Distribution of 3-methyl-glucose (MEG) and glucose between liver, muscle and plasma water in anaesthetized rats after an infusion of MEG-$^{14}$C

| Length of MEG infusion (min) | No. of experiments | Insulin | Average glucose concentration in plasma-$H_2O$ mg/100 ml | Distribution space of MEG, ml/g | | Ratio of glucose concentration in liver-$H_2O$/plasma-$H_2O$ [b] | Ratio of glucose concentration in 'available' liver-$H_2O$/plasma-$H_2O$ [c] |
|---|---|---|---|---|---|---|---|
| | | | | Liver [a] | Gastrocnemius | | |
| 60 | 5 | – | 82.9 | 0.787 ± 0.047 | 0.220 ± 0.019 | 1.395 ± 0.154 | 1.776 ± 0.196 |
| 120 | 4 | – | 76.2 | 0.919 ± 0.059 | 0.239 ± 0.014 | 1.506 ± 0.067 | 1.655 ± 0.115 |
| 60 | 5 | + | 27.7 | 0.864 ± 0.041 | 0.666 ± 0.058 | 2.258 ± 0.347 | 2.632 ± 0.432 |

Average values ± standard error of means are shown. [a] The overall average of the 2 groups not treated with insulin is 0.846 ± 0.042. [b] The overall average of the 2 groups not treated with insulin corresponds to an average ratio of 1.13 for mg glucose in g liver/mg glucose in ml plasma. [c] Equals the calculated apparent distribution volume of glucose/calculated distribution volume of MEG. The overall average of the groups not treated with insulin is 1.722 ± 0.112.

between their results and ours is due either to differences in the chemical methods used, or to the divergence between injection and steady state infusion methods in estimating distribution spaces[4]. In agreement with earlier investigators an increased distribution space of MEG was found in the gastrocnemius of rats treated with insulin[10].

The calculated apparent distribution space of glucose in the liver was larger than the MEG space. Assuming that the same volume of water is available to dissolve both sugars, the average concentration of glucose was 1.72 ± 0.11 times as high in this water as in plasma water. Since, however, in rats about 30% of the total liver water[11], and thus 36% of the distribution volume of MEG, is extracellular, the true concentration ratio of glucose between the glucose-containing compartment of the liver cell and extracellular water is likely to be about 2.0. In insulin induced hypoglycaemia the ratio of the concentrations of glucose in the MEG-space and plasma water was 2.63 ± 0.43, the estimated true intracellular glucose concentration being 3.3 times as high as in the plasma. Since insulin did not cause an accumulation of MEG in the liver, its effect on the calculated distribution space of glucose is not likely to be due to an alteration of permeability of the liver cell membrane. It appears that this increase is the result of the circumstances that the outward movement of glucose is not facile enough to prevent the existence of a considerable concentration gradient when net glucose flow out of the liver is going on[3], especially at an increased rate as in hypoglycaemia[12].

*Zusammenfassung.* Es wurde gefunden, dass 3-Methyl-Glukose (MEG) sich in 85% der gesamten Gewebeflüssigkeit der Rattenleber löst. Ist Glukose in gleicher Plasmamenge enthalten, so ist die berechnete Konzentration in der Leberzellflüssigkeit doppelt so hoch. Bei Insulinhypoglykämie stieg dieses Verhältnis weiter an, ohne aber eine Veränderung im Verteilungsvolumen von MEG zu ergeben. Die Leberzellmembran scheint die Penetration der Glukose aus der Zelle zu verzögern.

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## Similar Effects of Arginine-Vasopressin and Arginine-Vasotocin on Permeability of Toad Skin

Neurohypophysial peptides have been generally assayed on test objects which detect smooth muscle activity (uterus, blood pressure, and mammary gland) or fluid reabsorption (rat anti-diuresis and amphibian bladder), and the 2 types of response have been sometimes thought to be related to augmented sodium transport and to increased water permeability. While vasopressor activity has seemed to be associated with basicity of the amino acid in position 8[1], and oxytocic activity has seemed to depend on iso-leucine in position 3 and the integrity of the peptide ring[2], the responses of various tissues to natural or synthetic hormones have seldom been in agreement. BOURGUET and MAETZ[3] have shown for several peptides that there is a lack of correlation between natriferic and hydrosmotic activity. It was therefore of interest to examine the effect of various peptides

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on a test object which permits differentiation of the hydrosmotic and natriferic effects.

*Bufo melanostictus*, hydrated overnight and having the cloaca tied, was found when placed in distilled water to take up water through the skin at a rate of 17 $\mu$l/cm$^2$/h (at 29 °C). This rate remained constant for more than 24 h. When arginine$^8$-vasopressin was injected i.m., the rate of water uptake increased and then slowly returned to normal over the next few hours. Since the toads were in contact with distilled water this increase could not be ascribed to an alteration in sodium transport, but could be due only to increased permeability, and the method therefore provided a means of assessing and comparing the effect of various peptides on the water permeability of the toad skin. The rate of water uptake during the $^1/_2$ h immediately following the injection was measured and was expressed as % of the basic rate of water uptake. A range of doses was used, and dose-response curves were prepared for arginine$^8$-vasopressin, arginine$^8$-vasotocin, and oxytocin (Figure 1).

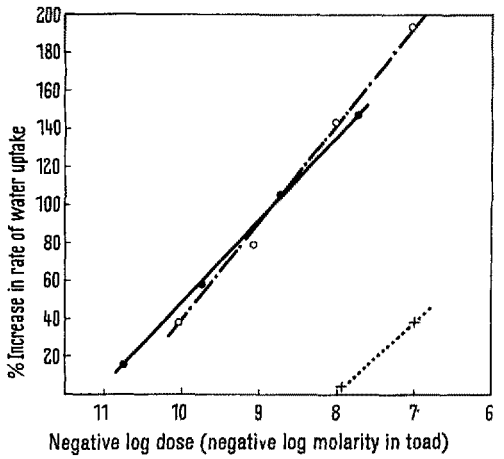


Fig. 1. Relationship between dose of peptide and % increase in water uptake during the $^1/_2$ h immediately following i.m. injection; toads bathed in distilled water. Lines drawn by eye. ●—● = arginine$^8$-vasotocin; o-·-·-o = arginine$^8$-vasopressin; + · · · + = oxytocin.

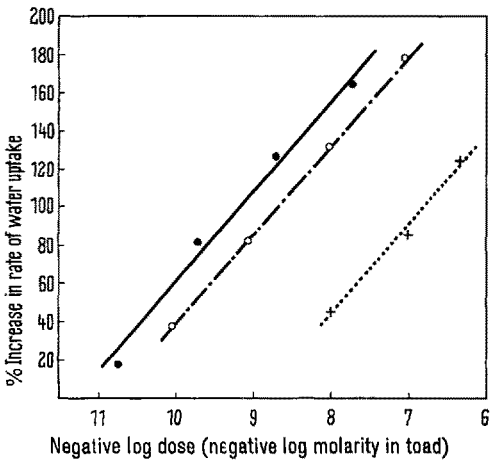


Fig. 2. Relationship between dose of peptide and % increase in water uptake during the $^1/_2$ h immediately following i.m. injection; toads bathed in 0.1% NaCl. Lines drawn by eye. ●—● = arginine$^8$-vasotocin; o-·-·-o = arginine$^8$-vasopressin; + ····· + = oxytocin.

Arginine$^8$-vasopressin and arginine$^8$-vasotocin in a wide range of doses produced closely similar effects, and each produced 100% increase in permeability when present within the toad in a concentration of $10^{-9}$ molar. Oxytocin produced only a very small effect on permeability and could not be given in a dose large enough to double the rate of water uptake (the toads died). Making a comparison of the dose of each peptide required to produce a 50% increase in the rate of water uptake, the action of arginine$^8$-vasopressin and arginine$^8$-vasotocin were seen to be closely similar, and both were about 2000 times more effective than oxytocin.

In another series of experiments the toads were placed in 0.1% NaCl solution, a situation from which there are 2 elements of water uptake – water movement in response to osmotic gradient, and water movement in pursuit of transported $Na^+$. Again a range of doses was used, and dose-response curves were obtained for the 3 peptides (Figure 2).

When NaCl was available on the outer aspect of the toad skin, arginine$^8$-vasotocin was found to be more potent at all dose levels. A 100% increase in the rate of fluid uptake was produced by a concentration within the toad of $2 \cdot 10^{-9}$ molar arginine$^8$-vasopressin or $0.6 \cdot 10^{-9}$ molar arginine$^8$-vasotocin. Oxytocin now produced a very much greater increase in the rate of water uptake. It was possible to compare the doses of each peptide which produced 100% increase in fluid uptake, and it was found that in the presence of sodium chloride, arginine$^8$-vasotocin was 3 times more potent than arginine$^8$-vasopressin, and they were respectively 120 times and 40 times more potent than oxytocin.

While arginine$^8$-vasotocin has been variously reported to be from 200–400 times more effective than arginine$^8$-vasopressin in its action on toad bladder[4–6], the measurements were always made in the presence of $Na^+$ ion. The present experiments suggest that although arginine$^8$-vasotocin and arginine$^8$-vasopressin differ in their effect on $Na^+$ transport, they produce an identical effect on the water permeability of toad skin, and the results lend further support to the suggestion of BERDE and his coworkers[7] that the basic arginine in position 8 is significant in the production of this permeability effect[8].

*Résumé.* Des crapauds ont été employés pour doser l'action des peptides neurohypophysaires sur la perméabilité de la peau. L'arginine$^8$-vasopressine et l'arginine$^8$-vasotocine ont en absence de $Na^+$ le même effet. Elles augmentent les mouvements de l'eau, à doses variées, et leur action à toutes deux est 2000 fois plus puissante que celle de l'oxytocine. En presence de $Na^+$ l'action de l'arginine$^8$-vasotocine est 3 fois plus grande que celle de l'arginine$^8$-vasopressine. Elles sont respectivement 120 fois et 40 fois plus puissantes que l'oxytocine.

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