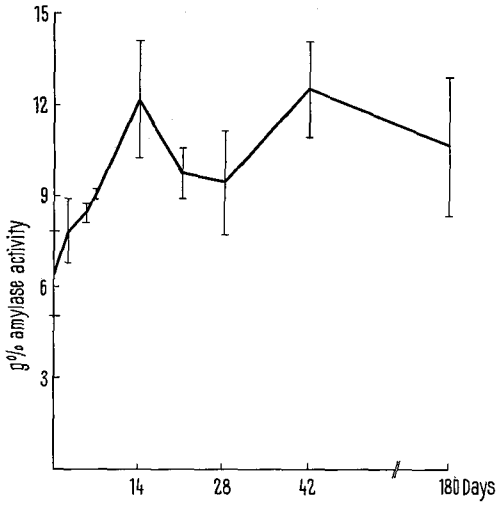


Amylase activity in the blood of Wistar rats in the first half year of life.

In our opinion this behaviour seems to indicate that the primary origin of the amylase occurring in the amniotic fluid is the urine excreted by the foetus.

Just as in the case of humans[7], the mean amylase activity rate in the blood of the rat is somewhat higher during gravidity.

*Zusammenfassung.* Die Amylaseaktivität im Blut von Wistarratten steigt bis zum Ende der Säugeperiode an. In der Amnionflüssigkeit fehlt sie zunächst, nimmt oberhalb eines Fötalgewichtes von 2 g auf ein Vielfaches der Blutwerte zu und fällt mit schwindender Amnionflüssigkeit am Ende der Gravidität stark ab.

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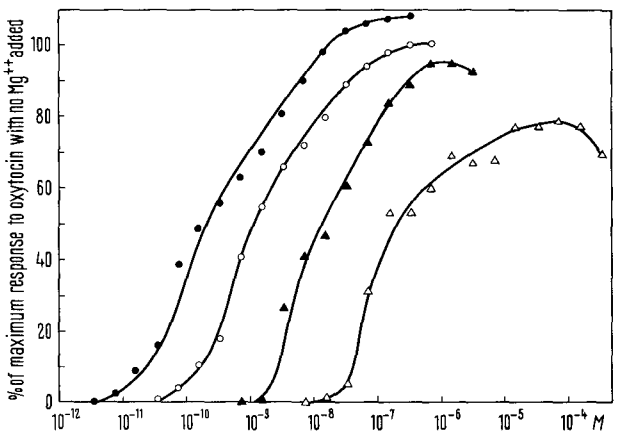
## Comparison of the Mode of Action of Oxytocin and Lysine-Vasopressin on the Isolated Rat Uterus

The primary structures of 7 natural neurohypophyseal octapeptides, isolated from a broad spectrum of vertebrate species, have been elucidated[1–4]. Point mutations in the genetic code(s) for the ancestral octapeptide molecule(s) resulted in alterations of the primary peptide sequence. Available evidence to date indicates that only substitutions in positions 3, 4 and 8 have been favored during the course of evolution. In this context it was desirable to explore whether, in addition to quantitative biological differences (affinity for a given target organ), also qualitative differences (ability to induce a response subsequent to receptor occupation) played a role in adaptation.

Hormones of the posterior pituitary gland possessing neutral alkyl amino acid residues in positions 3 and 8 (e.g., isoleucyl and leucyl, respectively, in the case of oxytocin) are potent contractile stimulants of the in vitro rat uterus; hormones, e.g. [lysine]-vasopressin, possessing in position 3 an aromatic amino acid residue (phenylalanyl) and in position 8 a basic alkyl amino acid residue (lysyl) exhibit low potencies in this bioassay. Usually the potencies of these hormones are determined by a 4-point design[5] in magnesium-free van Dyke-Hastings solution[6] and are expressed in terms of specific activities. To gain a deeper understanding of the mode of interaction of these hormones with the uterine receptor(s) we determined the cumulative dose-response curves[7] of [lysine]-vasopressin and oxytocin in magnesium-free solution (Figure, curves Ia and IIa). It is apparent from this study that these 2 neurohypophyseal hormones differ not only in their affinities (oxytocin, $pD_2$ 9.17 $\pm$ 0.08[8]; [lysine]-vasopressin, $pD_2$ 6.82 $\pm$ 0.12) for the smooth muscle receptor but, more significantly, in their maximum effects – a measure of intrinsic activity (oxytocin, $\alpha$ 1.00; [lysine]-vasopressin, $\alpha$ 0.83 $\pm$ 0.04). That the diminished intrinsic activity of [lysine]-vasopressin is associated with the presence of the phenylalanyl residue in position 3, and not with the presence of the lysyl residue in position 8, is suggested by recent findings[8] in this laboratory: dose-response studies with oxytocin analogs, which differ specifically in the substitution of the aliphatic isoleucyl residue in position 3 by the aromatic phenylalanyl residue (3-isoleucine-8-alanine-oxytocin *v.* 3-phenylalanine-8-alanine-oxytocin), showed that the side-chain in position 3 not only actively contributes to bind-




Cumulative dose-response curves of [lysine]-vasopressin and oxytocin on the in vitro rat uterus mounted in Munsick's fluid without added magnesium and with 0.5 m*M* added magnesium. Oxytocin without added magnesium (○——○) (Ia); oxytocin with added magnesium (●——●) (Ib); [lysine]-vasopressin without added magnesium (△——△) (IIa); [lysine]-vasopressin with added magnesium (▲——▲) (IIb). The experimental procedure followed for obtaining dose-response curves has been detailed in [8]. Each curve represents an average of at least 10 experiments with 6 different rats.

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 m$M$ 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 ($\alpha$ 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; $\alpha$ 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 m$M$ nicht nur, wie bisher angenommen, die Affinität der Hormone, sondern auch ihre maximale Aktivität bei Absättigung des Rezeptors beeinflussen.

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[10] Probability that the increase in intrinsic activity is due to chance, $P < 0.015$.
[11] Supported by NIH grant No. AM-10080 and the U.S. Atomic Energy Commission. P. EGGENA acknowledges an NIH postdoctoral Research Fellowship. We wish to thank Dr. J. MEIENHOFER of the Children's Cancer Research Foundation, Boston, for the generous supply of highly purified [lysine]-vasopressin.

## 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 m$M$ 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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