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THE EFFECTS OF MAMMALIAN POSTERIOR LOBE HORMONES ON THE SWELLING OF LIVER AND KIDNEY MITOCHONDRIA, IN THE RAT AND THE DOG

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SUMMARY

1. The activity of the neurohypophyseal hormones, arginine- and lysine-vasopressin and oxytocin, as mitochondrial swelling agents has been tested on the mitochondria from the liver and kidney of rat and from the cortex and medulla of dog kidney.

2. The most potent hormone in this respect is arginine-vasopressin and the least oxytocin. Rat liver was more sensitive to the swelling action of the hormones than kidney, and medullary mitochondria more sensitive than those from the cortex. Swelling activity could be detected with levels of arginine-vasopressin which approached the physiological range.

3. The role of the amino acid sequence and the disulphide group of hormones has been discussed in relation to the relative activity of the hormones and their target organ specificity.

INTRODUCTION

The recent demonstrations that many hormones can cause mitochondrial swelling[1-4] prompt the speculation that modification of the permeability of intracellular membranes may be a common mechanism by which such substances produce their action. The special actions of the hormones would then have to be accounted for in terms of the specificity of the receptor sites and the relative susceptibilities of the different target organs. In their study of the effects of the posterior lobe hormones on mitochondrial swelling LEHNINGER AND NEUBERT[4] have drawn attention to the fact that all the peptide and protein hormones which induce water uptake by mitochondria, with the single exception of adrenocorticotrophic hormone, contain disulphide linkages. As other disulphide compounds such as glutathione, exhibit similar swelling properties LEHNINGER AND NEUBERT[4] have suggested that it is the disulphide linkage which is the active group, its activity being modified by the adjacent peptide chain configuration.

The existence of two types of naturally occurring vasopressins, differing only by one amino acid (in position 8) but with different antidiuretic potency, and of oxytocin, differing from the vasopressins by two amino acids (positions 3 and 8)

allows a closer examination of the effect of these particular amino acids on the disulphide bond, and some study of the effect of these substitutions on the response of mitochondria from different tissues. The results described below show that although all three hormones are extremely effective swelling agents, there are significant differences both in their potency and in their effect on mitochondria from different organs.

METHODS

Materials

ATP (Na-salt) was obtained from the Sigma Chemical Co., St. Louis (U.S.A.). The Tris was a commercial preparation recrystallized before use. All other reagents were standard commercial preparations.

The arginine-vasopressin was a highly purified preparation which was received as a gift from Dr. M. PICKFORD who obtained it from Professor V. DU VIGNEAUD. Both lysine-vasopressin and oxytocin were synthetic products kindly made available by Messrs. Sandoz Ltd. of Basle. The lysine-vasopressin assayed 270 pressor units/mg and the oxytocin 450 units/mg.

Preparation of mitochondria

The liver and kidney mitochondria of adult male rats were prepared in 0.25 M sucrose containing 10^{-4} M EDTA.

The tissues were homogenized in a loose fitting Potter–Elvejhem homogenizer (clearance 18/1000 in) and the nuclei and debris removed by centrifugation (6000 $g \cdot$min). The mitochondria were subsequently isolated by centrifuging the liver homogenate at 10000 $\times$ g for 10 min and the kidney homogenate at 10000 $\times$ g for 15 min. They were washed twice with small volumes of 0.25 M sucrose and finally suspended in 0.125 M KCl–0.02 M Tris medium (pH 7.2) so that 1 g of tissue was equivalent to 2 ml of suspension.

The mitochondria from the cortex and medulla of dog kidney were isolated as follows. The kidneys were removed under pentothal anaesthesia and placed on ice. They were divided longitudinally and the pelvis removed. The medulla was dissected free from the cortex and the two tissues were treated separately. Each was first forced under pressure through a stainless-steel plate containing holes 0.5 mm in diameter and then homogenized in 10 volumes of 0.25 M sucrose–10^{-4} M EDTA in a Potter–Elvejhem homogenizer with a clearance of 18/1000 in. After removal of the nuclei and cellular debris, the mitochondria were isolated by centrifuging at $1.5 \cdot 10^5$ $g \cdot$min. The sedimented mitochondria were then treated as described above for rat mitochondria.

The swelling or contraction of the mitochondria was followed by measuring the changes in light absorption at 520 mμ as described by LEHNINGER[5]. The ATP solution used to reverse the swelling action of the hormones had the following composition[4]: ATP, 0.15 M; $MgCl_2$, 0.15 M; bovine serum albumin, 6.0 mg/ml (0.1 ml of this solution was added to a cuvette containing 2.0 ml).

RESULTS

Rat liver and kidney mitochondria

The effect of the three hormones on the swelling of rat liver and kidney mitochondria is shown in Table I.

Arginine-vasopressin was considerably more active than either of the other two hormones for both liver and kidney and oxytocin was the least active of the three. It is also clear that although the liver and kidney mitochondria responded to similar dilutions of both arginine- and lysine-vasopressins, the liver response was always more

TABLE I

THE EFFECT OF ARGININE^8- AND LYSINE^8-VASOPRESSIN AND OF OXYTOCIN ON THE SWELLING OF RAT LIVER AND KIDNEY MITOCHONDRIA

Final concentrations	*Liver*			*Kidney*		
	Arginine^8-vasopressin	*Lysine^8-vasopressin*	*Oxytocin*	*Arginine^8-vasopressin*	*Lysine^8-vasopressin*	*Oxytocin*
	$-\Delta A\ 520 \cdot 10^3$			$-\Delta A\ 520 \cdot 10^3$		
$3 \cdot 10^{-7}$ M	302	151	28	98	42	24
$3 \cdot 10^{-8}$ M	202	85	14	58	27	16
$3 \cdot 10^{-9}$ M	145	54	8	36	14	0
$3 \cdot 10^{-10}$ M	73	0	0	27	0	0
$3 \cdot 10^{-11}$ M	54	0	0	13	0	—
$3 \cdot 10^{-12}$ M	17	—	—	6	—	—
$3 \cdot 10^{-13}$ M	0	—	—	0	—	—
$3 \cdot 10^{-9}$ M* + 10^{-5} M GSH	—	90	46	—	38	8
$3 \cdot 10^{-12}$ M* + 10^{-5} M GSH	61	—	—	37	—	—

* 10^{-5} M GSH alone did not cause swelling.

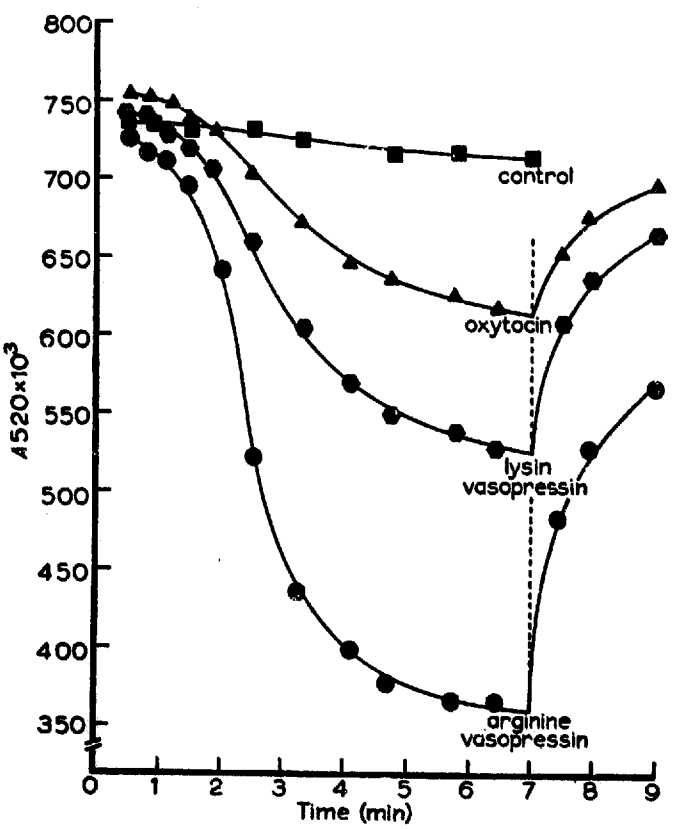

Fig. 1. The effect of argine-vasopressin (●—●), lysine-vasopressin (■—■) and oxytocin (▲—▲) on the swelling of rat-liver mitochondria. ATP (ATP, 5 mM; $MgCl_2$, 5 mM; bovine serum albumin 0.2 mg/ml final concentration) was added at the time marked by the arrow. Medium, 0.125 M KCl–0.02 M Tris. Temperature, 18°.

marked than that of the kidney. With oxytocin, however, the response appeared to be about the same for the two tissues. It is interesting to note that the lowest effective concentration of arginine-vasopressin (10^{-12} M) was of the order of concentration which approaches that encountered in biological fluids under physiological conditions. The addition of reduced glutathione potentiated the effect of all three hormones. This is in agreement with the observation of LEHNINGER AND NEUBERT[4], who first reported it. The addition of ATP (ATP, 5 mM; $MgCl_2$, 5 mM; bovine serum albumin, 0.2 mg/ml final concentration) reversed the effect of the swelling agents. The reversal was usually of the order of about 60 % of the observed fall in absorbancy caused by the hormones (Fig. 1).

It will be observed from Fig. 1 that swelling was usually complete in about 10 min and with the higher concentrations of arginine-vasopressin could be as short as 3 min. This is considerably more rapid than that described by LEHNINGER AND NEUBERT.

Dog-kidney mitochondria

Table II shows the effect of the same hormones on the swelling of mitochondria from the medulla or cortex of dog kidney.

The mitochondria from the medulla were about 1000 times more sensitive to the action of the hormones than those from the cortex. This is in agreement with the findings of BROWN AND PETKAS[6] who reported that while lysine-vasopressin enhanced the rate at which water entered the medullary mitochondria it had no effect on those from the cortex even at concentrations 10^3 times greater than those used here. The low sensitivity of their preparations, however, may be related to the fact that their experiments were carried out in 0.44 M sucrose, a concentration which LEHNINGER[5] has shown to inhibit mitochondrial swelling.

In the present experiments, arginine-vasopressin was about 100 times more potent than lysine-vasopressin which, in turn, was about 100 times more effective than oxytocin. In contrast to the results in rat, glutathione had practically no effect on the action of the hormones on dog-kidney-cortex mitochondria and only a slight one on those from the medulla. As was the case in the rat, all the swelling caused

TABLE II

THE EFFECT OF ARGININE[8]- AND LYSINE[8]-VASOPRESSIN AND OF OXYTOCIN ON THE SWELLING OF THE MITOCHONDRIA FROM THE CORTEX AND FROM THE MEDULLA OF THE DOG KIDNEY

Final concentration	*Cortex*			*Medulla*		
	$-\Delta A_{520 \cdot 10^3}$					
	Arginine[8]-vasopressin	*Lysine[8]-vasopressin*	*Oxytocin*	*Arginine[8]-vasopressin*	*Lysine[8]-vasopressin*	*Oxytocin*
$3 \cdot 10^{-8}$ M	31	17	15	160	121	90
$3 \cdot 10^{-9}$ M	12	6	6	75	57	41
$3 \cdot 10^{-10}$ M	0	0	0	64	30	22
$3 \cdot 10^{-11}$ M	—	—	—	45	12	3
$3 \cdot 10^{-8}$ M + 10^{-5} M GSH*	36	18	15	—	—	—
$3 \cdot 10^{-11}$ M + 10^{-5} M GSH*	—	—	—	56	23	13

* 10^{-5} M GSH alone had no effect on mitochondrial swelling.

by the addition of the hypophysial hormones to medullary or cortical mitochondria of the dog could be reversed up to some 60 % or so by the addition of the ATP–$MgCl_2$–bovine serum albumin solution. The rate of swelling of the mitochondria of the dog kidney was slower than that observed for the rat. The rate of swelling was fastest with arginine-vasopressin and slowest with oxytocin.

DISCUSSION

The results reported here confirm and extend those of LEHNINGER AND NEUBERT[4]. They differ, however, from those reported by these authors in that the uptake of water by the mitochondria was much more rapid and was observed for dilutions of the hormones very much greater than those reported by them, possibly by a factor of as much as 10^6.

The two main facts which emerge from the present experiments are that there is a definite element of target organ sensitivity and specificity, and that small modifications of the peptide chain of the hormone produce striking changes in potency. Between tissues it may be noted that liver mitochondria are more sensitive to the hormones of the neurohypophysis than those from the kidney (Table I) a fact which may be related to the previous observations of DICKER AND GREENBAUM[7] that liver binds and inactivates these hormones more rapidly than kidney. It may also be noted that differences exist within a single organ. Mitochondria isolated from the kidney medulla are much more sensitive than those isolated from the kidney cortex (Table II). This latter finding agrees well with the physiological observations that antidiuretic hormones act essentially on that part of the tubule which is located in the medulla.

If the suggestion[4] that the disulphide group in these hormones is the active group is valid then the results given above indicate clearly the important role played by the amino acid composition of the adjacent peptide chain. NEUBERT AND LEHNINGER[8] have studied the activity of a range of thiols and disulphides as swelling agents and found that while quite a number of them were active at concentrations of 10^{-2} M or 10^{-3} M, very few were more potent and only two had some swelling activity at 10^{-5} M. On the evidence of the present experiments oxytocin and arginine-vasopressin are 10^4 and 10^7 times more active respectively than the most active of the simple compounds tested by LEHNINGER AND NEUBERT[4]. Arginine- and lysine-vasopressins may be considered chemically, as well as in a pharmacological and evolutionary sense, as derivatives of oxytocin, *i.e.* phenylalanine^3–arginine^8-oxytocin and phenylalanine^3–lysine^8-oxytocin. Substitution of amino acids in positions 3 and 8 completely changes the pharmacological and swelling activity of these compounds while substitution of one basic amino acid for another at position 8 increases the potency of the hormone as a swelling agent 1000-fold. It seems possible therefore that these amino acids may be involved in the process of binding the hormones to the surface of the membranes, after which the possibility of mixed disulphides reactions occurring between the hormones and membrane disulphides, as envisaged by FONG, SILVER, CHRISTMAN AND SCHWARZ[9], could occur. The very large differences in activity between these three hormones and between the three hormones and the range of simpler disulphides emphasizes the point that if their common mode of action is by way of a disulphide group, then the potency of the hormones and their target specificity is governed by the adjacent peptide structure.

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