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THE RELEASE OF VASOPRESSIN IN RESPONSE TO HAEMORRHAGE AND ITS ROLE IN THE MECHANISM OF BLOOD PRESSURE REGULATION

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SUMMARY

1. The release of vasopressin in response to haemorrhage and the effects of vasopressin infusions on blood pressure and heart rate have been investigated in anaesthetized dogs. Haemorrhage was produced by the method of Lamson & de Türk (1945), which allows for a precise control of the changes in arterial blood pressure.

2. Blood samples were collected from an external jugular vein, from a femoral vein or from a femoral artery and extracted with alcohol; blood extracts were assayed for antidiuretic activity.

3. Haemorrhage experiments showed that vasopressin secretion is increased when the fall in diastolic blood pressure (diastolic ΔP) is 25 mm Hg or more. Mild hypotensions (diastolic ΔP ranging from 21 to 30 mm Hg) produce an average fourfold increase in the concentration of vasopressin in blood. Such increase is maintained throughout the oligaemic period. Severe hypotensions produce, in most cases, a biphasic secretory response, with an initial high peak followed by a lower, constant, secretory plateau. In all experiments, the retransfusion of blood restored vasopressin to control levels.

4. Vasopressin infusion experiments showed that the amounts of hormone secreted in response to haemorrhage are sufficient to cause vasopressor response, provided that the buffering action of blood pressure regulation mechanisms is suppressed. It was also found that the amounts of vasopressin secreted in response to haemorrhage are apparently adequate, if the function of such secretion is to combat the hypotension which follows haemorrhage.

5. The effect of hypophysectomy on the blood pressure of animals previously submitted to bilateral division of the vagi and sinus nerves

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(deafferented animals) was also investigated. It was found that hypophysectomy is followed by a fall in arterial blood pressure which is positively correlated to the previous existing amounts of vasopressin. The time course of this hypotension is similar to that following the stopping of an infusion in a deafferented hypophysectomized animal. In some experiments it was shown that, following hypophysectomy, blood pressure can be restored to its pre-hypophysectomy level by an adequate infusion of vasopressin.

6. It is proposed that the release of vasopressin in response to stimuli arising from cardiovascular sensory receptors plays a part in the mechanism of blood pressure regulation.

INTRODUCTION

It is well established that massive amounts of vasopressin are released in response to haemorrhage in the dog, cat and rat (Ginsburg & Brown, 1957; Weinstein, Berne & Sachs, 1960; Beleslin, Bisset, Haldar & Polak, 1967). It is also known that bilateral carotid occlusion results in vasopressin secretion (Share & Levy, 1962). It was recently demonstrated, in the cat, that both haemorrhage (Beleslin *et al.* 1967) and bilateral carotid occlusion (Clark & Rocha e Silva, 1966) release vasopressin without simultaneous release of oxytocin. Clark & Rocha e Silva (1967) showed that the release of vasopressin which follows haemorrhage is mediated through afferents in the vagi and sinus nerves, since it is reduced or abolished when these afferents are divided. Such results were soon afterwards confirmed, in the dog, by Share (1967*b*).

The present investigation, of which a preliminary account has been given (Rocha e Silva & Rosenberg, 1968) is an attempt to assess a possible role of vasopressin, secreted in response to haemorrhage, in the mechanism of blood pressure regulation. Belief in the existence of such a role is strengthened by the fact that hypophysectomized animals are more sensitive to the hypotensive effects of haemorrhage than normal animals (Braun Menendez, 1934; Frieden & Keller, 1954; Turner, 1963). In this connexion it is interesting to note:

(*a*) that the relations between baro- and chemoreceptors and the secretion of vasopressin are essentially similar to those existing between these same receptors and the sympathetic vasomotor pathway (Share & Levy, 1966*a*, *b*). This means that whenever this vasomotor pathway is reflexly activated, there is a simultaneous increase in the release of vasopressin. Conversely, when the vasomotor sympathetic tone is reflexly inhibited, vasopressin secretion is also inhibited;

(*b*) that the amounts of vasopressin which are released when this neurosecretory pathway is activated are massive, being at least one hundred times greater than the amounts required to produce maximal antidiuresis;

(*c*) that large amounts of vasopressin have a vasopressor action. This action seems to have two components, a direct one, on vascular smooth muscle, and an indirect one, through the potentiation of the vasopressor action of catecholamines (Bartelstone & Nasmyth, 1965).

These facts suggest that the release of vasopressin in response to stimuli from cardiovascular sensory receptors may play a part in the mechanism of blood pressure regulation. If such is the case, the massive release of vasopressin following hypotension should also provoke maximal antidiuresis. As it is, however, such release occurs in circumstances which are known to produce a radical reduction in glomerular filtration rate, as a consequence of haemodynamic changes. It is therefore conceivable that the release of vasopressin in response to haemorrhage does not play an important role in the control of extravascular volume through its effect on the transport of water by the distal nephron.

METHODS

Experiments were performed on thirty-eight mongrel dogs weighing between 5·5 and 17·0 kg, anaesthetized with pentobarbitone sodium (30 mg/kg) given intraperitoneally. In eight experiments, supplementary doses of a fifth of the initial dose were given intravenously whenever necessary. In all other experiments a constant intravenous infusion of pentobarbitone sodium was given to maintain the depth of anaesthesia at such a level as to ensure the absence of tremor and the presence of the leg stretching reflex in response to gentle manual compression of the quadriceps surae muscle. This reflex was tested at frequent intervals. In all experiments the trachea was cannulated and arterial blood pressure was measured from a cannulated femoral artery by means of a mercury manometer and a smoked drum (five experiments) or by means of a strain-gauge transducer (Statham P23AA) and one channel of a galvanometric recorder (Sanborn Poly Viso or EM Physiograph). Heart rate was estimated by direct counting of pulses on the blood pressure record and respiratory frequency by counting the negative deflexions of blood pressure associated with respiratory movements. Body temperature was kept constant at 37–39° C by means of electric heaters. In all experiments an interval of at least 60 min was allowed between the end of surgical procedures and the collection of the first blood sample.

Blood sampling. Blood samples (4 ml.) were collected from a cannulated external jugular vein (five experiments), from a femoral vein (nine experiments) or from a femoral artery (twenty-four experiments) over a period of 30 sec with simultaneous replacement of a plasma expander solution heated to body temperature.

Haemorrhage. Haemorrhage was produced by the method of Lamson & de Türk (1945), which allows for a precise control of the changes in blood pressure: a short polyethylene cannula is introduced into a femoral artery and connected to a large bore polyvinyl cannula, the first segment of which is made into a spiral and immersed into a bath at 39° C. The far end of this cannula is connected to a reservoir which can move vertically from −80 to +220 cm, with respect to the position of the heart of the dog. The system is filled with heparinized blood from a donor animal: blood is allowed to stand at 39° C for at least 4 hr to ensure inactivation of the vasopressin released during collection. The level of blood in the

reservoir is noted. After completion of all surgical procedures, a priming intravenous injection of heparin (500 u./kg) is given; supplementary doses of 500 u. are given intravenously every hour until the end of the experiment.

Following the collection of the first sample of blood, the femoral artery is connected to the reservoir, which is kept at its lowest position until the diastolic blood pressure has fallen to the desired level. Under such conditions, blood pressure falls very rapidly and a drop of 80 mm Hg is usually attained in less than 40 sec. The reservoir is then placed at such a height as will ensure the maintenance of diastolic blood pressure at the desired level. Blood samples are collected 5, 30 and 60 min after the establishment of hypotension. Re-transfusion of the shed blood is effected by lifting the reservoir to an adequate position. The level of blood in the reservoir is noted every 5 min during the period of oligaemia. Fourteen experiments were performed: nine animals were submitted to a single haemorrhage each and five to two haemorrhages each. These nineteen haemorrhages were separated into three groups (A, B and C), according to the severity of the hypotension: group A includes haemorrhages with an average fall in diastolic blood pressure (diastolic ΔP) of $24{\cdot}7 \pm 1{\cdot}5$ mm Hg (S.E. of mean; $n = 6$); group B includes haemorrhages with an average diastolic ΔP of $43{\cdot}0 \pm 1{\cdot}7$ mm Hg ($n = 7$) and group C, haemorrhages with an average diastolic ΔP of $81{\cdot}5 \pm 2{\cdot}8$ mm Hg ($n = 6$). In order to simplify the analysis of results, each of these groups was taken as being homogeneous with respect to diastolic ΔP, which was considered to be the independent variable.

Vasopressin infusions. Nineteen experiments were performed in which the effects of constant intravenous infusions of vasopressin on arterial blood pressure and heart rate were observed. Infusions were calculated to reproduce the blood levels of vasopressin observed in the haemorrhage experiments. If the half-life and the distribution space of vasopressin are known, it is easy to predetermine the blood concentration (B in u./ml.) to be expected from an infusion (I in u./min.kg) of vasopressin:

$$B = I/C,$$

where C = vasopressin clearance rate (ml./min.kg),

but $C = (D/T\frac{1}{2}) \ln 2$,

where D = distribution space of vasopressin (ml./kg)

and $T\frac{1}{2}$ = half life of vasopressin (min)

Lauson & Bocanegra (1961) have shown that the distribution space of vasopressin is 170 ml./kg and that the half-life is 5 min, in the dog; therefore

$$C = 23{\cdot}6 \text{ ml./min.kg}$$

and $$B = (I/23{\cdot}6) \text{ u./ml.}$$

Vasopressin infusions lasted 20 min and blood samples were collected before and 5 and 20 min after the start of an infusion. A final control sample was usually collected 30–60 min after the end of the infusion. Five experiments were performed on animals submitted to the basic surgical procedure only (control animals); four experiments were performed on reserpinized animals (1 mg/kg reserpine, given intraperitoneally, 16 hr before the experiment); ten experiments were performed on animals with bilateral division of the vagi and sinus nerves (deafferented animals). In these experiments the region of the carotid sinus on each side was exposed and the carotid bifurcation removed with all its nervous and vascular connexions. The vagi were divided on each side at the level of the nodose ganglion.

Hypophysectomy. Thirteen experiments were performed in which the hypophysis was rapidly removed at some stage during the experiment. Two to five days before the actual experiment, the hypophysis was exposed by removal of the sella turcica of the sphenoid bone, under pentobarbitone anaesthesia (McLean, 1928). On all occasions the vagi and the sinus nerves were divided bilaterally before hypophysectomy. The hypophysis was removed for two different purposes:

(*a*) to suppress endogenous secretion of vasopressin, which is high after bilateral division

of the vagi and sinus nerves (Share & Levy, 1962; Clark & Rocha e Silva, 1967). This allows for a more precise evaluation of the effects of infused vasopressin. Eight experiments were performed in which vasopressin was infused to deafferented hypophysectomized animals.

(*b*) to observe the effects of hypophysectomy on the blood pressure and heart rate of previously deafferented animals. In these experiments, an initial blood sample was collected and the nerves divided. As soon as blood pressure had become stable a second sample was collected and the hypophysis removed. Two more samples were collected 5 and 20 min after the removal of the hypophysis. This procedure was performed on eleven animals. On six occasions, the same animal was subsequently used for vasopressin infusions.

In every experiment involving hypophysectomy, the animal was treated with desoxycorticosterone acetate (5 mg, given intramuscularly, on the eve and on the day of the experiment) and with prednisolone succinate (100 μg/min, given by constant intravenous infusion throughout the experiment). The removal of the hypophysis was checked by post-mortem examination.

Extraction and assay of blood samples. Blood samples were extracted with alcohol according to the method of Bisset, Hilton & Poisner (1967), with one modification: the protein precipitate was resuspended in 3 ml. alcohol in water solution (75:25) and centrifuged; the supernatant was added to the alcoholic extract.

Antidiuretic activity was assayed by intravenous injection into the water-loaded, alcohol-anaesthetized rat, according to the method of Dicker (1953) and Bisset (1962), with the modifications described by Clark & Rocha e Silva (1967). The calculation of antidiuretic activity is made from the percentage reduction in urine flow produced during the 5 min period from the 2nd to the 6th min after injection (Bisset, 1962). Standard and blood extracts were injected in volumes of 0·05–0·4 ml.

Statistical treatment. The effects of graded haemorrhage on vasopressin secretion, heart rate and respiratory frequency were assessed through analysis of variance tests. The volume of blood removed from the animal was also analysed in this manner. The logarithm of vasopressin concentration in blood was used as a measure of vasopressin secretion. This is of course only an approximate measure of the rate of secretion, since the blood concentration is determined also by the distribution volume and clearance rate, both of which probably change as a result of haemorrhage. Most investigators, however, have taken blood concentration as an index of secretion, since it is virtually impossible to measure the rate of release and since changes in blood concentration are very large, in response to haemorrhage (for a discussion of the problem, see Share, 1967*a*).

The effects of vasopressin infusions on diastolic blood pressure were analysed according to a linear regression model: the rise in diastolic blood pressure during each infusion period was plotted against the respective logarithm of vasopressin concentration in blood. Only infusions performed on deafferented hypophysectomized dogs were submitted to statistical analysis.

The effects of the removal of the hypophysis on diastolic blood pressure were evaluated by plotting the fall in diastolic pressure during the first 15 min following hypophysectomy (Y) against the logarithm of the fall in vasopressin concentration in blood following hypophysectomy (X). The correlation coefficient between these two variables was determined. The slope of one of the regression equations associated with this correlation (Y on X) was compared with the slope of the regression equation computed for the infusion experiments, according to an analysis of variance model for divergence in slopes of regression equations (Bliss, 1952).

Materials. All cannulae, glassware and needles which were to be in contact with blood were siliconed to prevent kinin formation and to retard haemolysis and clotting in haemorrhage experiments. Extracts were assayed against pituitary (posterior lobe) extract (PPLE). A laboratory standard was prepared from a sample of Third International Standard for Oxytocic, Vasopressor and Antidiuretic Substances (Bangham & Musset, 1958), according

to the specifications of the British Pharmacopoeia. Vasopressin infusions were obtained from ampoules of pitressin (Parke Davis), diluted to 2 u./ml., according to the specifications of the British Pharmacopoeia. After dilution, each batch was tested for antidiuretic activity against PPLE. Recovery tests for vasopressin in blood were performed.

RESULTS

Recovery of vasopressin from samples of blood

Preliminary experiments showed that the recovery of antidiuretic activity from samples of dogs' blood after the addition of known amounts of PPLE was lower than that reported by Bisset, Hilton & Poisner (1967) for the cat. It was also found that the plasma protein precipitate contained appreciable amounts of antidiuretic activity, which could be recovered by the resuspension of the precipitate in a 75 % alcohol solution with subsequent centrifugation. The supernatant was therefore added to the original alcoholic extract. With this modification the recovery of PPLE rose to $76{\cdot}6 \pm 3{\cdot}5$ % (S.E. of mean; $n = 8$) and the recovery of vasopressin was found to be $80{\cdot}0 \pm 3{\cdot}0$ % ($n = 8$).

Estimation of the total clearance and half-life of vasopressin

The method of constant infusion, coupled with the determination of vasopressin concentration in blood, allows for an estimation of the total clearance of the hormone. If the distribution space for vasopressin is assumed to be 170 ml./kg (Lauson & Bocanegra, 1961), the half-life of the hormone can also be estimated. Vasopressin infusions were given to seventeen dogs, eight of which had been previously hypophysectomized. Table 1 shows that there are no significant differences between expected and measured concentrations of vasopressin in blood. The half-life of vasopressin was estimated at $4{\cdot}6 \pm 0{\cdot}5$ and $5{\cdot}2 \pm 0{\cdot}4$ min for non-hypophysectomized and hypophysectomized dogs, respectively. The differences between these two groups are not significant ($P > 0{\cdot}4$).

Haemorrhage

These experiments are divided into three groups (A, B and C) according to the severity of the hypotension. The effect of haemorrhage on vasopressin secretion is shown in Table 2. In experiments belonging to group A the average vasopressin concentration rose from a control value of 12·8 μu./ml. to 64·8 μu./ml. in the 5 min sample and declined steadily throughout the oligaemic period. Re-transfusion of blood was followed by a return of vasopressin to control levels. Experiments in groups B and C show a different pattern of secretory response: in both groups the level of vasopressin rises to a high peak in the 5 min sample (average 353 μu./ml.) and then drops

to a more or less constant concentration during the rest of the oligaemic period. In both groups, re-transfusion was followed by a return of vasopressin to control levels. This biphasic response, with an initial peak, followed by a secretory plateau can be seen in the majority of the thirteen experiments in groups B and C: on eight occasions (experiments 5B, 7, 9A, 9B, 10, 11, 12 and 14) the secretion followed the pattern described above. On two other occasions (experiments 6 and 13) secretion was also biphasic, but remained high until the 30 min sample. In three experiments

TABLE 1. Measurement of the total clearance rate and of the half-life of vasopressin in hypophysectomized and non-hypophysectomized dogs

Expt. no.	Infusion rate (m-u./min.kg)	Vasopressin in blood (μu./ml.) Expected	Measured	Expected/measured	Vasopressin clearance (ml./min.kg)	Half-life (min)
		Non-hypophysectomized dogs				
15	0·8	36	50	0·72	15·9	7·9
	2·4	100	170	0·59	14·1	—
16	1·6	65	60	1·08	26·6	4·4
17	4·0	170	130	1·31	30·8	3·9
18	2·4	60	90	0·67	26·6	4·4
19	6·0	255	165	1·54	36·2	3·3
20*	6·0	255	133	1·92	45·0	3·1
21*	1·5	65	61	1·07	24·6	5·3
	3·0	130	160	0·81	18·8	—
	6·0	255	255	1·00	23·5	—
22*	1·18	50	50	1·00	23·6	5·2
	2·36	100	110	1·10	21·4	—
	4·72	200	200	1·00	23·6	—
23*	4·72	200	130	1·54	28·6	3·6
	4·72	200	165	1·21	36·2	—
Mean				1·12		4·6
S.E.				0·08 ($P > 0·4$)†		0·5
		Hypophysectomized dogs				
26	2·36	100	60	1·67	39·4	4·4
	4·72	200	330	0·61	14·3	—
27	2·36	100	100	1·00	23·6	5·4
	4·72	200	235	0·85	20·0	—
28	1·18	50	70	0·72	16·9	7·4
	2·36	100	155	0·65	15·2	—
29	4·72	200	220	0·91	21·4	5·2
	9·45	400	395	1·01	23·9	—
30	1·18	50	48	1·04	24·6	4·8
	2·36	100	92	1·09	25·6	—
31	1·18	50	51	0·98	23·2	5·1
	2·36	100	100	1·00	23·6	—
32	4·72	200	220	0·91	21·4	5·4
	9·45	400	420	0·95	22·4	—
33	1·18	50	35	1·43	33·6	3·5
	2·36	100	70	1·43	33·6	—
Mean				1·02		5·2
S.E.				0·07 ($P > 0·8$)†		0·4 ($P > 0·4$)‡

* Reserpinized animals.

† Probability of accepting the null hypothesis within each group.

‡ Probability of accepting the null hypothesis between groups.

(1B, 3B and 8) no secretory peak was observed, blood levels of vasopressin remaining more or less constant throughout the oligaemic period. It is also to be noted that two instances of biphasic secretory response occur in group A (experiments 2B and 4).

TABLE 2. The effect of haemorrhage on vasopressin secretion

Group	Expt. no.	Diastolic ΔP* (mm Hg)	Vasopressin in blood (μu./ml.) Control	Haemorrhage 5 min	Haemorrhage 30 min	Haemorrhage 60 min	Control
A	1A	21	22	50	100	65	25
	2A	23	15	25	43	20	12
	2B	21	12	149	68	47	17
	3A	25	12	25	12	25	10
	4	28	6	110	50	60	13
	5A	30	10	30	18	27	14
	Mean	24·7	12·8	64·8	48·5	40·7	14·7
B	1B	43	12	322	405	331	22
	5B	44	12	354	147	145	10
	6	37	50	306	240	100	50
	7	40	51	232	180	119	35
	8	40	12	39	50	50	39
	9A	45	17	733	160	111	9
	10	51	31	310	69	62	26
	Mean	43·0	26·4	328·0	178·7	131·1	27·3
C	3B	84	10	100	100	93	40
	9B	19	9	960	87	75	25
	11	71	15	130	70	65	13
	12	78	9	216	75	80	12
	13	80	12	455	400	150	12
	14	86	6	440	130	99	7
	Mean	81·5	10·1	383·5	143·7	93·7	18·2

Analysis of variance of the effect of haemorrhage on vasopressin secretion

Source	Sum of squares	n†	Mean square	F	P
Between rows	5·44	18	—	—	—
Between groups	3·33	2	1·66	12·59	< 0·001
Within groups	2·11	16	0·13	—	—
Between columns	16·18	4	—	—	—
Between haemorrhage and control	14·95	1	14·95	36·28	< 0·01
Within haemorrhage and control	1·23	3	0·41	—	—
Residual	5·60	72	—	—	—
Total	27·22	94	—	—	—

* Average fall in diastolic blood pressure during the oligaemic period.

† Degrees of freedom.

The analysis of variance of these results shows that the secretory response to haemorrhage is highly significant. The difference between groups is also highly significant.

Figure 1 shows the effects of haemorrhage on the heart rate and on the respiratory frequency. It also shows the amounts of blood removed from

the animal and transferred to the reservoir throughout the oligaemic period. The analysis of variance of these results (Table 3) shows that:

(*a*) there is no significant alteration of the heart rate as a result of haemorrhage;

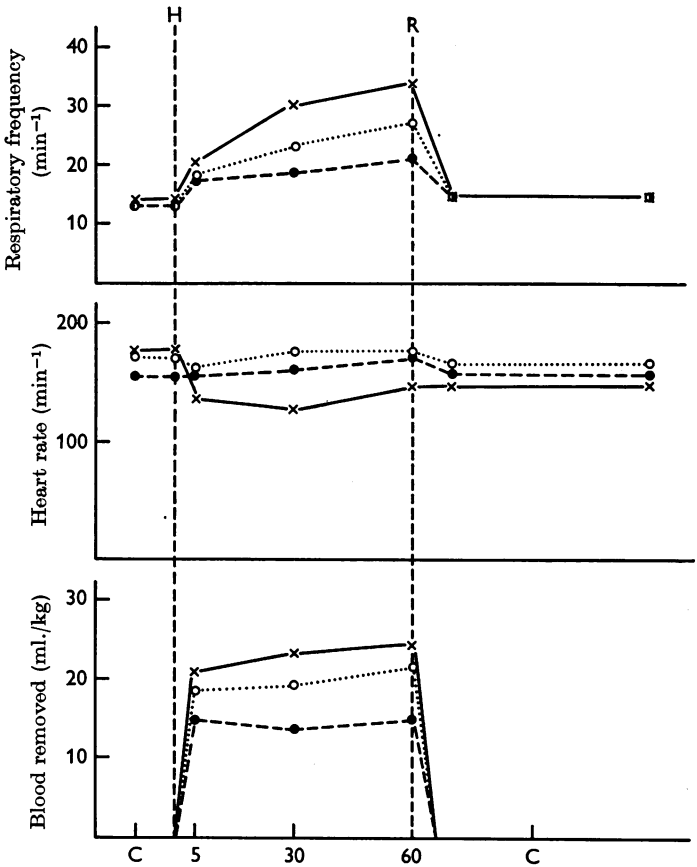

Fig. 1. The effects of haemorrhage on respiratory frequency and heart rate. The lower tracing represents the volume of blood removed from the animal during the oligaemic period. C: control periods; 5, 30, 60: time (min) from the onset of hypotension; H: haemorrhage; R: re-transfusion. Haemorrhage with diastolic ΔP = 25 mm Hg (- - - - -); 40 mm Hg(. . . . .); 80 mm Hg (——).

(*b*) respiratory frequency increases significantly as a result of haemorrhage; differences between groups are not significant;

(*c*) the amount of blood removed from the animal is not significantly related to the severity of the hypotension and remains essentially constant throughout the oligaemic period in each group.

Vasopressin infusions

Control animals. Figure 2 shows the effects of an infusion of vasopressin (4 m-u./min.kg) on arterial blood pressure and on blood levels of vasopressin. It can be seen that the effect of the infusion upon blood pressure is slight and transient. A total of six infusions, ranging from 0·8 to 6·0 m-u./min.kg were given to five control animals; measured blood concentrations of vasopressin ranged from 45 to 180 μu./ml.: in every case the

TABLE 3. Analysis of variance of the volume of blood removed during the oligaemic period and the effects of haemorrhage on the heart rate and respiratory frequency

Source	Sum of squares	*n**	Mean square	*F*	*P*
Blood removed					
Between rows	2,372·86	15	—	—	—
Between groups	495·89	2	247·95	1·72	> 0·2
Within groups	1,876·97	13	144·38	—	—
Between columns	23·20	2	11·50	1·41	> 0·2
Residual	245·70	30	8·19	—	—
Total	2,641·76	47	—	—	—
Heart rate					
Between rows	111·42	14	—	—	—
Between groups	6·28	2	3·14	0·36	> 0·2
Within groups	105·14	12	8·76	—	—
Between columns	2·01	4	—	—	—
Between haemorrhage and control	0·64	1	0·64	1·39	> 0·2
Within haemorrhage and control	1·37	3	0·46	—	—
Residual	15·27	56	—	—	—
Total	128·70	74	—	—	—
Respiratory frequency					
Between rows	6,889	18	—	—	—
Between groups	256	2	128	0·31	> 0·2
Within groups	6,633	16	415	—	—
Between columns	3,011	4	—	—	—
Between haemorrhage and control	2,507	1	2,507	14·92	< 0·05
Within haemorrhage and control	504	3	168	—	—
Residual	29,988	72	—	—	—
Total	12,838	94	—	—	—

* Degrees of freedom.

infusion produced a slight initial rise in blood pressure and a reduction in the heart rate. These effects lasted for 2–4 min and were followed by a restoration of control conditions. On stopping the infusion there was no further effect on these circulatory parameters.

Reserpinized animals. The effective suppression of sympathetic activity by reserpine was checked in two experiments of this group by tyramine injections. Figure 3 shows the effects of two infusions of vasopressin (6·0 m-u./min.kg) on arterial blood pressure, heart rate and vasopressin con-

centration in the blood. The first infusion produced a rise of 20 mm Hg in arterial blood pressure and an intense bradycardia, heart rate falling from a control value of 120 beats/min to 60 beats/min. These effects persisted until the infusion was stopped, when control conditions were restored. Between the first and second infusions the animal was atropinized; the second infusion caused a rise of 60 mm Hg in blood pressure with no change in heart rate.

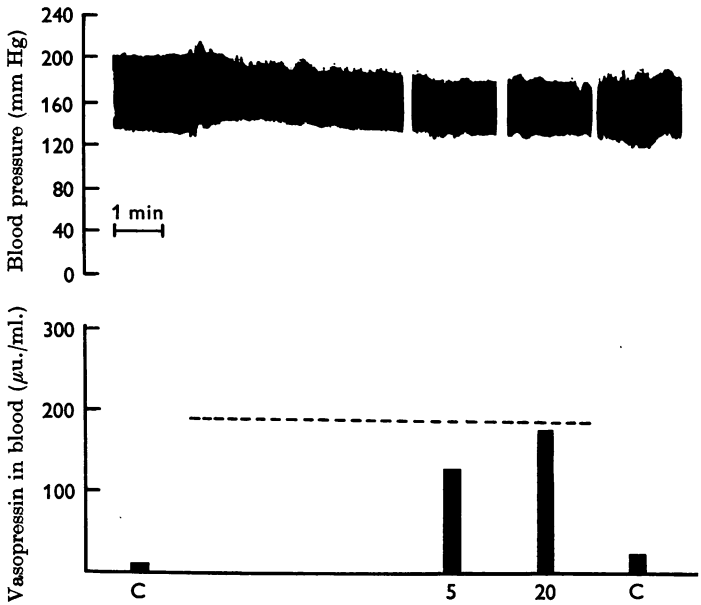

Fig. 2. The effect of an infusion of 4 m-u./min.kg vasopressin on the blood pressure and on the blood concentration of vasopressin. The interrupted line represents both the duration of the infusion and the expected blood concentration of vasopressin. Block diagrams represent measured concentrations of the hormone. C: control samples; 5, 20: time (min) from the onset of the infusion.

Figure 4 shows the effects of three infusions of vasopressin (1·18, 2·36 and 4·62 m-u./min.kg) given to a reserpinized atropinized dog; the resulting blood concentrations of vasopressin were 56, 110 and 200 μu./ml.; diastolic blood pressure increased 20, 36 and 56 mm Hg respectively. There was no change in the heart rate during any of these infusions. A total of ten infusions were performed, two in reserpinized dogs and eight in reserpinized atropinized dogs. Infusions ranged from 1·18 to 6·0 m-u./min.kg and the results agree in general with those presented in Figs. 3 and 4.

Deafferented animals. Ten experiments were performed in which vasopressin infusions were given to animals with bilateral division of the vagi and sinus nerves. Table 4 shows the results of two experiments in which the nerves were divided and vasopressin subsequently infused. In both

cases, division of the nerves resulted in an increase of vasopressin concentration in blood. Infusions further increased these concentrations as expected, but had little effect on blood pressure, even when massive doses of hormone were infused; during the last infusion of experiment 26, for

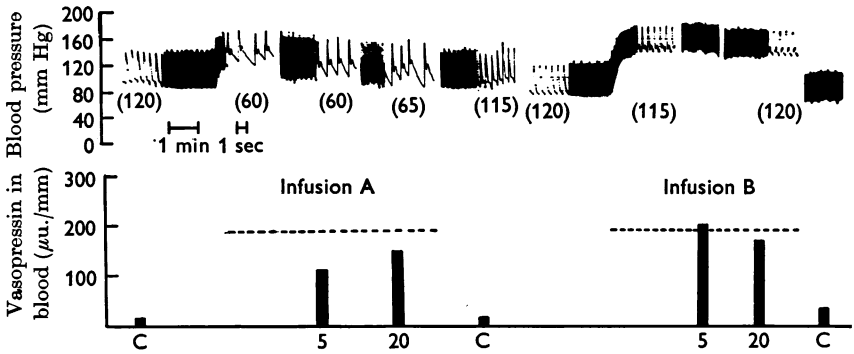

Fig. 3. The effects of infusions of vasopressin (6 m-u./min.kg) on the blood pressure, heart rate and blood concentration of vasopressin of a reserpinized dog. Between infusions A and B the animal was atropinized. Time calibration marks refer to low (1 min) and high (1 sec) blood pressure recording speeds. Figures between brackets refer to heart rates. Other conventions as in Fig. 2.

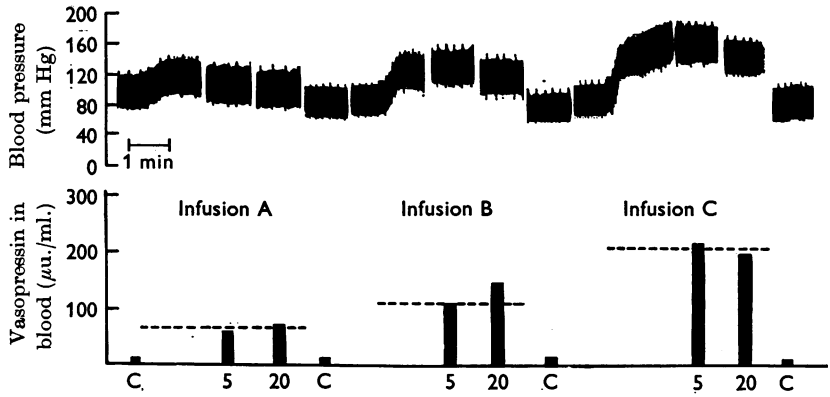

Fig. 4. The effects of vasopressin infusions on the blood pressure and on the blood concentration of vasopressin of a reserpinized atropinized dog. Infusion A: 1·18 m-u./min.kg; infusion B: 2·36 m-u./min.kg; infusion C: 4·72 m-u./min.kg. Other conventions as in Fig. 2.

instance, the concentration of hormone in blood rose to 650 μu./ml., but the effect on blood pressure was an increase of only 20 mm Hg.

To avoid the complication of a high endogenous blood level of vasopressin, infusion experiments were repeated on hypophysectomized dogs. In these, control samples contained no detectable amounts of the hormone;

nineteen infusions, ranging from 1·18 to 9·45 m-u./min.kg, were given. Blood concentrations of vasopressin ranged from 35 to 420 μu./ml. and in every case a clear cut vasomotor response, with blood pressure rising 20–50 mm Hg, was observed. The vasopressor effect of every infusion persisted until the end of the infusion, when control conditions were restored. In Fig. 5, the rise in diastolic blood pressure resulting from vasopressin infusions is plotted against the logarithm of the respective

TABLE 4. The effects of vasopressin infusions on the blood pressure of dogs with bilateral division of the vagi and sinus nerves

Expt. no.	Experimental sequence*	Vasopressin infusion (m-u./min.kg)	Vasopressin in blood (μu./ml.)	Blood pressure (mm Hg)	Diastolic ΔP† (mm Hg)
24	Control	—	25	140 × 80	—
	Bilateral division of the vagi and sinus nerves				
	Control	—	111	160 × 80	—
	5 min	2·36	153	160 × 90	10
	20 min	—	134	160 × 80	0
	Control	—	85	150 × 90	—
	5 min	4·72	400	180 × 110	20
	20 min	—	405	170 × 100	10
	Control	—	79	150 × 90	—
	5 min	9·45	605	200 × 130	40
	20 min	—	650	180 × 110	20
	Control	—	80	150 × 90	—
25	Control	—	15	200 × 140	—
	Bilateral division of the vagi and sinus nerves				
	Control	—	198	200 × 140	—
	5 min	1·18	200	200 × 140	0
	20 min	—	210	200 × 140	0
	Control	—	175	200 × 100	—
	5 min	2·36	270	200 × 110	10
	20 min	—	255	200 × 110	10
	Control	—	150	200 × 100	—
	5 min	4·72	440	220 × 120	20
	20 min	—	440	220 × 110	10
	Control	—	140	200 × 80	—

* Control: control samples; 5 and 20 min refer to blood samples collected during vasopressin infusions.

† Increase in diastolic blood pressure as compared to the pre-infusion level.

vasopressin concentration in blood. The computed regression equation of the rise in diastolic blood pressure (Y) on the logarithm of the hormone concentration in blood (X) was found to be $Y = -12{\cdot}28 \pm 20{\cdot}73X$ (equation A). This regression was found to be highly significant by analysis of variance ($F = 16{\cdot}29$, for $n_1 = 1$ and $n_2 = 16$ degrees of freedom; $P < 0{\cdot}001$).

Hypophysectomy

It has been shown above that the bilateral division of the vagi and sinus nerves results in an increased secretion of vasopressin. It was also demonstrated that deafferented hypophysectomized animals respond with a rise

in arterial blood pressure to infusions capable of maintaining blood concentrations of 50 μu./ml. or more. Such concentrations are ordinarily found after division of the nerves. The effect of the suppression of such endogenous secretion was therefore investigated. Eleven experiments were performed in which the vagi and sinus nerves were divided, blood pressure was allowed to become stable and the hypophysis rapidly removed. Blood samples were collected before division of the nerves, before and 30 min after hypophysectomy. To check whether the removal procedure did not cause excessive vasopressin secretion, a supplementary sample was

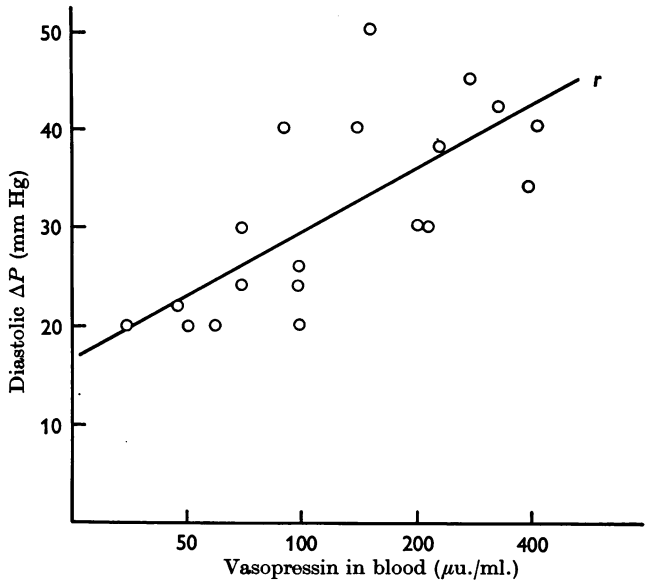

Fig. 5. The relation between the rise in diastolic blood pressure (diastolic ΔP) and the blood level of vasopressin resulting from infusions given to deafferented hypophysectomized dogs; each point represents a separate infusion; r is the regression of diastolic ΔP on the logarithm of blood concentration of vasopressin.

collected, 3–5 min after hypophysectomy, in some experiments. In all cases, this sample contained approximately half of the amount of hormone found in the pre-hypophysectomy sample. The average level of vasopressin in the first sample of these experiments was $16{\cdot}2 \pm 1{\cdot}5$ μu./ml. (S.E. of mean, eleven observations). After division of the nerves the level of hormone rose in nine out of eleven experiments to concentrations ranging from 40 to 515 μu./ml. On two occasions there was no rise. The removal of the hypophysis was followed by a slow fall in arterial blood pressure during the first 10–15 min. Afterwards, it became stable at the new level. Figure 6 illustrates this situation: the left-hand tracing shows the effect of hypophysectomy on blood pressure: after a transient hypotension at the

moment of removal of the gland, prehypophysectomy conditions were restored; during the next 10 min blood pressure fell gradually to settle at a new level. The right-hand tracing shows the effect of stopping a vasopressin infusion on the blood pressure of a deafferented hypophysectomized animal; it can be seen that the time course of the fall of blood pressure is very similar in these two situations; this suggests that the suppression of vasopressin secretion following hypophysectomy was the cause of the

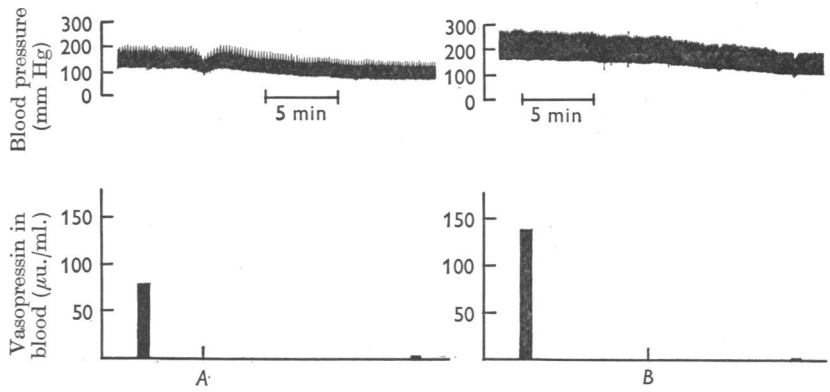

Fig. 6. The effects of hypophysectomy (*A*) and of the stopping of an infusion of vasopressin (4·72 m-u./min.kg) (*B*) on the blood pressure and on the blood concentration of vasopressin of deafferented animals.

observed hypotension. In Fig. 7, the fall in arterial blood pressure during the first 15 min following hypophysectomy is plotted against the logarithm of the fall in blood concentration of vasopressin during the same period. This fall is of course equal to the concentration of vasopressin in the sample of blood collected before the removal of the gland, since the hormone is not detectable in blood 20 min after hypophysectomy. The correlation coefficient between these two variables was found to be 0·835, which means that there is a highly significant positive correlation ($P < 0{\cdot}005$) between the fall in blood pressure and the fall in vasopressin concentration in blood. The computed regression equation of the fall in diastolic blood pressure (Y) on the logarithm of the fall in blood concentratoion of vasopressin (X) was found to be $Y = -36{\cdot}90 + 35{\cdot}84X$ (equation B). The slope of this equation ($b = 35{\cdot}84$) was compared to that of equation A ($b = 20{\cdot}73$) and it was found that there is no significant divergence between these slopes, by analysis of variance ($F = 1{\cdot}65$, for $n_1 = 1$ and $n_2 = 25$ degrees of freedom; $P > 0{\cdot}2$).

On six occasions hypophysectomy was performed as a preliminary stage of an infusion experiment. In these cases it is possible to compare the effect of the removal of the gland with the effect of an infusion of vasopressin on

blood pressure in the same animal. These results are presented in Table 5. From each experiment we have selected for presentation the infusion or infusions which most closely match the level of hormone found in the pre-hypophysectomy sample. In experiments 26, 29 and 31, hormone concentrations before removal of the gland were lower than those resulting from any of the infusions. The lowest infusion was selected, for comparison

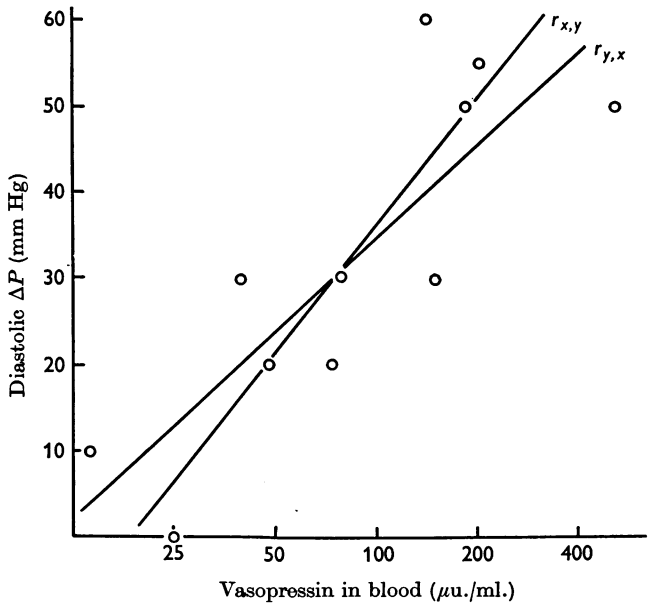

Fig. 7. The effect of hypophysectomy on the blood pressure of deafferented dogs. Ordinates represent the fall in the diastolic blood pressure (diastolic ΔP) during the first 15 min following hypophysectomy. Abscissae represent the fall in the concentration of vasopressin in the 30 min following the removal of the gland. Each point represents a separate experiment. $r_{x,y}$ and $r_{y,x}$ represent the regressions associated to this correlation.

and it can be seen that, in these experiments, the pressor effect of the infusion was greater, in absolute value, than the depressor effect of hypophysectomy. In experiment 28, the pre-hypophysectomy hormone level was higher than that resulting from any of the infusions. The highest infusion was selected for comparison and it can be seen that its pressor effect was smaller, in absolute value, than the depressor effect of hypophysectomy. In experiment 30, the pre-hypophysectomy concentration of hormone was intermediate between those resulting from two vasopressin infusions: the depressor effect of hypophysectomy was, in this case, intermediate, in absolute value, between the pressor effects of the two infusions. Experiment 27 presents a discrepancy: the pre-hypophysectomy

concentration of vasopressin was intermediate between those resulting from two infusions, but the resulting hypotension was greater, in absolute value, than the pressor effect of the highest infusion.

TABLE 5. A comparison between the vasodepressor effect of hypophysectomy and the vasopressor effect of vasopressin infusions in deafferented hypophysectomized dogs

Expt. no.	Vasopressin in blood* (μu./ml.)	Diastolic ΔP† (mm Hg)	Vasopressin in blood‡ (μu./ml.)	Diastolic ΔP§ (mm Hg)
26	14	10	60	20
27	140	60	100	24
—	—	—	235	38
28	210	55	155	50
29	150	30	220	35
30	60	30	48	22
—	—	—	92	40
31	25	0	51	20

* Vasopression concentration in blood immediately before hypophsectomy.

† Fall in diastolic blood pressure in the first 15 min following hypophysectomy.

‡ Vasopressin concentration in blood as a result of vasopressin infusion.

§ Rise in diastolic blood pressure as a result of vasopressin infusion.

DISCUSSION

The release of vasopressin in response to haemorrhage and to an acute reduction of the extracellular space is a well established fact for the cat, the dog and the rat (Ginsburg & Brown, 1957; Weinstein *et al.* 1960; Share, 1961; Beleslin *et al.* 1967). Beleslin *et al.* (1967) showed that, in the cat, such secretion occurs in the absence of oxytocin secretion. It had been earlier proposed that vasopressin secretion in response to haemorrhage was independent also in the rat (Ginsburg & Smith, 1959), but quite recently Fabian, Forsling, Jones & Lee (1968) have demonstrated that, in this species, some oxytocin is also secreted. The discrepancy must be attributed to the development of new and more sensitive methods for the assay of oxytocin in this interval (Fitzpatrick, 1961; Tindall & Yokoyama, 1962; Bisset, Clark, Haldar, Harris, Lewis & Rocha e Silva, 1967). Fabian *et al.* (1968) confirmed, however, the finding of independent release of vasopressin in the cat and, in addition, demonstrated that, in the dog, vasopressin secretion is also independent, even when the animal is bled to death. Clark & Rocha e Silva (1967) showed that the secretion of vasopressin in response to haemorrhage is mediated via afferents in the vagi sinus nerves, since such release is reduced or abolished when these nerves are divided. These results were soon afterwards confirmed by Share (1967*b*) in the dog. It seems therefore that vasopressin secretion in response to haemorrhage is mediated by similar neurosecretory reflex arcs in the dog and in the cat: the afferent pathway is the same and the release is inde-

pendent, suggesting that the efferent pathway might also be the same. The relative importance of the most likely afferents involved in this neurosecretory reflex has not been assessed: it is known that chemoreceptors (Share & Levy, 1966*a*), baroreceptors (Share & Levy, 1966*b*) and left atrial stretch receptors (Henry & Pearce, 1956; Baïsset & Montastruc, 1957; Share, 1965) are involved in the control of vasopressin secretion.

The efferent pathway of the reflex seems to have been well established by Bisset, Hilton & Poisner (1967), who showed that the electrical stimulation of the supraoptic nucleus causes independent release of vasopressin in the cat. It can therefore be assumed that inhibitory fibres arising from the left atrial stretch receptors and sino-aortic baroreceptors, as well as excitatory fibres arising from chemoreceptors impinge directly or indirectly upon the neurones of the supraoptic nucleus (Clark & Rocha e Silva, 1967). In this way, changes in blood pressure or blood volume would reflexly control the independent release of vasopressin. Such a reflex arc is well established for the cat and it is highly likely that a similar pathway might exist also in the dog.

The haemorrhage experiments described in this paper reveal some additional facts:

(*a*) the secretory response to haemorrhage in the dog has a much lower threshold than in the cat or rat: the present data show that vasopressin is released in response to a fall in arterial blood pressure of 25 mm Hg. The cat secretes vasopressin only if the fall of arterial blood pressure is 80 mm Hg or more (Beleslin *et al.* 1967); the rat secretes only when hypotension is greater than 50 mm Hg (Ginsburg & Brown, 1957);

(*b*) the secretory response becomes maximal when the fall in diastolic blood pressure is 40 mm Hg or more; in the present experiments, such hypotension usually brought diastolic blood pressure to the level of 60 mm Hg or less; this may be regarded as evidence in favour of a dominant role of baroreceptors in the genesis of the reflex, since these afferents become silent when blood pressure falls below 60 mm Hg in the dog (Heymans & Neil, 1958);

(*c*) the secretory response to severe haemorrhage (diastolic ΔP of 40 mm Hg or more usually goes through two phases: there is an initial secretory peak, followed by a secretory plateau, which lasts to the end of the oligaemic period. This can also be regarded as evidence in favour of a dominant role of baroreceptor afferents, since it is known that such receptors are specially sensitive to variations in blood pressure (Ead, Green & Neil, 1952). Therefore, the sudden change in blood pressure due to the onset of haemorrhage would be a much more powerful stimulus upon baroreceptors than the continued maintenance of hypotension;

(*d*) there is no significant change in the heart rate as a consequence of

hypotension; this is in good agreement with the findings of Wiggers (1950), who showed that the heart rate does not usually change, in response to haemorrhage, when it is high in the control period; such is the case in the present experiments, due to the use of pentobarbitone sodium as an anaesthetic;

(*e*) the increase in respiratory rate is a common finding in haemorrhage experiments and may be taken as evidence for the activation of chemoreceptor reflexes (Landgren & Neil, 1951).

(*f*) the volume of blood removed from the animal is not proportional to the degree of hypotension and remains essentially unchanged throughout the oligaemic period. The former fact may be due to the relatively short series of experiments, whereas the latter shows that under the conditions imposed in the present experimental setup, the irreversible stage of haemorrhagic shock has not been reached (Chien, 1967).

Infusion experiments represent an attempt to find out whether the amounts of vasopressin released in response to haemorrhage have any effect on the circulatory system. Observations were therefore limited to infusions which reproduce the blood levels of vasopressin observed in response to haemorrhage. The effects of such infusions on control animals were light and transient, which confirms the existing evidence that vasopressin only exhibits a pressor effect in normal animals at very high and unphysiological concentrations: if the half-life of vasopressin in the dog is 5 min, the threshold for vasopressor action (Nakano, 1967) is equivalent to blood concentrations of vasopressin of the order of 1 m-u./ml. Such concentrations seldom occur in the dog, even in the presence of the severest hypotension.

The normal animal however is a very inadequate model for the assessment of the effect of vasopressin on blood pressure of the hypotensive animal. This is due to the fact that the pressor action of infused hormone will be counteracted by a resetting of the sympathetic vasomotor tone in response to stimuli arising from cardiovascular sensory receptors (Gardier, Richards, James & Wheele, 1965). Vagal tone to the heart is also reset in a similar manner as is shown in the present experiments, where the slight rise in arterial blood pressure during the first minutes of infusion was always accompanied by bradycardia.

Infusions were therefore repeated in two different experimental conditions, having one feature in common, namely the suppression of the blood pressure regulating reflexes.

Experiments performed on reserpinized animals (i.e. with suppression of the efferent sympathetic vasomotor pathway) show a lower threshold for the vasopressor action of vasopressin. On the other hand they confirm the buffering role of blood pressure regulating mechanisms, since infusions

produce slight rises in blood pressure and sharp decreases in the heart rate. The bradycardic component of the response was abolished by atropine, thus revealing its vagal origin. Experiments performed on reserpinized atropinized animals (i.e. with complete suppression of the efferent pathways to the circulatory system) confirm the results of Nash (1965), who showed that the threshold for vasopressor action of vasopressin is lower in the reserpinized animal. They also show that the amounts of vasopressin secreted in response to haemorrhage are certainly sufficient to produce a vasopressor response in the reserpinized atropinized preparation.

These results cannot, however, be extrapolated to the haemorrhage situation without taking into account two important qualifying factors: on one hand the reserpinized preparation has a very low vasomotor tone, which renders it more sensitive to the action of vasopressor agents, whereas the hypotensive animal has a high vasomotor tone; from this point of view, the effects of vasopressin in the reserpinized animal must be regarded as an overestimation of the effect of vasopressin released in response to haemorrhage; on the other hand, it has been recently demonstrated that, apart from its direct vasopressor action, vasopressin has an indirect action on vasomotor tone, through the potentiation of the pressor actions of the catecholamines (Bartelstone & Nasmyth, 1965; Traber, Gary & Gardier, 1967); this effect is observed *in vivo* and *in vitro* and can be elicited with subpressor doses of vasopressin; from this point of view, the effects of vasopressin infusions to reserpinized animals represent an underestimation of the effect of vasopressin released in response to haemorrhage; this is because reserpinized animals have no stocks of catecholamines, whereas the hypotensive animal has large amounts of circulating epinephrine and norepinephrine (Watts, 1956; Millar & Benfey, 1958; Richardson, 1965).

These problems are solved by the suppression of the afferent pathway of the blood pressure regulating reflexes, i.e. by the division of the vagi and sinus nerves: the most important consequence of such a procedure is to release the sympathetic vasomotor tone from the inhibitory control of baroreceptors; the resulting preparation has a high vasomotor tone and no reflex regulation of blood pressure. This preparation is also known to be more sensitive to the vasopressor action of vasopressin (Gardier & Abreu, 1958); moreover, it has very important points of similarity with the animal submitted to haemorrhage: both have a high vasomotor tone and, in both, the baroreceptors are silent. It presents, however, one difficulty: the suppression of the inhibitory baroreceptor tone is reflected upon vasopressin secretion, which is also under its control (Share & Levy, 1962; Clark & Rocha e Silva, 1967). This results in increased vasopressin secretion; infusions given to this preparation will not reveal the entire magni-

tude of the vasopressor effect of vasopressin, since they will add themselves to a high endogenous blood level of the hormone. The problem is overcome by the removal of the hypophysis: in this condition it can be seen that infusions producing blood levels of vasopressin of 35–420 μu./ml. exhibit a clear cut vasomotor effect and that this effect is proportional to the logarithm of the blood concentration of the infused hormone.

It is also interesting to note that the amounts of vasopressin secreted in response to haemorrhage are apparently adequate, if the function of such secretion is to participate in the mechanism of blood pressure regulation. It was shown that haemorrhages with a diastolic ΔP of 25 mm Hg increase blood vasopressin to an average 65 μu./ml.; such a concentration, produced by infusion, increases blood pressure by 20 mm Hg; haemorrhages with a diastolic ΔP of 40 mm Hg are associated to blood levels of 150–350 μu./ml.; such levels, produced by infusion, increase diastolic blood pressure by 30–40 mm Hg.

Hypophysectomy experiments provide further evidence for the role of vasopressin in the mechanism of blood pressure regulation; they show that the high vasomotor tone which follows division of the vagi and sinus nerves is partly due to the presence of the hypophysis; the removal of the gland is followed by a fall in arterial blood pressure, the time course of which closely resembles that which follows the stopping of a vasopressin infusion. The high positive correlation between the fall in arterial blood pressure and the fall in vasopressin concentration following hypophysectomy, as well as the lack of significant divergence between the slopes of equations A and B, show that the cause of the hypotension is the suppression of vasopressin secretion. In six experiments, it was further demonstrated that adequate infusions of vasopressin restored blood pressure to pre-hypophysectomy levels. These experiments afford direct proof of the role of endogenously secreted vasopressin in the mechanism of blood pressure regulation.

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