δ-agonist DADLE with the nootropic drugs. Thus the results of this microionotophoretic study lead to the following conclusion. First, a high proportion of cerebral cortical neurons (about 70%) is sensitive to nootropic drugs, and this may lie at the basis of the mechanism of their action on the higher integrative functions of the brain. Second, among cerebral cortical neurons there is a small cell population on which antagonistic relations are exhibited between nootropic drugs and opioids.

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STUDY OF GABA-BENZODIAZEPINE RECEPTOR SYSTEMS OF THE HUMAN MYOMETRIUM

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Tocolytics of the β_2-adrenomimetic group and inhibitors of prostaglandin synthesis, used at the present time, have not solved the problem of treatment and prevention of the premature termination of pregnancy, and moreover, their adverse action on mother and fetus has been demonstrated [3, 4]. A search for new drugs with a more physiological mechanism of action, for the protection of pregnancy, and created on a basis of endogenous biologically active substances, is therefore necessary.

In the modern view, gamma-aminobutyric acid (GABA) is the leading neurotransmitter of inhibition in the CNS [1, 2] and at the periphery [6, 8, 9]. It has been suggested that GABA-positive drugs with an inhibitory type of action may be used as agents for the pathogenic treatment and prevention of threatened abortion, coexisting with GABA-deficient states. However, only sporadic studies of GABA-benzodiazepine (BD) receptor systems of the myometrium have been reported in the literature [5, 7, 11]. The existence of GABA-BD complexes in the human myometrium had not hitherto been studied. The aim of this investigation was to determine sites of specific binding of ^{14}C-GABA and ^{3}H-flunitrazepan in the plasma membranes (PM) of the human myometrium, and also to study the possibility of linking of these sites.

EXPERIMENTAL METHOD

Tissue from the histologically unchanged human myometrium, obtained in the course of hysterotomy for myoma, was used. The PM fraction was obtained by differential centrifugation and stepwise sucrose gradient followed by repeated washing [10]. The purity of the isolated PM fraction was estimated by enzyme analysis. To determine binding parameters of ^{14}C-GABA ("Amersham") and ^{3}H-flunitrazepam ("Amersham") with PM the labeled compounds were incubated, within the concentration range of 10-300 mM with 0.1 ml of a suspension of PM (1 mg/ml as protein) at 4°C for 2 h. At the end of incubation PM were precipitated on CF/F

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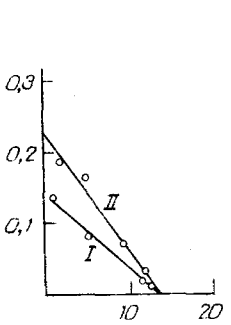

Fig. 1

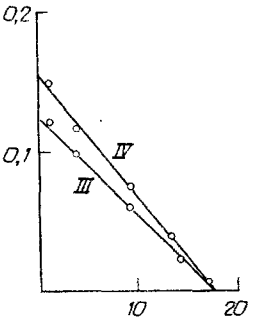

Fig. 2

Fig. 1. Specific binding of ^{14}C-GABA by PM of myometrium in absence (I) and presence (II) of flunitrazepam. Abscissa, quantity of bound ^{14}C-GABA (in fmoles/mg protein); ordinate, ratio of quantity of bound ^{14}C-GABA and concentration of free ^{14}C-GABA (in fmoles·mg protein^{-1}/nM).

Fig. 2. Specific binding of ^{3}H-flunitrazepam by myometrial PM in absence (III) and presence (IV) of GABA. Abscissa, quantity of bound ^{3}H-flunitrazepan (in fmoles/mg protein); ordinate, ratio of amount of bound ^{3}H-flunitrazepam to concentration of free ^{3}H-flunitrazepan (in fmoles·mg protein^{-1}/nM).

filters (Whatman). The filters were then placed in scintillation flasks and radiography measured. To isolated specific binding of ^{14}C-GABA and ^{3}H-flunitrazepam, PM were incubated with the labeled ligands in the presence of a 200-fold excess of GABA, bicuculline, and diazepam respectively. The results were analyzed by means of Scatchard plots.

Confidence intervals of the experimental data were calculated and the significance of differences between them estimated by Student's method at a level of significance of $p = 0.05$, by standard software for the EMG/666 computer.

EXPERIMENTAL RESULTS

The results of a study of binding of ^{14}C-GABA with PM of myometrial cells are shown on Scatchard plots in Fig. 1. Specific binding sites of ^{14}C-GABA are characterized by the following parameters: K_d (the equilibrium dissociation constant of the ligand–receptor complex) = 104 ± 12 mM; N (binding capacity) = 14 fmoles/mg protein. Competitive analysis of binding of ^{14}C-GABA with myometrial PM in the presence of unlabeled ligands of GABA receptors showed that GABA and bicuculline displace labeled GABA about equally from the complex with the receptor. The concentration of ligand at which inhibition of specific binding of ^{14}C-GABA by 50% is observed (IC_{50}) was 0.08 and 0.1 μM respectively for unlabeled GABA and bicuculline. The results are evidence that PM of the human myometrium contains peripheral bicuculline-sensitive $GABA_A$-receptors.

The study of the character of binding of the ligand of BD-receptors for ^{3}H-flunitrazepam with the human myometrium showed that PM of the myometrial cells contain specific binding sites of ^{3}H-flunitrazepam possessing high affinity (K_d = 22 ± 5 nM) for the ligand (Fig. 2) also. The highest concentration of binding sites of ^{3}H-flunitrazepam, calculated by Scatchard plot, was 17 fmoles/mg protein.

To determine relations between the specific binding sites of GABA and benzodiazepines thus revealed, specific binding of ^{14}C-GABA and ^{3}H-flunitrazepam with myometrial PM was studied when both were present together. Recording the double label (^{3}H and ^{14}C) revealed the presence of coupling between binding sites of GABA and of BD. For instance, during

incubation of ^{14}C-GABA with BM of myometrial cells in the presence of ^{3}H-flunitrazepam significant changes were observed in the binding parameters of the labeled ligands. Under these conditions K_d of the GABA-receptor complex was 60 ± 7 nM. The affinity of the benzodiazepine receptor sites also was increased (K_d = 11 ± 4 nM). The number of binding sites of the labeled ligands in the PM of the cells was virtually unchanged (Figs. 1 and 2). The changes we found indicate that specific binding sites of ^{14}C-GABA and ^{3}H-flunitrazepam are coupled, and evidently in the same way as the GABA-BD receptor-ionophore complexes, located in the CNS.

These results suggest that the GABA-ergic system is involved in the mechanism of peripheral regulation of uterine contractile function. The GABA-BD-receptor complexes identified in the myometrium can be used as molecular targets for potential drugs aimed at protecting the course of pregnanacy.

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NOOTROPIC ACTIVITY OF NICOTINAMIDE AND ITS STRUCTURAL ANALOGS

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KEY WORDS: nicotinamide, piracetam, nootropic activity, electron-structural analogy.

Nicotinamide is an endogenous ligand of benzodiazepine receptors and, under experimental conditions, possesses definite tranquilizing and stress-protective properties [1, 2, 4, 5, 7]. In the investigation described below nicotinamide and its structural analogs were studied as substances with nootropic activity.

EXPERIMENTAL METHOD

Experiments were carried out on noninbred male albino mice weighing 22-26 g. Nicotinamide in a dose of 250-1000 mg/kg and its electron-structural analogs nicomorpholine and acetylnicotinate in a dose of 10-20 mg/kg were injected intraperitoneally 30-40 min before the investigation. Acute hypobaric hypoxia was created in a chamber, and normobaric (3% oxygen and 97% nitrogen) and hemic (methemoglobin) hypoxia were created by the methods described in [3]. Antiamnesic activity was studied by the passive conditioned avoidance reflex (PCAR) method, using maximal electric shock as the factor inducing amnesia, by the method in [6]. The action of the test substances was compared with the effects of known nootropic agents: piracetam (250-1000 mg/kg), piritinol (100-200 mg/kg), and meclofenoxate (100-200 mg/kg).

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