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SPECIFIC BINDING OF ^{3}H-GABA BY SYNAPTIC MEMBRANES OF THE RAT HYPOTHALAMUS AND HIPPOCAMPUS AFTER ADRENALECTOMY AND HYDROCORTISONE AND ACTH ADMINISTRATION

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The concept of heterogeneity of GABA receptors has now been successfully elaborated [2], their structure elucidated [4], and many aspects of the function of the oligomeric GABA/benzodiazepine/Cl^--channel complex have been studied both under normal conditions [5] and in pathological states of the brain [3], and the role of GABA receptors in the mechanism of action of drugs has been investigated [8]. Meanwhile, the question of the effect of hormones and, in particular, those of the hypothalamo-hypophyseo-adrenal system, on the state of GABA receptors remains largely unexplained, although it is well known that any change in the corticosteroid level in the body is accompanied to a greater or lesser degree by the changes in the functional state of brain structures [1], which are directly linked with disturbances of ionic permeability of nerve cell membranes.

The aim of this investigation was to study the effect of adrenalectomy, and also of single or repeated injections of hydrocortisone and ACTH on specific binding of ^{3}H-GABA by synaptic membranes of the rat hypothalamus and hippocampus.

EXPERIMENTAL METHOD

Experiments were carried out on male Wistar rats weighing 150-200 g. The adrenals were removed under ether anesthesia 8 days before sacrifice of the animals. Hydrocortisone ("Gedeon Richter," Hungary) in a dose of 5 mg/kg, and ACTH and ACTH-zinc-phosphate (Kaunas Endocrine Preparations Factory), in a dose of 2.5 U/100 g, was injected intramuscularly in a single dose or daily for 7 days. The rats were decapitated 4 h after the single injection and 24 h after the last of the series of injections of the preparations. Rats undergoing a mock operation, and receiving injections of the corresponding volume of physiological saline, served as the control.

The synaptic membrane fraction was obtained from the coarse mitochondrial fraction (18,000*g*, 20 min). The residue of the fraction was suspended in 20 volumes of bidistilled water, frozen at –20°C for 18-20 h, thawed, and centrifuged (45,000*g*,

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TABLE 1. Specific Binding of ^{3}H-GABA (in picomoles ^{3}H-GABA/g original tissue) by Synaptic Membranes of Rat Hypothalamus and Hippocampus after Adrenalectomy or Injection of Hydrocortisone and ACTH ($M \pm m$, $n = 5.6$)

Experimental conditions	Hypothalamus	Hippocampus
Physiological saline, 4 h	926,3±155,4	221,2±22,8
Hydrocortisone, 4 h	1910,0±350,5*	214,2±25,8
ACTH, 4 h	1849,9±252,3*	551,9±87,5*
Physiological saline, 7 days	463,5±61,8	244,9±18,8
Hydrocortisone, 7 days	543,6±172,4	286,3±36,5
ACTH, 7 days	105,9±35,5*	478,0±144,6*
Mock adrenalectomy	520,4±154,5	269,4±94,0
Adrenalectomy	426,7±65,5	306,1±63,6
Adrenalectomy + hydrocortisone, 4 h	1382,0±62,2**	182,7±44,7

Legend. *$p < 0.05$ Compared with corresponding control, **$p < 0.05$ compared with adrenalectomy.

20 min, 4°C). The synaptic membranes thus obtained were washed 3 times with 50 mM Tris-citrate buffer, pH 7.4, followed by sedimentation at 45,000*g*, and allowed to stand at —20°C. Immediately before the experiment the membrane fraction was resuspended in fresh Tris-citrate buffer. To determine Na-independent binding of ^{3}H-GABA, 30-60 μg (as protein) of synaptic membranes was incubated for 10 min at 0°C with Tris-citrate buffer, containing 5 nM ^{3}H-GABA ("Izotop," specific radioactivity 869 GBq/mmole). The volume of the sample was 1 ml. Specific binding of ^{3}H-GABA was determined as the difference between the total (in the absence) and nonspecific (in the presence of 10^{-3} M unlabeled GABA) binding. The bound ^{3}H-GABA was separated from the free ^{3}H-GABA by rapid filtration of the samples through nitrocellulose filters with pore diameter of 0.3-0.4 μm ("Synpor," Czechoslovakia). Synaptic membranes on the filters were washed with cold buffer, and after drying the filters were placed in flasks for subsequent counting of radioactivity on a liquid scintillation counter. The results were subjected to statistical analysis by parametric and nonparametric tests.

EXPERIMENTAL RESULTS

Binding of ^{3}H-GABA by synaptic membranes in the control was greater in the hypothalamus than in the hippocampus (Table 1). A single injection of hydrocortisone caused a sharp increase in binding of ^{3}H-GABA by hypothalamic membranes, and in response to injection of ACTH, an increase in binding was observed in both parts of the brain tested. Repeated injections of hydorcortisone did not change the binding of ^{3}H-GABA by synaptic membranes of the hypothalamus or hippocampus. Repeated injections of ACTH led to a marked lowering of the level of binding of the mediator by hypothalamic membranes and raised the level of its binding by hippocampal membranes. Adrenalectomy did not affect binding of ^{3}H-GABA by membranes in the parts of the brain studied, but a single injection of hydrocortisone into adrenalectomized rats increased binding of ^{3}H-GABA by synaptic membranes of the hypothalamus only.

Consequently, disturbance of the corticosteroid level in the body is accompanied by changes in GABA binding with synaptic membrane receptors in the hypothalamus and hippocampus. The significant increase in binding of ^{3}H-GABA in the hypothalamus after a single injection of hydrocortisone into intact and adrenalectomized rats, i.e., under conditions of a short-term rise of the exogenous hydrocortisone level in the body and during the time of its maximal accumulation in the brain [6], will be noted. The action of glucocorticoids on the brain is considered to be mediated not only through mechanisms of steroid reception in the cytosol, but also through membrane receptors, whose location is assumed to be in the oligomeric GABA/benzodiazepine/Cl^--channel receptor complex [10].

Meanwhile, GABA reception by hippocampal synaptic membranes was unchanged after injection of hydrocortisone. It was shown previously that a single injection of dexamethasone does not change binding of ^{3}H-GABA in the hippocampus and cerebellum, reduces it in the thalamus, and increases it in the corpus striatum [9], whereas injection of corticosterone into intact or hypophysectomized rats does not affect GABA reception in the midbrain [7].

The absence of changes in ^{3}H-GABA binding by hypothalamic membranes in response to repeated injections, and hippocampal membranes in response to single and repeated injections of hydrocortisone and the clearly defined effect of ACTH on binding of the mediator in both parts of the brain (in the same direction after a single injection, in opposite directions after repeated injections), may perhaps be evidence of an independent and direct effect of ACTH on GABA reception. This hypothesis is confirmed by data showing that adrenalectomy increases, whereas hypophysectomy decreases ^{3}H-GABA binding by membranes in the midbrain, and that injection of ACTH into intact or hypophysectomized rats increases GABA reception in this part of the brain due to an increase in the number of low-affinity binding sites [7]. Thus, both glucocorticoids and ACTH are involved in the regulation of GABA binding with receptors in the limbic structures of the brain, and this may be one mechanism aimed at monitoring the functional state of these structures when the balance between these hormones in the body is disturbed.

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