ing of the hormone but also participates in the catalytic function of the hormone receptor complex.

It has been known for some time that the response of the isolated rat uterus to neurohypophyseal hormones is potentiated in the presence of magnesium ions in the ambient solution[6,9]. However it was demonstrated recently that this augmentation of the oxytocic activity can result not only from an increase in hormone-receptor affinity, but as well from an alteration of intrinsic activity[8]. Therefore we investigated the effect of magnesium on the rat uterotonic response to increasing (cumulative) doses of [lysine]-vasopressin. We found that magnesium at a concentration of 0.5 mM not only increased the affinity of [lysine]-vasopressin for the rat uterine receptor (pD_2 7.82 $\pm$ 0.12) but, in addition, altered the intrinsic hormonal activity (α 0.96 $\pm$ 0.03[10]; Figure, IIa *v.* IIb); this increase in affinity and intrinsic activity for [lysine]-vasopressin is significantly greater than the magnesium-induced changes in these parameters in the case of oxytocin (pD_2, 9.87 $\pm$ 0.08; α 1.07 $\pm$ 0.01[8]; Figure, Ia *v.* Ib)[11].

Zusammenfassung. Ein der Rezeptorentheorie zugrunde liegender Vergleich der Dosis-Wirkungs-Beziehung zwischen Oxytocin und Lysine-vasopressin an der isolierten Rattengebärmutter hat gezeigt, dass während der Evolution die Oktapeptide der Neurohypophyse sowohl ihre Affinität für den Rezeptor als auch ihre maximale Aktivität bei Absättigung des Rezeptors, «intrinsic activity», verändert haben. Weiterhin wurde gefunden, dass Magnesium-Ionen in einer Konzentration von 0.5 mM nicht nur, wie bisher angenommen, die Affinität der Hormone, sondern auch ihre maximale Aktivität bei Absättigung des Rezeptors beeinflussen.

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Regulation of Leucine Incorporation into Cardiac Protein by Work Loads

When an increase in mass of the heart, cardiac hypertrophy, is induced in experimental animals by raising the resistance to the output of the heart, net increase in cellular RNA and protein occurs[1,2]. Since the synthesis of messenger (m) and ribosomal (r) RNA in mammals is a relatively slow process[3–5], a question arises as to how rapidly a message from the physical parameters of muscle contraction is realized at the chemical level. There is a growing body of evidence indicating the existence of a control of protein synthesis at the level of the ribosomes[6–8]. In other words, the synthesis may be regulated by mechanisms involving the translation of mRNA, rather than modulating mRNA synthesis. Therefore, when the work load to the heart is changed, factors such as the flux of ionized calcium in the cell, the conformational change in endoplasmic reticulum, or changes in the high energy phosphate potential of compartments may regulate the incorporation of amino acids into myocardial protein. Indeed, by using the heart-lung preparation of rats, in which a precise control of hemodynamic parameters is possible, it is seen that cytoplasmic protein synthesis varies directly with a change in the cardiac work level.

Rats of Wistar strain, 200–250 g, were anaesthetized with ether and used for the heart-lung preparation[9]. The circulating solution consisted of 25 ml of donor's blood and 75 ml of Ringer-Locke solution containing 18 amino acids (0.1 mM each): L-arginine, L-aspartic acid, L-cystein, L-glutamic acid, glycine, L-histidine, hydroxy-L-proline, DL-isoleucine, L-leucine, L-lysine, L-methionine, DL-phenylalanine, L-proline, DL-serine, L-threonine, L-tryptophane, L-tyrosine and L-valine. In

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addition, C-14 labelled leucine (10 μc) was added to the reservoir. 2 levels of work load were imposed on the heart for periods varying up to 180 min; for a 'high' work level, left ventricular outflow (cardiac output minus coronary flow) was maintained at 36 ml/min and the mean arterial pressure at 64 mmHg, while for a 'low' work level, 15 ml/min and 32 mmHg, respectively, were selected[9]. Inhibitors of protein synthesis were administered according to the following schema: actinomycin D (1 mg/kg) was injected i.p. 1 h prior to the experiment. Puromycin was added to the reservoir at a concentration of 10 mg per 100 ml[10]. Cycloheximide (25 mg/kg) was injected i.p. 1 h prior to experiment; in addition, 5 mg of the same substance were mixed with the perfusate[10]. At the end of each experiment, the coronary bed was rinsed with Ringer-Locke solution. Pieces of tissue from the left and right ventricles were separately weighed and digested in 30% KOH. The protein was precipitated with trichloroacetic acid 3 times, and, after having been dissolved by NCS solubilizer (Nuclear Chicago Corp.), was counted. Aliquots were taken for protein determination[11]. Radioactivity in the perfusing solution was determined using BRAY's solution[12].

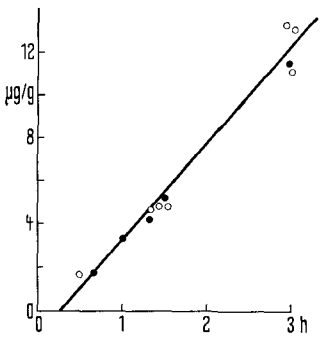

Fig. 1. Leucine incorporation into left ventricular protein. The peripheral resistance and the venous pressure in the rat heart-lung preparation were adjusted to produce a mean blood pressure of 64 mmHg and a cardiac output of 36 ml/min. The perfusate contained a mixture of non-labelled amino acids in addition to leucine-^{14}C. Experiments were terminated at various intervals (abscissa) and left ventricular protein was analyzed. The extent of leucine incorporation was expressed as μg leucine/g wet weight of left ventricle. Circles represent the experiments without, and dots those with actinomycin (1 mg/kg, 1 h prior to the experiments).

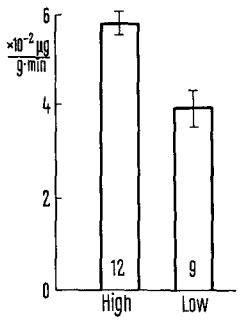

Fig. 2. The rates of leucine incorporation into protein of the left ventricle working at 2 different levels of work load. The work levels: the mean blood pressure of 64 mmHg and the cardiac output of 36 ml/min (high), and 32 mmHg and 15 ml/min (low). The values were expressed as means ± S.E. 10^{-8} g leucine/g of left ventricle/min, and the number of experiments is in columns. The difference was significant ($p < 0.001$).

Hemodynamic characteristics of the heart in the heart-lung preparation have previously been published[9]. In the present study, spontaneous failure, when judged by the incidence of pulmonary edema, did not occur until 5–6 h after the experiment had begun. The dryweight/wetweight ratio and protein content of the ventricles did not change during the experimental period of 3 h, regardless of whether or not amino acids were added. Radioactivity in the supernatant of the perfusate gradually decreased during the experiment. As shown in Figure 1, the rate of incorporation of leucine-^{14}C into cardiac protein was essentially linear at a given working level of the ventricle. The slope was less steep at a low work load. Consequently, the difference in the rate of incorporation between 2 work levels selected was statistically significant ($p < 0.001$) (Figure 2). Pretreatment with actinomycin D did not influence the leucine incorporation at a high (Figure 1) or a low work level. On the other hand, about 95% of suppression of protein synthesis occurred when puromycin was added (Table). Similarly, a high dose of cycloheximide inhibited amino acid incorporation (Table). With these inhibitors, early occurrence of pulmonary edema was frequent.

The heart-lung preparation was used in this study in order to exclude the possibility that oxygenation become a rate limiting factor[9,13]. Furthermore, in a few experiments in which variations in cardiac power (the product of mean arterial pressure and left ventricular outflow) were produced by different combinations of pressure and flow, the relationship between cardiac power and leucine incorporation was found to be similar to that observed under the standard conditions described in methods, suggesting that coronary perfusion per unit mass of muscle is not a primary determinant in this relationship.

A fourfold increase in cardiac work load resulted in 50% increase in leucine incorporation (from 0.039 to 0.058 μg/g left ventricle/min) (Figure 2). A greater difference in protein synthesis was reported by using guinea-pig hearts perfused with a fortified bicarbonate buffer solution[14]. There, however, the rate of incorporation was not linear[14]. At the present time, no study is available which deals with the changes in mRNA or rRNA in relation to the cardiac work level, although indirect evidence was presented to suggest an increase in rRNA, 24 h or more after aortic constriction[15]. The present study strongly suggests that,

Leucine incorporation into cardiac protein (3 h low load)

	dpm/mg protein	
	Right ventricle	Left ventricle
No inhibitor	37.0 ± 2.5	31.6 ± 4.0
Puromycin (10 mg/100 ml)	0.82	0.56
Cycloheximide (25 mg/kg)	5.0	5.3

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at an early stage in the changing work level, the regulation of amino acid incorporation in the heart is not related to the synthesis of mRNA or rRNA, since the dose and the mode of administration of actinomycin D used in this study is more than effective in preventing the synthesis of RNA[16].

Upon the addition of puromycin and of cycloheximide, the control of synthesis was inhibited (Table). It is therefore postulated that the site regulating amino acid incorporation is at the level of the membrane-ribosome complex. This contention is further supported by the fact that there was a very brief time lag for a detectable difference in the rate of incorporation. Reports from various laboratories show that the life time both of mRNA and of rRNA and the response time for nuclear RNA polymerase are all in excess of 3 h[3-5,17,18]. In contrast, there has been evidence indicating an extremely rapid control of protein synthesis at the level of translation of preformed template RNA in response to hormones, feeding, partial hepatectomy or stimulation of a skeletal muscle[6-8,10,18-20]. However, whether or not the control observed in this study is a result of a specific protein[10], an inhibitor[7] or some other means of regulation, requires further experimentation[21].

Zusammenfassung. Die Einbaugeschwindigkeit der Aminosäure steigt mit erhöhter Herzbelastung an. Bei Vorbehandlung mit Actinomycin D bleibt dieses Verhältnis unbeeinflusst, Puromycin oder Cycloheximid indessen hemmen die Proteinsynthese.

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Response of Respiration to Selective Heating of the Spinal Cord Below Partial Transection

Afferent conduction of warmth stimuli applied to the spinal cord has been proved by the finding that thermal panting is evoked in conscious dogs by selectively heating the spinal cord[1]. Partial cordotomy involving ventrolateral or dorsal funiculi has been performed in the present investigation in order to find out the afferent pathway by which spinal thermal stimuli are conducted to the supraspinal thermoregulatory effectors.

Method. In 20 out of 26 nembutalized rabbits, partial cordotomy of both dorsal funiculi was performed between the fourth and eleventh thoracic segment; in the remaining cases the ventrolateral funiculi were cut on either side. – Heating of the spinal cord below the transection level was performed by means of a thermode which had been implanted into the peridural space between the level of transection and the seventh lumbar vertebra. The thermode was perfused with hot (46–52 °C) water for 2–10 min at constant ambient temperatures between 24–28 °C. – Electromyograms were recorded from the lower lumbar dorsal trunk muscles using a pair of needle electrodes. Respiratory movements were recorded by displaying on a recorder the changes of electric resistance in an electrolyte-filled, distensible rubber tube, which had been tied round the rabbit's chest. The temperatures within the rectum and the peridural space of the lumbar vertebral canal and the skin temperature of one ear were measured by thermocouples. Several days after the cordotomy, the animals were sacrificed. The spinal cord was fixed with Müller's solution, and Marchi's stain was performed.

Results. In Figure 1, the response of respiration and muscular activity to spinal cord heating below the level of bilateral transection of both dorsal funiculi is demonstrated. Partial cordotomy had been performed at the sixth thoracal segment as indicated by the hatched part of the inset Figure. At an ambient air temperature of 24 °C and a rectal temperature of 38.2 °C, slight cold shivering was observed in this lightly anaesthetized animal before the spinal cord was heated (left side of the Figure). After 4 min of spinal cord heating (middle part), vertebral canal temperature had risen to 41.8 °C. Shivering had disappeared, and respiratory rate had increased to a tachypnoic level. Further, cutaneous vasodilatation is indicated by the rise of ear skin temperature at constant ambient and rectal temperatures. This combined response to heating – inhibition of shivering, thermal tachypnea and cutaneous vasodilatation – was abolished 4 min after the end of spinal cord heating, when vertebral canal temperature had reached a normal value.

Six days after the partial cordotomy, the animal was killed, and Marchi's stain was performed of the spinal cord. Figure 2 shows a transverse section of the cord, 2 segments rostral to the transection level. In Figure 2A, numerous dark, small clots, which correspond to the myelin of degenerated fibers, cover the area of both dorsal funiculi. A few grey spots are interspersed between the degenerated myelin, especially at the left side medial to the entrance of a dorsal root. Apparently, they represent intact fibers, which have entered through the dorsal roots above the transection. The dark clots are, however, almost lacking in the adjacent parts of the lateral funiculi. The histological differences between these intact lateral funiculi and the degenerated dorsal funiculi are demonstrated in detail in Figure 2B. The clots of degenerated myelin are stained intensely, while the myelin sheaths of the intact fibers are visible as faintly stained annular structures.

Such a combined response to spinal cord heating below the level of bilateral transection of the dorsal funiculi has been confirmed in 19 out of 20 investigated animals. However, in all cases – up to now 6 animals – in which the ventrolateral funiculi had been cut bilaterally, afferent influences of spinal cord heating below transection on respiration and on cutaneous blood flow of the ear

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