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FATE AND EXCRETION OF THE PRESSOR ACTIVITY OF VASOPRESSIN IN RATS

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The fate of the antidiuretic activity of vasopressin has been investigated in experiments *in vivo* (Burn & Singh Grewal, 1951; Heller, 1952, 1953; Ginsburg & Heller, 1953; Dicker, 1954; Dicker & Greenbaum, 1954) and in experiments *in vitro* (Heller & Urban, 1935; Birnie, 1953; Dicker & Greenbaum, 1956). The antidiuretic activity of vasopressin appears to be inactivated by both liver and kidneys though the manner in which the inactivation takes place is still not clear. As for the metabolism of the pressor activity of vasopressin, little is known about its fate in the body beyond the fact that the pressor activity of post-pituitary gland extracts appears to be less stable than the antidiuretic activity (Heller, 1939).

Since vasopressin has been synthesized there can be no doubt that both activities belong to the same octapeptide molecule. According to van Dyke (1955) the pharmacological activities of the vasopressin are abolished by cleavage of the disulphide bond. As, however, the two activities of vasopressin are so different, there is a possibility that the metabolism and the rate of excretion of the pressor activity are different from those of the antidiuretic activity. The present investigation is a contribution to this problem.

METHODS

Male albino rats of approximately 250 g were used. They had been bred and reared in the Department's animal house and fed on a standard diet containing: protein 14 g, fat 4 g, and soluble carbohydrate 49 g/100 g.

The pressor activity was assayed on rats injected with urethane and dibenamine (Dekanski, 1952). The errors of the method fell within the limits stated previously (Dicker & Nunn, 1957).

Nephrectomy and evisceration were performed on rats under ethanol anaesthesia, induced by the administration of 5 ml./100 g of a 12% (v/v) ethanol solution, and maintained, if necessary, by intravenous injections of 1 ml. of a 10% ethanol solution. The 'evisceration' consisted in the removal of the whole of the gastro-intestinal tract with the pancreas and spleen: in what follows the word is used with this special meaning. The liver, however, remained *in situ*, but its whole blood supply, except that from the vena cava, was stopped by ligation of the coeliac and the superior mesenteric arteries and their branches, and of the portal vein and its branches.

The drug or urine was infused with a motor-driven tuberculin syringe, delivering 0·0083 ml./min. At this rate, it takes 60 min to infuse 0·5 ml.

Drug. A solution of vasopressin (Pitressin, Parke Davis and Co; batch LS 883H) was used throughout.

Statistical treatment. Student's *t* test was applied for estimations of the significance of differences of means, using the 'small sample' method (Mainland, 1938).

RESULTS

Dicker & Greenbaum (1956) have shown that in experiments *in vitro* the antidiuretic activity of vasopressin was partly inactivated by tissue homogenates of kidney, liver, spleen and duodenum. To see whether these tissues were involved in the destruction or inactivation of the pressor activity of vasopressin in experiments *in vivo*, the following operations were performed: (*a*) double nephrectomy, (*b*) evisceration, (*c*) double nephrectomy and evisceration. The animals were allowed to recover from the operation for at least 1 hr. When the blood pressure was constant, random doses of 5, 10 and 20 m-u. vasopressin were injected intravenously, and both the pressor effect and its duration were compared with those obtained in control and in sham-operated animals. In *all* operated animals the rise of blood pressure was markedly smaller than in control rats. The duration of the pressor effect, however, was reduced only in animals which had undergone both evisceration and nephrectomy. It was practically unaffected in sham-operated and in nephrectomized animals. The interpretation of these results was difficult, in view of the fact that after operation the blood pressure was no longer a reliable index of concentration of the hormone in blood.

Similar operations were carried out to investigate changes in the rate of disappearance of pressor activity from the blood. After recovery from operative shock, rats were slowly injected (duration: 2 min) with 400 m-u. vasopressin. Blood was taken by dividing both jugular and carotid vessels, either 5 min after the end of the injection, i.e. at the time of the maximum blood pressure rise, or between 18 and 22 min after the end of the injection when the blood pressure was back to its pre-injection level.

Blood was collected in heparinized chilled tubes, centrifuged at 4° C and its plasma assayed within 15 min. Similar experiments were carried out on sham-operated and control rats. The mean concentrations of pressor activity in the plasma of control rats were $11{\cdot}7 \pm 1{\cdot}82$ (7) m-u./ml. and $6{\cdot}0 \pm 2{\cdot}2$ (6) m-u./ml., 5 and 20 min after the end of the injection respectively. The values obtained in operated rats could not be distinguished from those in control animals (Fig. 1). In all cases, the blood pressure was back to its pre-injection level in $20{\cdot}7 \pm 0{\cdot}50$ (10) min, in spite of the fact that the pressor activity in plasma was still equivalent to $5{\cdot}0 \pm 0{\cdot}41$ (17) m-u. vasopressin/ml.

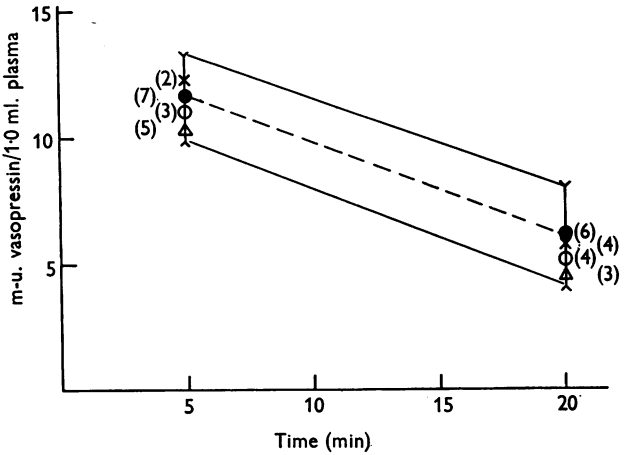

Fig. 1. Disappearance of pressor activity from the blood plasma, in control and operated animals. ● — — ●, mean of results for control rats; vertical lines, S.E. of the mean: ○, mean for nephrectomized rats; ×, eviscerated rats; △, mean for eviscerated-nephrectomized rats. The S.E. of the means for operated animals not given, as they would have obscured the S.E. of the controls. In brackets, number of animals in each series.

Urinary excretion of pressor activity

Rats under ethanol anaesthesia were injected with doses of vasopressin ranging from 40 to 400 m-u. The collection of urine lasted between 1 and 4 hr, according to the duration of the antidiuresis. The urine was mixed with one-tenth of its volume of a 3% (v/v) acetic acid solution and assayed for its pressor activity. There was a regular increase of the excretion of the pressor activity from $9{\cdot}0 \pm 1{\cdot}85$ (6) m-u. for doses of 40 m-u. vasopressin to $27{\cdot}7 \pm 3{\cdot}50$ (6) m-u. for doses of 120 m-u. vasopressin (Table 1). After the injection of 400 m-u. vasopressin, however, the excretion of pressor activity was $25{\cdot}5 \pm 2{\cdot}56$ (6) which is not statistically different from that observed after the injection of 120 m-u. ($t = 0{\cdot}920$, $P = 0{\cdot}4$). When vasopressin was *infused* over 1 hr instead of being injected there was a regular increase of the pressor activity excreted with increasing amounts of the drug (Tables 1 and 2), though the proportion of pressor activity excreted remained the same, $21{\cdot}0 \pm 1{\cdot}87$ (24)%. The pressor response to the infusion of 400 m-u. over 1 hr (i.e. at a rate of 6·7 m-u./min) can be described as follows: no change for about 7 min, then a slow rise of some 10 mm Hg, which is smaller than the pressor response to a single injection of 5 m-u.; this remained more or less unchanged for the next 40 min but was followed before the whole amount had been infused by a fall of blood pressure which eventually settled down at a level slightly below the pre-infusion level.

The question next investigated was whether the pressor activity was

TABLE 1. Amounts of pressor activity recovered from the urine of rats injected intravenously with vasopressin

Amounts injected: (m-u.) ...	40		80		120	
	(m-u.)	(%)	(m-u.)	(%)	(m-u.)	(%)
Amounts recovered	9·5	23·8	19·4	24·2	35·4	29·5
	10·9	27·3	27·8	34·8	20·5	17·1
	3·5	9·0	13·8	17·3	19·0	15·8
	3·8	10·0	25·0	31·3	21·0	17·5
	10·7	26·8	8·2	10·3	16·5	14·0
	15·2	38·0	19·5	24·4	24·0	20·0
	—	—	16·0	20·0	—	—
	—	—	14·4	18·0	—	—
Mean	9·0	22·5	18·0	22·5	27·7	19·0
S.E.	±1·85	±4·73	±2·23	±2·79	±3·50	±2·24

TABLE 2. Recovery of pressor activity from urine in rats infused with large amounts of vasopressin

Total amounts of vasopressin administered (m-u.)	Duration of infusion (min)	Amount of pressor activity in urine (m-u.)	Amount of pressor activity in urine (% of amount given)
250	60	65	26
250	60	55	22
400	60	100	25
650	90	165	25
			Mean 24·5

Rats had received 3 ml. water/100 g, followed 1 hr later by 5 ml. of 12% (v/v) ethanol solution/100 g. Infusion made through the jugular vein. Urine samples collected *before* the onset of the infusion were all devoid of pressor activity.

TABLE 3. Urinary excretion of pressor activity of rats infused with vasopressin

Description of rat A	Amount of vasopressin infused (m-u.)	Amount of pressor activity in urine of rat A (m-u.)	(%)	Amount of pressor activity infused in rat B (m-u.)	Description of rat B	Amount of pressor activity in urine of rat B (m-u.)	(%)
Normal*	400	110	24	—	—	—	—
Eviscerated (7)	120	?	?	—	—	—	—
(11)	1000	50	5	—	—	—	—
Normal (6)	400	80	20	36	Normal	21·0	59
Eviscerated (8)	1000	50	5	200**	Eviscerated	9·2	4·6
Eviscerated (4)	1000	45	4·5	180**	Normal	38·3	21·2

All animals were under ethanol anaesthesia. The results are given as means; number of experiments in parenthesis (for details see text). Experiments * represent the mean values as detailed in Table 2. Amounts marked ** were obtained by pooling urine from four rats A.

excreted in its original or in a modified form. Rats under ethanol anaesthesia were *infused* with 400 m-u. vasopressin. Their urine was assayed, and injected into other rats. The urine of the latter was likewise assayed. The mean pressor activity in the urine of the first rat was equivalent to 20% of the amount of vasopressin administered (Table 3). The pressor activity of the urine of the

second rats, however, varied widely. In the six experiments it was 100, 71, 66, 50, 40 and 25% respectively, with a mean of $59{\cdot}0 \pm 10{\cdot}6$ (6)%. Thus in five out of the six experiments the pressor activity of the urine injected into another animal was excreted at twice the rate of that of vasopressin. This suggested that the pressor activity in the urine might have been a modified form of the drug.

To ascertain whether this modification could take place in the liver, eviscerated rats, under ethanol anaesthesia, were infused with doses of vasopressin varying from 120 to 1000 m-u. With the larger doses (up to 1000 m-u.) there was a recovery equivalent to $4{\cdot}5 \pm 0{\cdot}55$ (11)% of the amount infused (Table 3). This was very different from the recovery of $21{\cdot}0 \pm 1{\cdot}87$% observed in control rats. With small amounts of vasopressin, however, no pressor activity could be detected in the urine. This was attributed to the fact that the amounts excreted were too small.

The possibility was next examined that the pressor activity found in the urine of eviscerated rats was vasopressin. Four eviscerated rats were infused with 1000 m-u. vasopressin each. The pressor activity of their pooled urines was equivalent to that of 197 m-u. vasopressin; i.e. 4·9% of the total amount administered. This now was infused into another eviscerated rat. The urine of the latter contained the equivalent of 9·0 m-u. vasopressin; i.e. 4·6% of the amount administered. In four similar experiments the mean pressor activity of the urine of the second 'recipient' eviscerated rats was $4{\cdot}7 \pm 0{\cdot}82$% of the urinary pressor activity infused (Table 3). As the injection of urine does not affect the excretion of vasopressin simultaneously administered (Dicker & Nunn, 1957), these results suggested that the 'recipient' eviscerated rat dealt with the pressor activity of the urine from the eviscerated 'donor' in the same way as the latter had done with infused vasopressin. This was also suggested by the following experiment: four eviscerated rats were each infused with 1000 m-u. vasopressin. Their pooled urines contained a pressor activity equivalent to 180 m-u. This was now infused into a control rat. The amount of pressor activity excreted by the latter was found to be equivalent to 38·3 m-u.; i.e. to 21·2% of the pressor activity administered, which is of the same order as that observed in control rats infused with vasopressin (Table 3).

DISCUSSION

It would appear from these results that the pressor activity found in the urine of *eviscerated* rats infused with vasopressin was due to the unmodified drug. As only about 5% of the administered vasopressin appears to be excreted, it follows that the remaining 95% must have been destroyed, inactivated or adsorbed somewhere in the body. Assuming that vasopressin is almost entirely inactivated by the kidneys, how does it fit in with the findings that control rats excrete a much greater proportion of the infused pressor activity

of vasopressin than eviscerated animals? There would seem to be one possible interpretation only, namely that in control rats some of the administered vasopressin has been transformed into a product which is excreted, but not inactivated, by the kidneys. Such a transformation could take place in the liver, the intestine, the pancreas or the spleen, or in all of them. If this is so, the pressor activity found in the urine of control rats injected with 100 m-u. vasopressin would consist of two fractions: one of 5 m-u. vasopressin and one with a pressor activity equivalent to 15 m-u. It can be calculated that should such a urine be injected into another control rat, it will excrete a urine containing about 75% of the administered pressor activity. The experimental value of the excretion of pressor activity by the second rat was $59 \pm 10{\cdot}6$% of the injected activity, a figure which is not very different from the calculated value.

If, however, the kidneys inactivate almost all the injected vasopressin, how is it possible that the rate of disappearance of vasopressin from the blood is the same in control, in eviscerated and in nephrectomized rats, during the 20 min period of observation following the administration of the drug? Five minutes after the *injection* of 400 m-u. vasopressin, the mean plasma concentration of pressor activity is $11{\cdot}7 \pm 1{\cdot}82$ m-u./ml. If none of the injected drug has been destroyed, as in rats of which both the kidneys and the alimentary viscera have been removed, this concentration would correspond to a 34-fold dilution of the drug. The total extracellular fluid phase of a rat of 250 g is however of the order of 65 ml. If the 400 m-u. vasopressin had been equally distributed throughout the extracellular fluid phase, its concentration in the plasma would be of the order of 6·0 m-u./ml., which is the value observed 20 min after the injection. It would then seem that in a rat without kidneys and alimentary viscera, it will take 20 min for vasopressin to be equally distributed in the body; hence the rate of disappearance of vasopressin from the blood, during the period, would represent the rate of distribution of the drug in the extracellular space. The length of time is not unreasonable when it is remembered that it takes about 1 hr for a single injection of inulin to be equally distributed between plasma and extracellular fluid (Schachter, Freinkel & Schwartz, 1950). The molecular weight of inulin is 5000, whereas that of vasopressin is only 1070. If this hypothesis is correct the rate of inactivation by the kidneys in control rats must be slow. This can also be concluded from the following experiments which belong to another line of research. Eviscerated rats infused with 1000 m-u. vasopressin were killed either immediately after the end of an infusion or 2 hr later. At the end of the infusion the pressor activity in the plasma was equal to 7·0 m-u./ml.; 2 hr later it was still 1·2 m-u./ml. The rate of disappearance of the pressor activity from the blood would appear to be only of the order of 2·7 m-u./min. Furthermore, if at the end of the infusion plasma and urine were collected and muscles and liver removed and homogenized,

it could be shown that (*a*) there was no pressor activity in the urine, (*b*) the liver contained traces of pressor activity only, (*c*) the 100 g of wet muscle, representing 40 % of the body weight, had a pressor activity equivalent to some 570 m-u., of which 114 m-u. were estimated to be in the extracellular fluid phase. The pressor activity in the plasma was equivalent to 7·0 m-u./ml., hence the total extracellular fluid, calculated as 25 % of the body weight, contained 430 m-u. vasopressin. The total recovery of pressor activity was 900 m-u., of which some 570 m-u. must have been adsorbed by the muscles.

It would then appear that after being injected or infused, vasopressin will be distributed slowly throughout the extracellular fluid phase of the body and plasma. The processes of equilibration may be complicated by the fact that a substantial portion of the drug may be adsorbed on tissues (Dicker & Greenbaum, 1956). The kidneys inactivate 95 % of the vasopressin that reaches them, and may excrete 5 % of it. It is reasonable to assume that the vasopressin adsorbed on muscles will be released in time, as there is no evidence of inactivation of vasopressin by muscle homogenates (Dicker & Greenbaum, 1956). As for the vasopressin that reaches the liver, it would seem that it is transformed into a product which is ultimately excreted but not inactivated by the kidneys. The pressor activity of the urine will therefore represent a mixture of true and of modified vasopressin.

SUMMARY

1. Intravenous injections of graded doses of vasopressin up to 20 m-u. into anaesthetized rats result in graded rise of blood pressure of graded duration. The rise of blood pressure following injections of standard amounts of vasopressin is smaller in nephrectomized, in 'eviscerated' and in sham-operated animals. The duration of the effect, however, is decreased in eviscerated rats only.

2. The rate of disappearance of the pressor activity of vasopressin from the blood from the 5th to the 20th min after administration is the same in nephrectomized, in eviscerated, in nephrectomized–eviscerated animals, and in control rats. It is due to the passage of vasopressin into the extracellular fluid phase.

3. Control rats under ethanol anaesthesia excrete in their urine 21·0 ± 1·87 % of the pressor activity administered: after evisceration, however, the urinary excretion contains only 4·5 ± 0·55 % of the pressor activity administered.

4. When the urine of eviscerated rats, with its content of 5 % of the pressor activity of the administered drug, is injected into control rats, 20 % of it is excreted, but when the urine of control rats, with its content of 21 % of the pressor activity of the administered drug is injected into another control, 60 % of it is excreted.

5. The kidneys appear to inactivate almost all the vasopressin that reaches them, whereas the liver transforms some portion of the drug into a more stable substance which is excreted by the kidneys. Thus the pressor activity found in the urine of control rats, injected with vasopressin, represents a mixture of two substances: a true and modified vasopressin.

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