CHANGES IN LUTEINIZING HORMONE RELEASING HORMONE LEVELS IN SYNAPTOSOMAL FRACTION OF THE MALE RAT MEDIOBASAL HYPOTHALAMUS INDUCED BY MONOAMINES

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The study of mechanisms of interaction of neurotransmitters and gonadotropin (luteinizing hormone) releasing hormone (LHRH) is an important problem in the neuroendocrine control of pituitary gonadotropic function. According to data in the literature, most LHRH is found in nerve terminals of neurons whose bodies lie in the preoptic region, the anterior hypothalamus, and the arcuate nuclei [8, 9, 12]. It has been shown that LHRH also is present in synaptosomes in homogenates of rat hypothalamus [3, 10]. In this connection synaptosomal preparations of the hypothalamus may provide a convenient model with which to study synthesis and secretion of LHRH, with the aim of discovering the mechanisms of interaction of the nervous and endocrine systems.

This paper gives data on the effect of monoamines on the LHRH level in synaptosome-rich fraction obtained from the mediobasal hypothalamus (NBH) of intact and castrated male rats. These data are compared with changes in blood levels of LHRH and luteinizing hormone (LH).

EXPERIMENTAL METHOD

Experiments were carried out on male rats weighing 200-250 g. Some of the animals were castrated two weeks before the experiment. Testosterone propionate (TP) in a dose of 250 μg daily was injected twice into the group of castrated rats two days before sacrifice as replacement therapy. Under urethane-chloralose anesthesia the control animals received an injection of physiological saline, and the experimental animals an injection of one of the following amines: noradrenalin bitarate (NA), dopamine (DA) and serotonin creatine-sulfate (5-HT) in a dose of 20 μg, dissolved in 4 μl of physiological saline, into the 3rd ventricle by means of a stereotaxic apparatus. The animals were decapitated 15 min after the injection, and the brain was removed and MBH isolated. A weighed sample of MBH from 5 animals (25-28 mg) was treated by Hajos' method [6] and the synaptosome-rich fraction was isolated. The presence of synaptosomes in the isolated fraction was verified electron-microscopically (Fig. 1). The LHRH concentration in the synaptosomes was determined by radioimmunoassay [2]. Protein was determined by Bradford's method [5]. The blood LH level also was determined radioimmunologically [1].

EXPERIMENTAL RESULTS

The results showed (Table 1) that the OHRH content in the synaptosomes of MBH was increased ($P < 0.01$) 2 weeks after castration. The blood LH level, meanwhile, was raised by 2.5 times ($P < 0.01$). Preliminary injection of TP into castrated animals sharply reduced the LHRH concentration in the synaptosomes compared with that in castrated control animals. The same rule also was observed with respect to the blood LH level in the animals of these groups. It can be postulated on the basis of these results that against the background of castration, i.e., removal of the influence of sex steroids and of the negative feedback mechanism, the supply of LHRH to the nerve terminals and, from them, to the portal system is increased, with the result that release of LH from the pituitary is increased.

Injection of NA into intact male rats caused a decrease in the LHRH concentration in the synaptosomal fraction of MBH by one-third compared with the intact control, and at the

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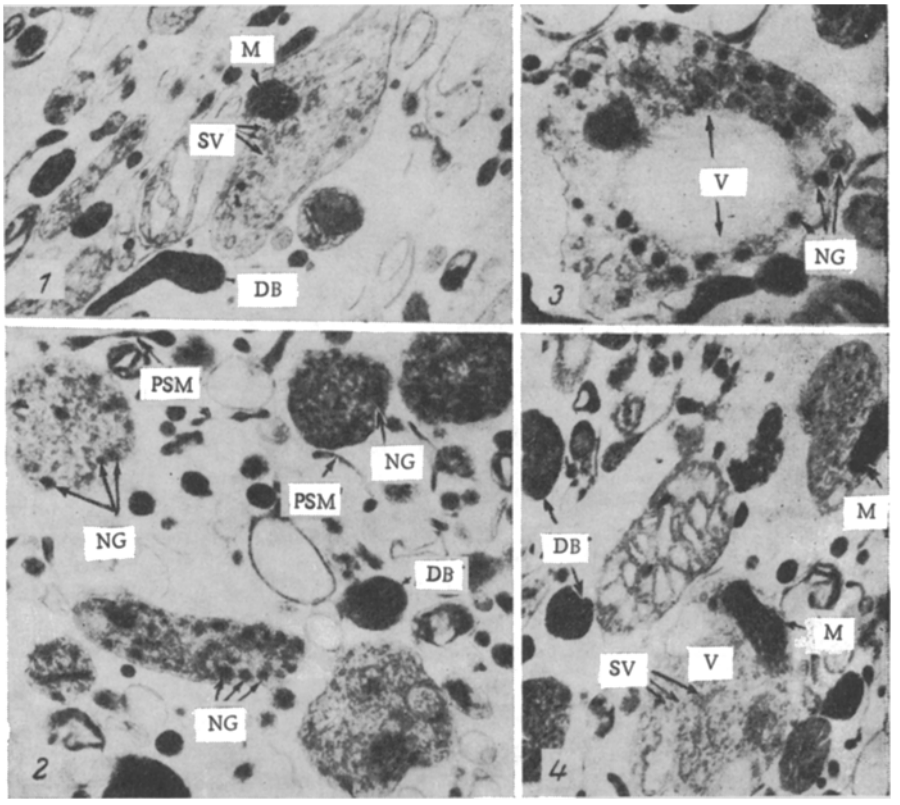

Fig. 1. Synaptosomal fraction of rat hypothalamus: 1) intact nerve ending containing synaptic vesicles (SV) and mitochondria (M), 23,000 ×; 2) nerve endings containing large neurosecretory granules (NG). Remnants of degenerating nerve endings in the form of dense bodies (DB) and freely lying postsynaptic membranes (PS), 22,000 ×; 3) ultrastructure of large nerve ending with neurosecretory granules (NG) and vacuole (V), formed as a result of preparative isolation of synaptosomes, 22,000 ×; 4) presynaptic nerve terminals and remnants of degenerating nerve endings in synaptosomal fraction, 7,000 ×.

same time it caused an increase in the blood LH level. These changes were evidently connected with increased LHRH secretion. The stimulating action of NA on LHRH release from the synaptosomal fraction of the rat hypothalamus in experiments *in vitro* was observed by other workers also [4].

Injection of NA into the castrated animals almost doubled the LHRH concentration in the synaptosomes compared with its value in intact rats ($P < 0.01$). Similar changes took place in the blood LH level ($P < 0.01$).

It can accordingly be tentatively suggested that NA potentiates LHRH synthesis and transport into the region of axon terminals, and from thence into the blood, as a result of which large quantities of LH are released from the pituitary. The stimulating action of NA is increased dramatically in the absence of any inhibitory action of sex steroids.

If DA was injected into intact animals the LHRH content in the synaptosomes remained at the same level as in the intact control. Meanwhile the LHRH level, in the castrated animals fell by half after injection of DA compared with that in castrated males of the control group ($P < 0.02$). At the same time no significant changes were found in the LHRH level in intact and castrated animals receiving injections of DA.

No difference likewise was found in the blood LH level of intact animals of the control group and intact rats receiving DA. Kamberti et al. [7], however, showed that injection of DA into the 3rd ventricle of intact male rats raises the blood LH concentration.

Injection of DA into castrated animals lowered the LH level by 1.7 times compared with that in the castrated control rats.

TABLE 1. LHRH Content in Synaptosomal Fractions of MBH and Blood LH Level in Intact and Castrated Male Rats after Injection of Monoamines into 3rd Ventricle

Experimental conditions	Group of experimental animals	LHRH level in synaptosomes of MBH, pg/μg protein	Blood LH level, ng/ml
Control	Intact	32,86±3,99 (n=16)	46,8±3,6 (n=29)
	Castrated	54,1±6,19 (n=12)	121,9±7,9 (n=22)
	Castrated + testosterone	26,04±5,38 (n=5)	63,9±5,15 (n=3)
NA	Intact	21,17±2,31 (n=7)	68,1±5,2 (n=4)
	Castrated	41,87±6,1 (n=5)	147,1±17,3 (n=8)
	Castrated + testosterone	25,3±1,6 (n=5)	56,0±9,2 (n=7)
DA	Intact	31,3±5,07 (n=8)	51,0±5,4 (n=12)
	Castrated	24,72±5,14 (n=8)	75,1±7,7 (n=8)
	Castrated + testosterone	29,1±2,16 (n=6)	48,9±4,8 (n=5)
5-HT	Intact	20,67±2,77 (n=6)	40,8±6,4 (n=8)
	Castrated	11,53±0,95 (n=5)	68,3±8,4 (n=9)
	Castrated + testosterone	28,8±4,04 (n=6)	54,0±6,9 (n=4)

Legend. n) Number of investigations.

Although no change could be found in the LHRH level in the synaptosomal fraction of MBH in response to injection of DA, a significant increase in the LH concentration was observed in the castrated animals under these circumstances compared with that in intact rats ($P < 0.05$). It can be tentatively suggested that in the absence of sex steroids DA can be liberated directly into the median eminence and transported through the primary plexus of the pituitary portal system into the pituitary gland, where it stimulates LH release.

Comparison of the action of 5-HT in intact and castrated animals reveals a fall in the LHRH concentration in the synaptosomal fractions of MBH in rats of both groups, which was particularly marked in the castrated rats ($P < 0.02$). Injection of 5-HT into the castrated animals evidently inhibited the secretion of LHRH to some degree, but this was accompanied by a small rise in the blood LH level.

A temporary stimulating action of 5-HT on LH secretion, when injected intraventricularly into intact female rats was demonstrated by Schneider et al. [11], whereas Kamberi et al. [7] found that 5-HT has an inhibitory action.

The results of the present investigation suggest that 5-HT has an inhibitory action on LHRH synthesis and secretion in the synaptosomal fraction of MBH.

Determination of the blood LHRH concentration in rats of all groups revealed no significant differences, for it was at the intact control level under all experimental conditions (31.1 pg/ml). The absence of any change in the blood LHRH concentration is most probably due to the more rapid utilization of this hormone. More objective results for changes in the LHRH concentration might be observed in blood draining from the pituitary stalk.

It can be concluded from these results that each of the monoamines studied performs its own particular role and, perhaps, may correlate the action of other monoamines in a given hormonal situation. The effect of monoamines on neurons containing LHRH is steroid-dependent. Interaction of monoamines and LHRH in the hypothalamus undergoes a specifically synaptic form of structural and functional organization.

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EFFECT OF ANTIDIURETIC HORMONE ON THE PLASMA ALDOSTERONE LEVEL IN RATS WITH CHRONIC HIGH WATER AND RESTRICTED SODIUM INTAKE

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The phenomenon of functional mismatching between components of the renin–angiotensin–aldosterone system under conditions of a high water intake has been described. The peripheral blood aldosterone level is disporportionately low relative to the increased level of activity of the renin–angiotensin component of the system in albino rats drinking excessively [4], in homozygous Brattleboro rats [9], and also in patients with untreated diabetes insipidus [8]. The present writer previously demonstrated marked disparity between high aldosterone production by the adrenals and its relatively low blood level in rats on a sodium-deficient diet combined with chronic high water intake [5]. In all the situations mentioned above, functionally or genetically determined inhibition of antidiuretic hormone secretion evidently takes place.

The aim of this investigation was to verify the hypothesis that antidiuretic hormone (ADH) may have a regulatory influence on the blood aldosterone level.

EXPERIMENTAL METHOD

Male rats weighing 250-300 g were kept for 12 days on a fluid diet with low sodium and high water intake (0.5 meq and 900 ± 50 ml/kg body weight/day respectively). Rats of different groups received a subcutaneous injection 1 h before sacrifice of the following substances (per 100 g body weight): 1) 0.2 ml of distilled water; 2) 0.1 mU pituitrin; 3) 0.5 mU pituitrin; 4) 5 U ACTH; 5) 5 U ACTH + 0.5 mU pituitrin. Groups 4 and 5 were included in the experiment because of previous data showing that ADH may affect the glucocorticoid and mineralocorticoid function of the adrenal cortex indirectly through stimulation of ACTH secretion by the pituitary [11], and the fact that the water loading model used in the present investigation has not previously been studied from this standpoint. The rats were decapitated and blood plasma separated by centrifugation, after which its aldosterone concentration was measured by radioimmunoassay (using the appropriate kit from CEA-IRE-Sorin) and the corticosterone level was measured flurometrically. The adrenals were pooled 5 at a time for each test and incubated *in vitro*. The method of incubation and quantitative assay of the hormones was described previously [5]. The results were subjected to statistical analysis by Student's test.

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