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IDENTIFICATION OF GABA RECEPTOR BINDING SITES IN RAT AND RABBIT UTERUS

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The present study provides evidence for the presence of gamma-aminobutyric acid (GABA) and L-glutamate decarboxylase in the uteri of the rat and the rabbit. Furthermore, it has been demonstrated for the first time that the uterine tissue contains high-affinity $GABA_A$ receptor binding sites in a high density. The present findings indicate that GABA may have a role in the uterine functions.

Gamma-aminobutyric acid[1] (GABA) is the major inhibitory neurotransmitter ir the vertebrate central nervous system (1) and several lines of evidence suggest that GABA may also be a neurotransmitter in the enteric nervous system of mammals (2,3). The occurrence of GABA and L-glutamate decarboxylase (EC 4.1.1.15), the enzyme primarily responsible for GABA formation in the brain, in peripheral tissues other than the gut has been reported (4-10). Particularly high GABA levels have been found in the pancreatic islets (7) and the Fallopian tube (9,10) of the rat. Furthermore, specific, GABA receptor mediated contractile responses of various smooth muscle tissues such as the gut (11), the vas deferens (12), the urinary bladder (13), certain blood vessels (14) and the Fallopian tube (15) have been observed. Considering that the oviduct has been found to contain high GABA concentrations synthesized in situ, at least in part, by glutamate decarboxylase (16,17) and specific GABA receptors mediating contractile

[1] Abbreviation used: GABA, gamma-aminobutyric acid

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response (15), it seemed resonable to examine whether a GABAergic system resembling that in the Fallopian tube is present in the uterus or not.

Earlier data have indicated that the amount of GABA and the activity of glutamate decarboxylase in the uterus are very low or not detectable (5, 9). Taking into account, however, that in certain peripheral tissues, such as the gut, even the poor GABA supply may have a physiological importance (3, 11), the present study attempted to reexamine the GABA levels and the glutamate decarboxylase activity and to identify specific GABA receptor binding sites in the uterine tissue.

METHODS AND MATERIALS

Animals: Diestrous, female Wistar rats (200-240 g) and virgin female New Zealand rabbits (3-4 kg) were used. The animals were killed by a blow on the head and the whole uterine horns were rapidly dissected, cleaned of connective tissues, and placed on dry-ice. In a separate experiment the rapid freezing proved to be sufficient for prevention of the postmortem increase of uterine GABA levels (Erdő, unpublished data).

Measurement of GABA levels and glutamate decarboxylase activity: Tissue extracts were prepared from uteri as described earlier (18) and the GABA concentration was determined by the radio-receptor assay method of Enna and Snyder (19). Glutamate decarboxylase activity was estimated in crude homogenates of the uterine tissue by the measurement of [^{14}C] GABA formation from universally labelled [^{14}C] L-glutamic acid. The enzyme preparation and the assay procedure were performed as described previously (16, 17). Estimates of protein content were made according to the method of Lowry et al (20).

Measurement of [^{3}H] GABA binding: Extensively washed, frozen-thawed membranes were prepared from the uterine tissue as described earlier in detail (18). The receptor binding assay was carried out according to the centrifugation method of Enna and Snyder (21). In brief, membranes suspended in Tris-citrate buffer (50 mM, pH 7.1) were incubated for 10 min at 4^{o}C in the presence of 10 nM [^{3}H] GABA (displacement studies) or of increasing concentrations (0.1 to 200 nM) of the same radio-ligand (saturation experiments). Specific binding was calculated by subtracting the nonspecific (in the presence of 0.5 mM unlabelled GABA) from the total binding (in the absence of unlabelled GABA).

Statistics and calculations: The data for GABA levels and enzyme activity were calculated as mean values of 6-8 independent determinations, each from a different specimen, ±S.E.M. The values obtained in the receptor binding experiments represent the mean of three independent determinations in

duplicates or triplicates. The binding parameters, i.e. the equilibrium dissociation constant (K_d) and the maximum binding capacity (B_{max}) were estimated by the appropiate intercepts of Scatchard plots, after Scatchard analysis of the data of saturation experiments. Concentrations of the model compounds used producing 50 percent displacement of the specific [^{3}H] GABA binding (IC_{50} values) were determined by log-probit analysis.

Chemicals used: [^{3}H] GABA (41.5 Ci/mmol, MTA Izotóp Intézet), [^{14}C] L-glutamic acid (200 mCi/mmol, New England Nuclear Co.), muscimol (Fluka), (+)-bicuculline (Sigma), (-)-bicuculline methiodide (Sigma), L-diaminobutyric acid (Fluka), (±)baclofen (Ciba-Geigy), isoguvacine HCl (kindly provided by Dr Povl Krogsgaard-Larsen). Other chemicals were of analytical grade and obtained from commercial origin.

RESULTS

Detectable amounts of GABA were found in the uterine tissue of the two species examined. The GABA levels in the rat and the rabbit uteri were 29.8 ± 7.6 and 16.3 ± 5.3 nmol/g tissue, respectively. Values are mean ± S.E.M. (n=8).

Glutamate decarboxylase activity could also be measured in both tissues. The estimated values for the enzyme activity in the rat and the rabbit uteri were 0.68 ± 0.21 and 0.98 ± 0.19 nmol/mg protein/h, respectively (n=6, mean ± S.E.M.).

In receptor binding experiments, the equilibrium of the specific binding was achieved within 10 min of incubation at 4°C (data not shown). As shown in Figure 1, a single population

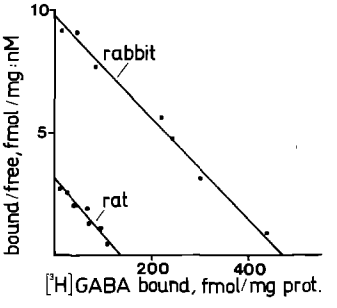

FIGURE 1. Saturation of [^{3}H] GABA binding to membranes of the rat and the rabbit uterus. Membranes were incubated for 10 min at 4°C, in the presence of increasing [^{3}H] GABA concentrations (0.1 to 200 nM). Each point in the Scatchard plots represents the mean of three independent determinations in duplicates. The K_d values for the rat and the rabbit uterus were 42 and 48 nM, respectively. The B_{max} values for the rat and the rabbit tissues were 131 and 467 fmol/mg protein, respectively.

of high-affinity binding sites was identified in the rat and the rabbit uterus. The K_d values for both tissues were about 40-50 nM, but the density of binding sites was over three times higher in the rabbit than in the rat uterus (see Legend to Figure 1).

In displacement experiments, the sensitivity of the binding sites to various model compounds was consistent in the rat and the rabbit tissue (Table 1). The binding could be displaced by bicuculline in a stereoselective fashion, by $GABA_A$ receptor agonists, i.e. muscimol and isoguvacine, however, it can not be influenced even by high concentrations

TABLE 1. Displacement of specifically bound [^{3}H]GABA from membranes of the rat and the rabbit uterus

Compound	IC_{50}, μM	
	Rat	Rabbit
Muscimol	0.02	0.03
GABA	0.09	0.07
Izoguvacine	0.19	0.18
(+)-Bicuculline	31	37
(-)-Bicuculline methiodide	>300	>300
(±)-Baclofen	>300	>300
L-Diaminobutyric acid	>300	>300

Concentrations of the compounds producing 50 percent displacement of the specific binding (IC_{50} values) were determined by log-probit analysis of the data of inhibition curves. Each compound was tested in at least 4 different concentrations. Values are the mean of 3 independent determinations in triplicates.

of baclofen (an agonist of $GABA_B$ receptors) or L-diamino-butyric acid (a selective blocker of neuronal GABA uptake).

DISCUSSION

The low levels of GABA and glutamate decarboxylase in the uterus, demonstrated in the present experiments are consistent with previous data (5, 9). The glutamate decarboxylase activity in the uterus presented here may well be responsible for the formation of the GABA content of this tissue.

Although the formation rate and the tissue level of GABA are as low as about 1 percent of the respective cerebral values (17), even the low GABA content may have a functional significance if specific, high-affinity GABA recognition sites are present in this organ.

Previous studies of this laboratory have revealed the presence of high-affinity $GABA_A$ receptor binding sites in the oviduct and ovary of the rat and the human (18, 22, 23, 24). Therefore the conditions of the receptor binding experiments presented here were choosen so that $GABA_A$ sites be detected. In the Tris-citrate buffer used only $GABA_A$ binding sites can be specifically labelled with [^{3}H] GABA, and $GABA_B$ recognition sites requiring the presence of bivalent cations (25) as well as GABA uptake sites requiring the presence of sodium ions (26) can not be detected.

The single, high-affinity binding sites in the rat and the rabbit uteri showed K_d values of 40-50 nM, which are consistent with the K_d values for the GABA binding sites in the rat and the human ovary and Fallopian tubes (18, 22, 23, 24). The maximum density of the GABA recognition sites in the rabbit uterus exceeded that in the respective rat tissue, and

is in the same order of magnitude as the number of GABA receptors in the human ovary and oviduct (18, 24).

The displacement experiments have revealed that the uterine GABA binding sites show the properties of a $GABA_A$ site. They are sensitive to bicuculline in a stereoselective fashion for the (+)enantiomer, and to the $GABA_A$ receptor agonists muscimol and izoguvacin. The lack of activity of baclofen and L-diaminobutyric acid confirms the above statement.

In summary, the occurrence of GABA and its biosynthetic enzyme glutamate decarboxylase in the rat and the rabbit uteri has been confirmed in the present study. Furthermore, it has been demonstrated for the first time, that specific, high--affinity GABA receptor sites are present in the uterine tissue of both species. These findings indicate that the GABA-ergic system in the uterus may be of functional importance. In order to elucidate the putative role of GABA, experiments attempting to characterize the effects of GABAergic model compounds on the contractility of isolated uterine strips are in progress in this laboratory.

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