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THE DEGREE OF INACTIVATION OF THE ANTIDIURETIC ACTIVITY OF VASOPRESSIN BY THE KIDNEYS AND THE LIVER OF RATS

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Heller (1937) examining the fate of 'Pituitrin' in animals, claimed that 'When small amounts of pituitrin are intravenously injected into rabbits a large proportion is excreted in the urine in a free state'. Shannon (1942), summarizing results of experiments in animals injected with commercial preparations of posterior pituitary extracts, regarded it as established that 'the kidney can excrete the antidiuretic hormone'. No information exists which would indicate how the posterior pituitary preparations are excreted beyond Heller's (1937) conclusions that 'as the amount of hormone injected is increased the proportion of the hormone which is excreted in the urine decreases'. This would suggest that glomerular filtration might be a contributory factor, as the filtration rate of rabbits would be very small after large doses.

More recently it has been found that in rats infused with amounts of vasopressin ranging from 300 to 1500 μU/100 g, the antidiuretic activity of the urine was equivalent to $8{\cdot}1 \pm 1{\cdot}23\%$ of the dose administered (Dicker, 1954). The antidiuretic activity present in the urine was unaffected by the height of the water load, and hence by changes in glomerular filtration or by the rate of urine flow. In hypophysectomized dogs, O'Connor (1951) showed that the rate of excretion of antidiuretic activity in the urine was equivalent to 12% of the rate at which vasopressin was infused, and was, as in rats, independent of the volume of urine excreted. Finally, in man, the antidiuretic activity of the urine was found to be equivalent to 12·8% of the amount of vasopressin infused (Noble & Taylor, 1953). There can thus be little doubt that the antidiuretic activity found in the urine could account for only 10% of the activity of the vasopressin administered. Therefore vasopressin antidiuretic activity is largely destroyed in the body. This paper is concerned with the nature of this inactivation process, and incidentally demonstrates that the small amount of

antidiuretic activity which is found in the urine is not due to vasopressin but to a transformation product. A preliminary account of this work has been communicated to the Physiological Society (Dicker & Greenbaum, 1954).

METHODS

The male albino rats used for the antidiuretic assays and for the preparation of tissue slices varied in weight from 180 to 250 g. Those used for infusion of antidiuretic material were heavier (300–350 g).

Kidney slices, approximately 0·3 mm thick, were cut on a Stadie-Riggs (1944) microtome. All tissue slices were incubated in a Mg- and Ca-free Krebs-Ringer phosphate buffer solution pH 7·4 (NaCl 0·154 M, 100 parts; KCl 0·154 M, 4 parts; KH_2PO_4 0·154 M, 1 part; phosphate buffer (pH 7·4) 0·1 M, 12 parts; gassed with O_2), at 37° C. Mg and Ca were omitted, as it was found that they formed a precipitate when the solution was boiled, which adsorbed some of the antidiuretic material. The amount of antidiuretic material added to this medium never exceeded 1/10 of the total volume of 2·0 ml. of the solution in which tissue slices were incubated.

At the end of the incubation period, the reaction was stopped by plunging the tubes into boiling water for 3 min. After centrifugation the supernatant fluid was assayed for its antidiuretic activity, by intravenous injection into rats in ethanol anaesthesia, with a constant water load (Dicker, 1953). Each assay consisted of four injections, two doses of the standard and two of the unknown, the ratio, high to low dose, being the same for standard and unknown solutions. Results were expressed in terms of the antidiuretic activity of a standard solution of vasopressin.

Before rats were infused with antidiuretic substances or with urine, the animals were given an oral dose of 3·0 ml. of water/100 g body weight followed an hour later by 5·0 ml. of 12% ethanol (v/v) /100 g body weight. When the animal was anesthetized a polyethylene cannula was fixed in a jugular vein, and a glass catheter in the bladder. Urine lost during the preparation of the rat was made good by feeding the animal with the required dose of a 2% ethanol solution. The water load (8·0 ml./100 g) was kept constant during the infusion (Boura & Dicker, 1953). Infusions were made with an apparatus delivering 0·008 ml./min. Total amounts of vasopressin infused varied from 200 to 300 mU/100 g. Urine collections were made until the inhibitory effect on the urine flow had disappeared.

All experiments on animals were conducted in a thermo-regulated room, at a temperature of 23° C.

Drug. Pitressin (Parke Davis and Co.) solutions of the same batch, E. 374472, were used for the incubation of tissues, for intravenous infusions, and as standard for the estimation of antidiuretic effects.

Definitions. mU = milliunit; μU = microunit = activity of 10^{-7} ml. injectio Vasopressini B.P. The antidiuretic activity of the drug Pitressin has been referred to as 'vasopressin'.

RESULTS

In vitro *experiments.* Kidney slices (mean wet weight 50 mg) were incubated with 2·0 mU of vasopressin in the modified Krebs-Ringer solution (total volume 2·0 ml.) for 5 hr and the antidiuretic activity of the supernatant fluid was assayed at hourly intervals. In all experiments, there was a decrease of the antidiuretic activity over the first 3 hr, after which it remained constant. The mean amounts of antidiuretic activity after 3, 4 and 5 hr were equivalent to $456 \pm 19{\cdot}5$ μU (S.E. of five estimations); $451 \pm 15{\cdot}5(4)$ μU; and $452 \pm 34{\cdot}9$ μU, respectively (Fig. 1).

The constancy of the antidiuretic titre, after 3 hr of incubation, was not due

to a loss of activity on the part of the tissue slices. Kidney slices, which had been incubated with vasopressin for 4 and 5 hr and had reduced its activity to a constant level were still able to inactivate a fresh solution of vasopressin. The addition moreover of fresh kidney slices to a solution of previously inactivated vasopressin did not result in any further decrease of the antidiuretic

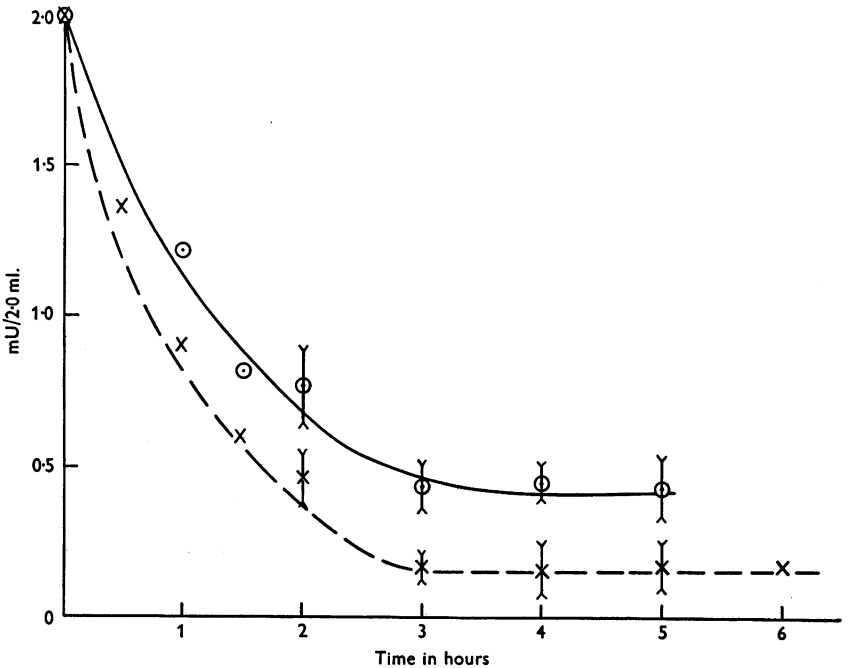

Fig. 1. Comparison of degree of inactivation of vasopressin by kidney slices and by liver slices. Inactivation by kidney slices: ⊙—⊙. Inactivation by liver slices: × – – – ×. The values for liver slices have all been corrected for the presence of an endogenous liver antidiuretic activity.

activity (Table 1A). Doubling the amount of kidney slices and incubating it with the same amount of vasopressin had no effect on the final level of residual antidiuretic activity. The time taken to reach the constant level of about 20% of the initial activity was, however, roughly halved.

As control experiments, vasopressin was incubated in the modified Krebs-Ringer solution for 5 hr in the absence of kidney slices and kidney slices were incubated for the same length of time without the addition of vasopressin. There was no decrease of the antidiuretic titre in the first series of controls, and no antidiuretic activity was found in the latter series.

Similar experiments were performed with liver slices. The inactivation of 2 mU of vasopressin by liver slices (mean wet weight 55 mg) followed a pattern comparable to that described for the kidney. The antidiuretic activity

decreased over the first 3 hr, after which it remained constant. The mean levels of antidiuretic activity after 3, 4 and 5 hr were equivalent to $185 \pm 16{\cdot}4(4)$ μU; $190 \pm 27{\cdot}2(4)$ μU; and $190 \pm 19{\cdot}1(4)$ μU—respectively. Addition of fresh liver did not produce any further decrease (Table 1 A). In contrast with kidney, however, when incubated in Krebs-Ringer solution without added vasopressin, liver slices liberated a substance which exhibited antidiuretic activity. This necessitated the determination of 'blank values' for each estimation of the antidiuretic activity of supernatant samples.

TABLE 1. The effect of adding fresh tissue (kidney or liver) to a vasopressin solution which has already been partly inactivated by tissues

Amounts of antidiuretic activity found after incubation, expressed as μU vasopressin

	1st period of incubation for 3 hr		2nd period of incubation for 3 hr	
A	2000 μU vasopressin and kidney slices	420 450 468 478 440	Addition of fresh kidney slices	430 480
			Addition of fresh liver slices	60* 60* 100*
	2000 μU vasopressin + liver slices	190 170 180 185 170	Addition of fresh liver slices	200 150
			Addition of fresh kidney slices	40* 80* 80*
	1st period of incubation for 6 hr		2nd period of incubation for 3 hr	
B	10,000 μU vasopressin + kidney slices	1990	Addition of fresh liver slices	60
	—		Liver slices (control) without vasopressin	70
	40,000 μU vasopressin + liver slices	2700	Addition of fresh kidney slices	120
	Liver slices (control) without vasopressin	100	—	

* These values could not be distinguished from the endogenous antidiuretic activity liberated by the liver (see text).

Mean wet weight of kidney slices: 50 mg (range from 45 to 54 mg)
Mean wet weight of liver slices: 55 mg (range from 52 to 58 mg).
All incubations at 37° C; in gas phase air.

The fact that the final levels of inactivation reached by kidney and liver slices were significantly different ($t = 13{\cdot}74$, $P < 0{\cdot}001$), suggested that the mechanism of inactivation must be different in the two tissues. This was unequivocally shown (*a*) by adding fresh kidney slices to a solution of vasopressin which had been inactivated by liver slices, (*b*) by adding fresh liver slices to a solution of vasopressin which had been inactivated by kidney slices (Table 1 A). In both cases, the remaining antidiuretic activity decreased to a level which could not be distinguished from that produced by the endogenous antidiuretic activity liberated by the liver. It was not possible to evaluate the degree of inactivation by the tissue slices in the second incubation. To decide whether the residual activity was vasopressin itself or partially inactivated vasopressin,

the same type of experiment was repeated using larger amounts of vasopressin (Table 1 B). If the substance after the first incubation had been residual vasopressin, final values of about 200 and 500 μU would have been expected after the second incubation. As the values obtained showed a virtually complete inactivation, the material responsible for the antidiuretic activity after the first incubation cannot have been vasopressin. Similar results were obtained when a mixture of both kidney and liver slices were incubated with vasopressin for 4 hr.

In vivo *experiments*. As the results of *in vitro* experiments suggested that both kidney and liver slices transformed vasopressin into substances of lower antidiuretic activity, it was of interest to see whether the same process of inactivation obtained *in vivo*. Four rats were given intravenous infusions of vasopressin, in amounts varying from 200 to 300 mU/100 g. Urine was collected from the onset of the infusion, until complete disappearance of the inhibitory effect of the urine flow, i.e. until 1 to 2 hr after the end of the infusion. The urine collected was assayed for its antidiuretic activity and then incubated for 4 hr either with kidney slices or with liver slices. When incubated with kidney slices the antidiuretic activity of the urine remained unchanged. When incubated with liver slices, however, it disappeared almost entirely, i.e. the antidiuretic activity decreased to a level where it was indistinguishable from that produced by the liver itself. Both kidney and liver tissues were still able to inactivate fresh vasopressin added to the urine (Table 2).

TABLE 2. Effect of incubation with liver and kidney slices on the antidiuretic activity of the urine of rats infused with vasopressin

Rats, under ethanol anaesthesia. Water load: 8 ml./100 g. Amounts of vasopressin infused: 200–300 mU/100 g. Duration of infusion: 2 hr. Urine collection from the onset of infusion until 2 hr after its end.

Treatment	Procedure	Amounts of antidiuretic expressed as μU vasopressin			
		A	*B*	*C*	*D*
	0·1 ml. urine collected from each rat contained (control)	580	1000	600	710
Incubation for 4 hr at 37° C	0·1 ml. urine + 1·9 ml. Krebs-Ringer solution	570	1030	580	720
	0·1 ml. urine + 1·9 ml. Krebs-Ringer solution + kidney slices	560	980	600	700
	0·1 ml. urine + 1·9 ml. Krebs-Ringer solution + kidney slices + 2·0 mU vasopressin	930	1450	1020	1170
	0·1 ml. urine + 1·9 ml. Krebs-Ringer solution + liver slices	240	—	—	175
	0·1 ml. urine + 1·9 ml. Krebs-Ringer solution + liver slices + 2·0 mU vasopressin	445	—	—	390
	2·0 ml. Krebs-Ringer solution + liver slices	200	—	—	180

In an attempt to see whether *in vitro* and *in vivo* experiments yielded similar results, urine obtained from a rat which had been infused with vasopressin was injected intravenously into a second rat, and both the urine infused and the urine excreted by the second rat were assayed for antidiuretic

activity. In four experiments the antidiuretic activity of the urine of the recipient was found to be equivalent to 48, 50, 54 and 59% of that of the donor (Table 3). These results would agree with the assumption that the liver destroyed the antidiuretic activity of the urine of the donor (as in *in vitro* experiments), whereas the kidneys excreted it unchanged.

TABLE 3. Amounts of antidiuretic activity excreted by a rat infused with urine collected from another rat injected with vasopressin

Rat *A*: 325 g. Ethanol anaesthesia; water load: 8 ml./100 g. Polyethylene cannula in jugular vein, catheter in bladder. Amount of vasopressin infused: 400 mU. Urine volume collected: 2·5 ml. This was made up to 5·0 ml. with H_2O, of which 0·1 ml. was used for antidiuretic activity estimation.

Rat *B*: 335 g. Ethanol anaesthesia; water load: 7·6 ml./100 g. Prepared as rat *A*. Intravenous infusion of 4·9 ml. urine from rat *A*. Urine volume collected: 7·7 ml.

Treatment			Amounts of antidiuretic activity expressed in terms of mU vasopressin
After intravenous infusion of	Rat *A*: 400 mU vasopressin	0·1 ml. urine *A*	0·800
		Thus: 4·9 ml. urine which was infused into rat *B* had a total antidiuretic activity equivalent to	39·200
	Rat *B*: 4·9 ml. urine of rat *A*	0·1 ml. urine *B*	0·025
		Thus: 7·7 ml. urine had a total antidiuretic activity equivalent to	19·250

DISCUSSION

Birnie (1953) investigated the degree of inactivation of vasopressin by kidney and liver homogenates and found, as in the present series of results, that kidney homogenates inactivate that substance only in part. He claimed that vasopressin is completely destroyed by liver homogenates. His technique of estimation, however, does not fully warrant such a conclusion, as the sensitivity of the method he used is only 2·0 mU vasopressin/100 g body weight (Burn, 1931; Heller & Blackmore, 1952). The method used in the present investigation has a sensitivity of 5 μU vasopressin/100 g (Dicker, 1953).

It will be recalled that after the injection or infusion of vasopressin into rats, dogs or man, the proportionate excretion of antidiuretic activity was about the same (O'Connor, 1951; Ginsburg & Heller, 1953; Noble & Taylor, 1953; Dicker, 1954) and was unaffected by changes in glomerular filtration or variations in urine flow (O'Connor, 1951) Assuming that the degree of inactivation of vasopressin by kidney and liver was of the same order of magnitude in both *in vitro* and *in vivo* experiments, it may be possible now to gain some insight into the fate of injected vasopressin in normal rats. Blood plasma does not seem to inactivate vasopressin (Dicker & Ginsburg, 1950) and according to Heller & Urban (1935) and to Birnie (1953) other tissues seem to be fairly inactive in that respect. As kidney slices inactivated vasopressin to a level of antidiuretic activity equivalent to about 20% of the initial amount,

and as the antidiuretic activity of urine was equivalent to about 10% only of the dose of vasopressin injected, it follows that the kidneys were able to 'clear' about half the amount of vasopressin administered. This agrees with previous findings (Ginsburg & Heller, 1953; Dicker, 1954). Further support for this conclusion could be derived from the results of experiments in which urine of a rat injected with vasopressin was infused into a second rat. The antidiuretic activity of the urine of the latter was only about half that present in the infused urine. Since the antidiuretic activity of urine from a rat injected with vasopressin was destroyed by incubation with liver slices, but unaffected by kidney tissue, it would seem reasonable to suppose that the same obtained *in vivo*, i.e. that the halving of the amount of antidiuretic activity in the urine was due to its destruction by the liver, the other half being excreted unaffected by the kidneys.

It would thus seem that in normal rats, both liver and kidneys may share about equally in the 'clearing' of injected vasopressin and that the antidiuretic activity of urine is not due to the excretion of vasopressin itself but to a substance which has an antidiuretic activity equivalent to approximately 10% of that of the dose injected. This interpretation, however, is valid only if it is accepted that the vasopressin used contained a single active substance and did not consist of fractions which could be inactivated separately by kidney or liver. Whether the same partition between liver and kidney clearances, and the same proportionate excretion of antidiuretic activity exist in cases of dehydration, of oedema secondary to malnutrition or after adrenalectomy is now being investigated.

SUMMARY

1. Kidney slices and liver slices of rats were incubated in a modified Krebs-Ringer phosphate buffer solution pH 7·4 with 2·0 mU vasopressin, at 37° C for periods of up to 6 hr, and the antidiuretic activity of the supernatant fluid assayed at hourly intervals by intravenous injections into rats in ethanol anaesthesia.

2. In the presence of both liver and kidney slices the antidiuretic activity of the supernatant fluid decreased over the first 3 hr, after which it remained constant. The final level of antidiuretic activity was equivalent to approximately 20% of the initial activity for the kidney and 10% for the liver. Liver slices alone on incubation liberated a substance which also exhibited antidiuretic activity.

3. The addition of fresh kidney or fresh liver slices to a solution of vasopressin previously inactivated by kidney or liver slices resulted in no further decrease of the antidiuretic activity. Slices of either kidney or liver which had reduced the level of vasopressin activity in one solution to a constant level could, if transferred after 5 hr, do the same to another fresh solution of vasopressin.

4. The addition of fresh kidney slices to a solution of vasopressin previously inactivated by liver, or the addition of fresh liver slices to a solution of vasopressin previously inactivated by kidney, both resulted in a further decrease of the antidiuretic activity to a level which could not be distinguished from that produced by the unidentified endogenous antidiuretic activity released by the liver.

5. The antidiuretic activity of urine collected from rats in ethanol anaesthesia during and after infusion of vasopressin remained unchanged after incubation with kidney slices. It was almost entirely destroyed after incubation with liver slices.

6. The antidiuretic activity of urine collected from rats which had been injected with urine collected from rats previously infused with vasopressin represented about 50% of the antidiuretic activity of the later.

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