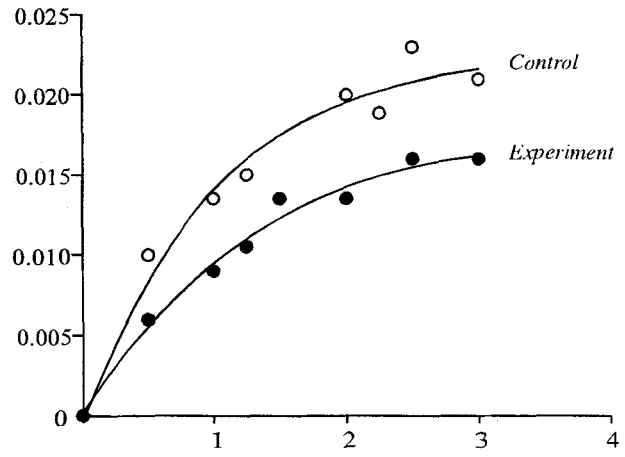

Fig. 3. Effects of morphine on specific binding of ^{14}C-NAD to synaptic membranes. Abscissa: free ligand, mM; ordinate: bound ligand, nmol/100 μg protein.

state are determined, among other factors, by its interaction with the NAD receptor system [2]. Therefore, we studied specific binding of ^{14}C-NAD to synaptic membranes under conditions of morphine dependence and revealed an approximately 20% decrease (Fig. 3).

Excessive doses of nicotinamide are known to increase the body content of NAD and GABA [4]. When administered during chronic morphine intake, nicotinamide abolished the effects of morphine on specific ^{3}H-muscimol binding, measured in the presence of 6 nM free ligand (Table 1).

The decrease in ^{3}H-flunitrazepam binding resulting from chronic morphine intake was not reversed by these doses of nicotinamide, which can be explained by its competition with ^{3}H-flunitrazepam for BD binding sites.

Our findings together with the data on the ability of nicotinamide to compete for BD binding sites on the GABA/BD receptor complex and to elevate the brain content of NAD and GABA [3] indicate that nicotinamide prevents dysfunction of the GABA/BD complex caused from chronic morphine intake.

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