EFFECT OF A CALCIUM CHANNEL BLOCKER ON PITUITARY LUTEINIZING HORMONE SECRETION IN INTACT AND CASTRATED MALE AND FEMALE RATS

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The basic principle of cell function, namely coupling of stimulus with response, is effected with the aid of calcium ions. This applies in full measure to the endocrine glands also and, in particular, to the pituitary [7, 13, 16]. For instance, releasing hormones, stimulators of secretion, realize their action through a change in calcium transport across the cell membrane [6, 11, 12, 15]. The mechanism of secretion of leuteinizing hormone (LH) with the aid of calcium is still being elucidated [2, 14]. However, in current medical practice, especially cardiology, preparations blocking calcium transport are being used therapeutically without regard to their possible side effects on the endocrine glands. There are no data in the literature on this problem.

For the reasons given above and to determine the role of calcium in the physiological mechanism of LH secretion, due to changes in the sex steroid level, it was decided to study the effect of a calcium channel blocker on LH secretion, and the investigation described below was undertaken for this purpose.

EXPERIMENTAL METHOD

Experiments were carried out on noninbred sexually mature albino rats weighing 200-250 g. The 67 females and 22 males were kept under conditions of artificial illumination (light from 5 a.m. to 7 p.m.). The estrous cycle of the females was monitored by the study of vaginal smears. Animals in which a 4-day cycle was observed at least three consecutive times were used for the experiments. Castration was carried out on the rats at the age of 30 days under ether anesthesia, 6-7 weeks before the experiment. Animals undergoing mock operations served as the control. For a few days before the experiment began the rats were adapted by injection of 0.5 ml of water into them through a gastric tube. Verapamil was injected into the stomach in a dose of 10 mg/100 g body weight 2 h before sacrifice. The animals were decapitated, blood was collected, and serum obtained by centrifugation. The adenohypophyses were isolated and homogenized in physiological saline. The sera and homogenates were kept at −30°C until required for LH assay. LH was determined by radioimmunoassay by the method in [1] in two or three parallel tests and in two dilutions. The results were subjected to statistical analysis.

EXPERIMENTAL RESULTS

It will be clear from Table 1 that a significant rise of the serum LH level of the rats in the proestrus stage was observed as early as at 3 p.m. ($P < 0.05$). The maximal increase in the intensity of secretion, by about 50-fold, was observed by 5 p.m. ($P < 0.001$). Administration of verapamil 2 h before the LH peak (Table 2) lowered its level by more than two-thirds ($P < 0.001$). Verapamil caused a small but significant fall in the LH concentration in the pituitary gland of these animals ($P < 0.05$). At other stages of the cycle (diestrus I and estrus) verpamil had no significant effect on LH secretion. No effect of verapamil either on the blood level of LH or on its concentration in the pituitary likewise was found in ovaricetomized rats.

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TABLE 1. Serum LH Concentration in Intact Female Rats

Stage of cycle	Serum LH, ng/ml	P
Diestrus I	46,7±10,2	
Proestrus (3 p.m.)	888±275	<0,05
Proestrus (5 p.m.)	2418±246	<0,001

TABLE 2. Effect of Verapamil on LH Level in Rat Blood Serum and Pituitary Gland

Group of animals	Undergoing mock operation	Blood LH level, ng/ml	P	LH in pituitary, μg/mg	P
		Females			
Undergoing mock operation (proestrus)	—	2417±378		59,6±7,8	
	+	748±126	<0,001	38,7±2,5	<0,05
Undergoing mock operation	—	785±34		168±52	
	+	817±104	>0,05	159±42	>0,05
		Males			
Undergoing mock operation	—	126±28		71,8*	
	+	145±49	>0,05	73,8	
Gonadectomized	—	590±41		181,3	
	+	629±32	>0,05	180,8	

Legend. Asterisk indicates that samples of sera were pooled for each group.

It will be clear from Table 2 that verapamil had no significant effect on the serum LH level of the control animals (males) or on the LH concentration in the pituitary. Orchidectomy lowered the blood LH level by about 75% and more than doubled its concentration in the tissue of the adenohypophysis. Verapamil had no significant effect on the parameters studied in these rats.

The experiments thus showed that verapamil inhibits LH secretion into the blood stream and also reduces the LH concentration in the pituitary gland of female rats only at the proestrus stage of the estrous cycle. In ovariectomized rats varapamil had no effect. The calcium channel blocker likewise had no effect on intact or castrated male rats. If gonadectomy sharply reduced the blood level of sex hormones and increased LH secretion through the secretion of luliberum (LH releasing hormone) by the hypothalamus, verapamil might be expected to inhibit the intensified LH secretion, having regard to data in the literature showing that calcium-dependent gonadotropin secretion is dependent on luliberin [10]. The results do not suggest that increased secretion of LH is always due to mobilization of extracellular calcium. Inhibition of LH secretion by verapamil, which we observed in female rats in the proestrus stage of the estrous cycle during the evening, is evidence not only that extracellular calcium must participate in preovulatory LH secretion, but also that it depends on a transient rise of the estradiol level. Indirect confirmation of this hypothesis is given by the absence of effect of verapamil in animals of the remaining groups. In clinical trials verapamil was found to have a weak inhibitory action on gonadotropin secretion, and this also was more marked in women [3]. There is also evidence that estradiol has a stronger action than testosterone on LH secretion in a culture of pituitary cells, activated by caclium ions [9]. The present experiments showed that verapamil reduced the LH concentration in the pituitary. It is possible that the calcium-mediated action of estradiol also is realized at the hypothalamic level. Although the role of calcium in gonadotropin secretion is not disputed and investigations to determine the precise pathways and methods by which calcium ions enter the pituitary cells from outside are continuing [4, 5, 8], the further study of the physiological principles governing preovulatory LH secretion in animals require further study and, in particular, the degree to which intracellular calcium mechanisms at different levels of hypothalamo-hypophyseal regulation participate in it, need clarification.

On the basis of the facts described above the writers suggest that verapamil may disturb preovulatory LH secretion in man, and some caution is therefore necessary when women of fertile age are treated with this preparation.

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CLONAL NATURE OF FIBROBLAST COLONIES FORMED BY BONE MARROW STROMAL CELLS IN CULTURE

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After explantation of suspensions of hematopoietic cells into monolayer cultures the stromal colony-forming units (CFU_f) present in the bone marrow form discrete fibroblast colonies [3, 8-10].

In mixed cultures of bone marrow cells from male and female guinea pigs, dividing fibroblasts of each stromal colony have a karyotype which is only male or only female, in agreement with the hypothesis of the clonal nature of the colonies [3]. However, this has been demonstrated for cells of autobred animals and the possibility cannot be ruled out that progenies of several syngeneic, but not of allogeneic CFU_f, are combined in the colony.

The aim of this investigation was to study whether colonies of fibroblasts formed by CFU_f are in fact cell clones. Experiments were carried out to take into account three possible sources of error when interpreting the results of fibroblast karyotyping in mixed cultures, namely: cells of stromal aggregates, not dissociated into separate cells, may be found in the explanted suspensions, only syngeneic but not allogeneic fibroblasts may be combined into common colonies, and colonies may be contaminated by macrophages and hematopoietic cells.

EXPERIMENTAL METHOD

Bone marrow cells from CBA and CBAT6T6 mice were used for explantation. Contents of the medullary cavity of the femora were expelled by means of a syringe into medium DMEM with 15 mM HEPES, and single-cell suspensions were prepared either by mechanical disaggregation (passage through syringes with needles of diminishing diameter), or by trypsinization [2]. All cell suspensions were filtered through four layers of kapron and centrifuged for 10 min at 400g; the cell residues were resuspended in fresh culture medium. The efficiency

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