quency was reduced, and after 2 min it was the same as it was initially. Changes in the amplitude and duration of the discharges were not significant. Changes in SAP reached a maximum 10 sec after the beginning of stimulation. The initial level was restored after 2 min. In 11 experiments stimulation of the nucleus was accompanied (Fig. 2) by a reduction in the frequency of the volleys by 36% ($P < 0.01$) and 30% ($P < 0.05$). Under these circumstances the level of SAP fell from 121 ± 3.5 to 98 ± 6.3 mm Hg ($P < 0.05$).

Stimulation of the paramedian and ventral reticular nuclei thus leads to regular and marked changes in efferent activity of the renal and splenic nerves. The different character of the changes in efferent activity and SAP during stimulation of the nuclei by similar doses of ACh is evidence of the diffuse distribution of pressor and depressor neurons within these structures. Stimulation of the ventral reticular nucleus was accompanied by more marked changes in efferent activity in the splenic nerve, whereas microinjection of ACh into the paramedian reticular nucleus gave more marked changes in the renal nerve. Changes in efferent activity in the sympathetic nerve were much more marked after stimulation of the ventral reticular nucleus, possibly on account of the greater density of cholinergic neurons in this region than in the paramedian reticular nucleus.

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EFFECT OF LUTEINIZING HORMONE RELEASING HORMONE ON CALCIUM-ACCUMULATING CAPACITY OF RAT MYOCARDIAL MEMBRANES

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The writers showed previously that some releasing factors, notably luteinizing hormone releasing hormone (LRH), have a cardiotrophic as well as a hypophyseotrophic action [3]. Changes in the activity of Na,K-ATPase in the sarcolemma [4] and of NADH-oxidase activity of submitochondrial particles [1] under the influence of LRH are evidence that the mechanism of regulation of cardiac activity by this peptide evidently proceeds through its influence on the function of the various myocardial membranes. At the same time we know that LRH can alter the permeability of hypophyseal membranes for calcium [7]. During muscular contraction Ca^{++} is the link between excitation of the muscle and activation of myofibrillary ATPase.

Accordingly, the present investigation was undertaken to study the effect of LRH on the calcium-accumulating capacity of the sarcolemma, the sarcoplasmic reticulum, and the mitochondria of the rat myocardium.

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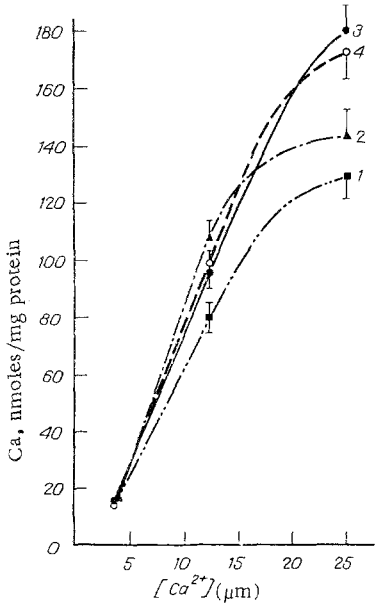

Fig. 1

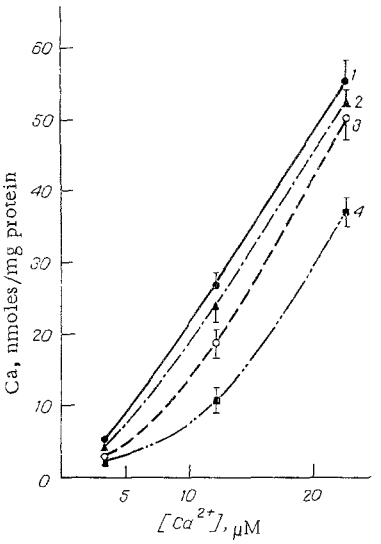

Fig. 2

Fig. 1. Effect of LRH on Ca-accumulating capacity of mitochondria. 1) Control; 2, 3, 4) in presence of 3, 30, and 300 nM LRH, respectively.

Fig. 2. Effect of LRH on Ca-accumulating capacity of sarcoplasmic reticulum. 1) Control; 2, 3, 4) in presence of 30 and 300 nM and 3 μM LRH, respectively.

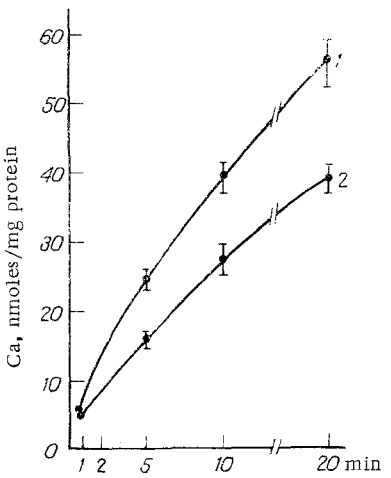

Fig. 3. Dependence of Ca-accumulating capacity of sarcoplasmic reticulum in the presence of LRH on incubation time (concentration of free Ca^{++} 25 μM). 1) Control; 2) in presence of 3 μM LRH.

EXPERIMENTAL METHOD

The sarcolemma, sarcoplasmic reticulum, and mitochondria were isolated from the myocardium of Wistar rats weighing 160-200 g by the method described previously, which simultaneously yields three fractions of membranes with preserved Ca-accumulating capacity [5]. The purity of the fractions was tested by electron microscopy and by determining activity of marker enzymes [5]. The protein concentration was determined by Lowry's method [6]. The Ca-accumulating capacity of the membranes was estimated by the isotope exchange method in a medium of the following composition (in mM): imidazole 40, KCl 100, NaCl 20,

$MgCl_2$ 5, ATP-Na_2 4, $^{45}CaCl_2$ 1.5 μCi/ml (pH 7.0). In experiments with the sarcoplasmic reticulum and mitochondria, the incubation medium contained, in addition to the components already mentioned, oxalate (5 mM) and succinate (2 mM), respectively. The total calcium concentration in the incubation medium varied between 10 and 100 μM; the free calcium concentration was determined by means of the F-2112 Ca-selective electrode with K-4112 comparison electrode on a type PHM-64 millivoltmeter (Radiometer, Denmark). The samples were incubated for 20 min at 37°C and applied to HA filters (Millipore, USA), previously soaked in 1M KCl and washed immediately before the experiment with 4 ml of 0.25 M sucrose. Filtration was carried out under a vacuum of 20 cm Hg; the samples were washed three times with 4 ml of 0.25 M sucrose, cooled to 0-2° C. The radioactivity of the samples was determined in Bray's solution on the SL-4002 liquid scintillation counter (Intertechnique, France). The radioactivity of the filters after application of an aliquot of buffer without additions of the membranes, washed under the same conditions, served as the control. The LRH used was obtained from the firms of Beckman and Serva.

The experimental results were subjected to statistical analysis. Arithmetic mean values and standard deviations are given in Figs. 1-3.

EXPERIMENTAL RESULTS

The experimental results show that LRH acts in different directions on the mitochondria, sarcoplasmic reticulum, and sarcolemma. In a concentration of 3×10^{-9} to 3×10^{-7}M it increased the Ca-accumulating capacity of the mitochondria, although no strict dose dependence on the LRH concentration was observed (Fig. 1). ATP-dependent calcium uptake by the sarcolemma was reduced in the presence of the peptide (3×10^{-6}M) by 15% ($P < 0.05$) if the free Ca^{++} concentration in the incubation medium was 7 μM. Similar results also were obtained in the experiments with the sarcoplasmic reticulum (Fig. 2). The Ca-accumulating capacity of the latter was reduced with an increase in the hormone concentration (3×10^{-8} to 3×10^{-6}M).

The high initial rate of calcium accumulation by the sarcoplasmic reticulum leads to a rapid decrease in the free Ca^{++} concentration during rhythmic work of the heart [5]. It was interesting to study the effect of LRH on the rate of calcium uptake by the sarcoplasmic reticulum. Since the greatest inhibition of the Ca-accumulating capacity of the sarcoplasmic reticulum under the influence of LRH (by 35%) was observed when the free Ca^{++} concentration was 25 μM, the subsequent experiments were performed with this Ca^{++} concentration in the medium. As will be clear from Fig. 3, LRH (3×10^{-6} M) slowed the rate of calcium accumulation by the reticulum. The decrease in the rate of calcium accumulation in the sarcoplasmic reticulum under the influence of LRH led to lengthening of the period required for complete relaxation of the myocardium. These disturbances of calcium transport may be the cause of the bradycardia which arises under the influence of LRH in the isolated rat heart [3]. The physiological role of slowing of calcium accumulation by the sarcolemma and of its quickening by the mitochondria in the presence of LRH is not yet clear.

The absence of any effect of LRH in experiments in vitro on adenylate cyclase and phosphodiesterase activity of rat heart homogenate [2] and the results of the present investigation indicate that the action of the peptide on rhythmic work of the heart [3] is based, not on a change in the system of cyclic nucleotides, but on modification of the intracellular calcium distribution.

LRH is thus evidently an intracellular regulator of cardiac activity, the action of which is realized through its effect both on the plasma membranes and on the intracellular membranes of the myocardium.

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