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PHARMACOLOGY

GABA-Ergic Receptor System of the Myometrium: a Basis for a Clinical Study of GABA-Positive Substances as Gravidoprotectors

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γ-Aminobutyric acid (GABA) is known to take part in reproductive function regulation by controlling gonadotropin secretion by the hypothalamo-pituitary system [2,6,7]. GABA itself [5,9], its receptors [1,3,9], and also the benzodiazepine binding sites [4,8] have been identified in rat and rabbit ovaries and uterus.

The aim of this study was to investigate the function of the uterine GABA- and benzodiazepine (BD) receptors and to determine the influence of GABA-ergic substances on uterine contractility.

MATERIALS AND METHODS

Three series of experiments were performed. In the first series (35 experiments) the radioligand method was used to study the affinity of GABA and BD receptors of the nonpregnant uterus. We used a histologically normal myometrium obtained after uterine myoma resection. ^{14}C-GABA and ^{3}H-flunitrazepam (BD receptor ligand) were used for analysis. The results were processed by the Scatchard method. In the experiments of the second series (69 rats) radioligands were used to study the affinity of nonpregnant rats in the diestrus phase, of rats in the first half of pregnancy (10 days), and of rats in the second half of pregnancy (10-20 days). In the third series a pharmacological analysis of the GABA-BD receptor systems was performed. The experiments were carried out on the basis of the spontaneous contractility model on isolated fragments of uterine horns. We used 12 ovariectomized rabbits, 8 nonpregnant rabbits, and 12 rats (40 experiments). The $GABA_A$ agonists muscimol and ethylenediamine, the $GABA_A$ an-

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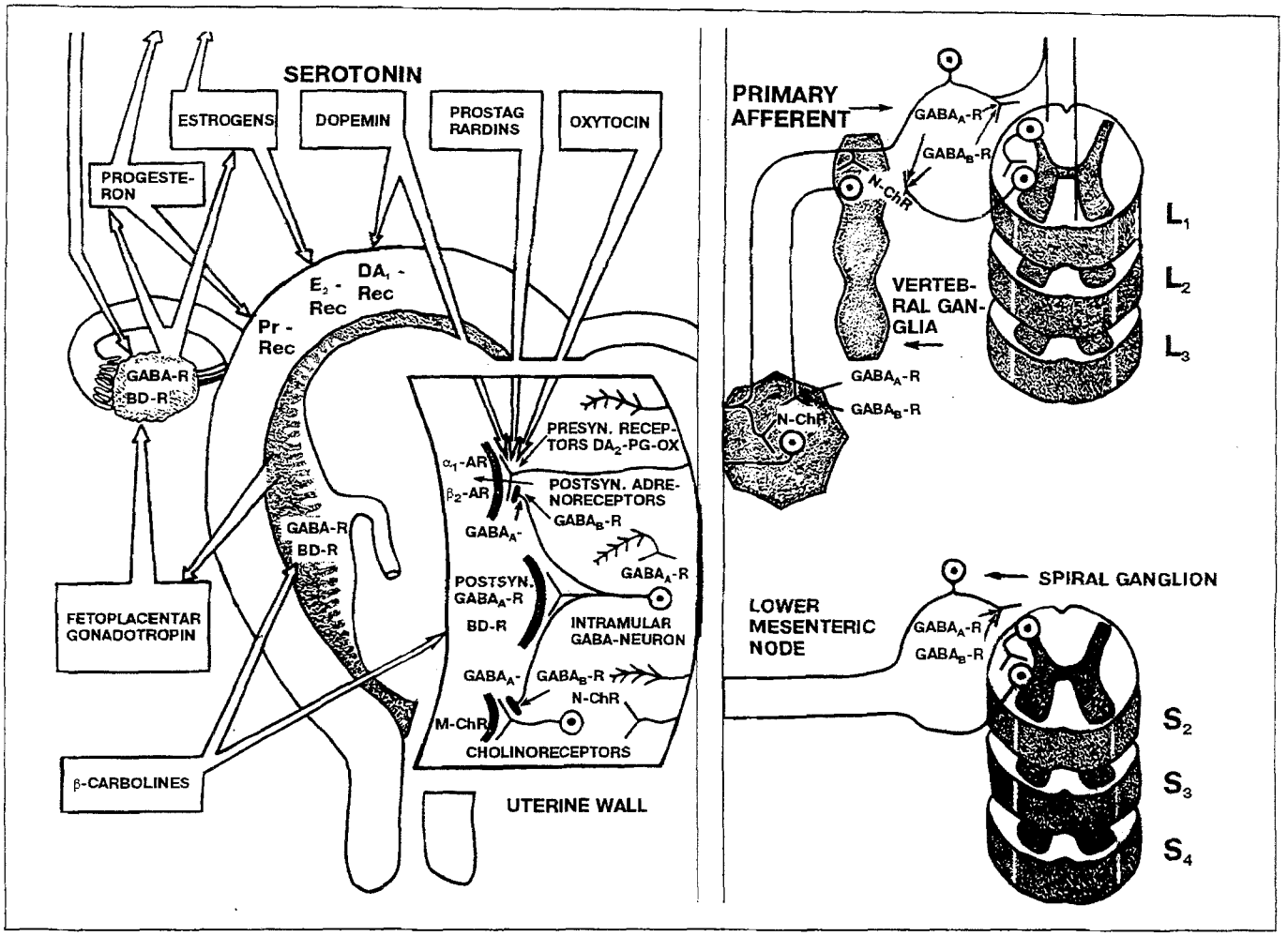

Fig. 1. Scheme of the GABA-ergic system of the myometrium. An intramural efferent GABA neuron is presented in the centre on a fragment of the uterine wall. Some endings of the axon form an axosomatic GABA-ergic synapse, where the postsynaptic $GABA_A$ inhibitory receptors are localized. The other terminals are integrated with adrenergic and cholinergic endings and form axoaxonal synapses. Presynaptic inhibitory $GABA_B$ and stimulatory $GABA_A$ heteroreceptors are localized here. Peripheral reflex closure can occur at different levels of regulation (intramural neurons, lower mesenteric node, vertebral ganglia, lateral horns of spinal column, and other upper levels). GABA can either inhibit the nervous impulse via the $GABA_B$ receptors or facilitate transmission via the $GABA_A$ stimulatory receptors.

tagonist bicuculline, the chlorine channel receptor complex blocker picrotoxin, the $GABA_B$ ligand baclofen, the BD receptor agonist seduxen (sibason), the inhibitor of GABA enzyme biosynthesis thiosemicarbazide, and the GABA metabolism inhibitor aminooxybutyric acid (AOBA) were used for analysis.

RESULTS

The experiments of the first series showed that ^{14}C-GABA binds with the plasma membrane (PM) of the myometrial cells with a K_D of 104±12 nM and with a binding capacity of 14 fM per mg protein. Unlabeled GABA and bicuculline forced the labeled GABA out of the bond with the PM (IC_{50} 0.08 and 0.1 μM, respectively). ^{3}H-flunitrazepam binds with the PM with K_D=22±5 nM and with a binding site concentration of 17 fM per mg protein. For the combined use of ^{14}C-GABA and ^{3}H-flunitrazepam with a double-label recording an increase of GABA and BD receptor-site affinity was recorded (K_D 60±7 nM and 11±4 nM, respectively). The total number of binding sites did not change significantly. These data show that myometrial cell membranes contain conjugated bicuculline-sensitive GABA-BD receptor complexes.

The radioligand analysis of GABA-BD receptor complexes in rats during various sexual states and in different pregnancy terms showed that the PM of nonpregnant rats bind ^{14}C-GABA with K_D=83±4 nM; K_D=110±9 nM during the first half of pregnancy and K_D=64±6 nM during the last pregnancy term. These data show that GABA-receptor affinity is lower at the beginning of pregnancy and higher at its end than in nonpregnant

animals. The analysis of ^{3}H-flunitrazepam specific binding showed that the affinity of BD receptors does not change significantly during different sexual states and pregnancy terms. K_D of specific ^{3}H-flunitrazepam binding was 18±4 nM in nonpregnant rats, 22±3 nM in rats during the first half of pregnancy and 23±4 nM during the second half. However, the K_D of specific ^{3}H-flunitrazepam binding was lowered 2-fold during all the sexual states and pregnancy terms in the presence of labeled GABA. ^{14}C-GABA raised the conjugation level and affinity of GABA-BD receptors to GABA and ^{3}H-flunitrazepam. The highest sensitivity of GABA-BD receptors to the ligands was recorded in rats during the second half of pregnancy.

The pharmacological analysis of the myometrium GABA-BD receptor system demonstrated that muscimol (1.7×10^{-4} M) and ethylenediamine hydrochloride (6.6×10^{-3} M) inhibit the contractility of isolated rat and rabbit myometrium. Bicuculline (5.4×10^{-6}-5.4×10^{-5} M) and picrotoxin (7.1×10^{-6}-1.4×10^{-4} M) stimulate uterine smooth muscle contractility. $GABA_A$-receptor antagonists in equally effective concentrations reverse the uterostimulatory effects of muscimol and ethylenediamine. Both muscimol and ethylenediamine suppress the same effects of bicuculline and picrotoxin. The two-sided competitive antagonism between $GABA_A$ receptor agonists and antagonists acting upon isolated uterine muscle contractility shows that the uterotropic mechanism of muscimol, ethylenediamine, bicuculline, and picrotoxin is realized via the peripheral $GABA_A$-inhibitory myometrium receptors.

The $GABA_B$-positive substance baclofen (2×10^{-3} M) suppresses the contractility of the isolated animal uterus and counteracts the uterostimulatory effect of bicuculline. Bicuculline, however, in an equally effective concentration does not reverse the effects of baclofen. The one-sided antagonism shows that baclofen and bicuculline have different action sites. These data prove that in the rat and rabbit myometrium there are inhibitory $GABA_B$ heteroreceptors which take part in the regulation of uterine contractility .

The BD agonist seduxen in a concentration of 7×10^{-5} M inhibits uterine contractility. Bicuculline and picrotoxin only partially reverse the effect of seduxen, suggesting that $GABA_A$ antagonists and benzodiazepine act upon different subunits of the GABA-BD receptor complex of the myometrium.

Experiments to identify endogenous GABA in the myometrium showed that thiosemicarbazide (4.4×10^{-3} M) causes a slowly increasing stimulation of uterine contractility. AOBA in a concentration of 7.9×10^{-4} M inhibits it. Bicuculline and AOBA show a two-sided competitive antagonism. These results prove that a system of biosynthesis and metabolism of endogenous GABA is present in the rabbit and rat myometrium.

Thus, as a result of radioligand and pharmacological analysis, conjugated GABA-BD receptor complexes, $GABA_B$ heteroreceptors, and endogenous GABA were identified in the human, rabbit, and rat myometrium. They are parts of one global GABA-ergic system, which is presented schematically in Fig.1. The affinity level of the GABA-BD receptor complex depends on the sexual maturation period and pregnancy term; $GABA_A$, $GABA_B$, and BD agonists and antagonists have a direct influence on isolated uterine muscle contractility. This proves that the ligands of the GABA-BD receptor complex of the myometrium take part in regulating uterine contractility during pregnancy. The GABA-positive substances muscimol, ethylenediamine, baclofen, AOBA, and seduxen have a suppressive effect which is mediated via the peripheral inhibitory $GABA_A$, $GABA_B$, and BD myometrial receptors. These data provide grounds for a clinical study of GABA-positive substances as gravidoprotectors in cases of at-risk pregnancies.

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