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AN AFFERENT PATHWAY FOR THE SELECTIVE RELEASE OF VASOPRESSIN IN RESPONSE TO CAROTID OCCLUSION AND HAEMORRHAGE IN THE CAT

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SUMMARY

1. The release of neurohypophysial hormones in response to carotid occlusion and haemorrhage has been studied in anaesthetized cats. Samples of jugular venous blood were extracted with alcohol and the extracts assayed for antidiuretic and milk-ejecting activity.

2. The release of vasopressin in response to bilateral occlusion of the common carotid arteries has been confirmed in the cat; this effect was abolished when the sinus nerves were divided.

3. Using a new sensitive preparation for the assay of milk-ejecting activity in blood extracts, it has been shown that no oxytocin accompanies the release of vasopressin during carotid occlusion.

4. The independent release of vasopressin without oxytocin during haemorrhage has also been confirmed, and the role of the sinus nerves and vagi in this response investigated. Bilateral division of either nerve reduced the response, but the vagus appeared to be of greater importance than the sinus nerve.

5. A reflex arc for the selective release of vasopressin has been proposed, of which the fibres of the sinus nerves and vagi form the afferent component and the neurones of the supraoptic nucleus the efferent component.

INTRODUCTION

It is known that in the rat and cat, haemorrhage results in a release of vasopressin from the neurohypophysis without a simultaneous release of oxytocin, and it is thought that the stimulus for the release is the fall in blood pressure (Ginsburg & Smith, 1959; Beleslin, Bisset, Haldar & Polak, 1967). A fall in blood pressure would be expected to stimulate chemo- and

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baroreceptors and this in turn might result in stimulation of the supraoptic and paraventricular nuclei of the hypothalamus via afferent fibres in the sinus nerves and vagi. Belief in the existence of such a pathway is strengthened by the finding that carotid occlusion in the vagotomized dog causes the release of vasopressin and that this release is abolished by cutting the sinus nerves (Share & Levy, 1962).

The present investigation, of which a preliminary account has been given (Clark & Rocha e Silva, 1966), is based on the assay of both vasopressin and oxytocin in the same sample of blood (Bisset, Hilton & Poisner, 1967). The work was designed to determine whether, like haemorrhage, carotid occlusion results in a selective release of vasopressin; and in addition, to determine the effect of section of the sinus nerves and vagi on the release of vasopressin in response to haemorrhage.

METHODS

Experiments were performed on twenty-two female cats weighing between 2·5 and 3·0 kg, all but two of which were anaesthetized with pentobarbitone sodium (36 mg/kg) given intraperitoneally. Supplementary doses of a fifth of the initial dose were given intravenously whenever necessary. In two experiments, anaesthesia was induced with ethyl chloride and ether, and then maintained with chloralose (60 mg/kg) given intravenously. In all experiments the trachea was cannulated and arterial blood pressure was recorded from a cannulated femoral artery by means of a strain gauge tranducer (Statham P 23 A) and a potentiometric recorder (Goerz type RE 511).

Blood sampling. Blood samples were collected in most experiments from a cannulated internal jugular vein. The contralateral internal jugular vein was ligated at the beginning of each experiment and both external jugular veins were occluded during blood sampling. In six animals where no internal jugular vein was present, blood was collected from an external jugular vein with occlusion of the contralateral vessel. Since each animal served as its own control, these experiments have not been dealt with separately. Each sample of blood (4 ml.) was withdrawn at a steady rate over a period of 2 min with simultaneous replacement into a femoral vein of an equal volume of dextran solution heated to body temperature. Repeated blood sampling in control animals did not alter the blood pressure, nor did it provoke a release of hormone.

Carotid occlusion. In the dog, release of vasopressin during carotid occlusion has been found to occur only after vagotomy (Share & Levy, 1962); since in the cat the aortic nerve occasionally runs in the sympathetic sheath (Agostini, Chinnock, Daly & Murray, 1957), both the vagi and the sympathetic trunks were divided in the mid cervical region in each experiment. One hour after the completion of surgery, both common carotid arteries were occluded with 'bulldog' arterial clamps below the level of the superior thyroid arteries for a period of 5 min. Blood samples were collected 5 min before and 2 min after applying the clamps. In some experiments a further sample of blood was collected 40 min after releasing the clamps.

Division of the sinus nerves. The region of the carotid sinus on each side was exposed under a dissecting microscope and the sinus nerve identified and divided. The sinus and the carotid body were then painted with 4% phenol solution. Following denervation, an interval of 1 hr was allowed to elapse before repeating carotid occlusion.

Haemorrhage. Fourteen experiments were performed in which blood was rapidly withdrawn from a cannulated femoral artery into a syringe containing 0·5 ml. of 0·1% Heparin,

in sufficient volume to cause an abrupt fall in arterial blood pressure of 90–120 mm Hg within 20–30 sec. It has been found that a fall of at least 80 mm Hg is required in the cat to stimulate the release of vasopressin (Beleslin *et al.* 1967). Blood samples were collected 5 min before and 5 and 20 min after blood withdrawal. The blood was then slowly reinjected into a cannulated femoral vein. This procedure was performed 3 times in each animal. In one group of four cats one haemorrhage was performed before, and a second after bilateral division of the sinus nerves, care being taken to preserve the blood supply to the nodose ganglion, and a third after bilateral division of the vagosympathetic trunks. In a second group of four cats the procedure was reversed, i.e. the vagosympathetic trunks were divided first, and in a third group all three haemorrhages were performed in animals with these nerves intact.

Since the release of vasopressin is considered to be more closely related to the degree of hypotension than to alterations in blood volume, in each experiment the fall in blood pressure in the first haemorrhage was matched by that in the second and third. Where possible, similar volumes of blood were withdrawn in all three haemorrhages. If, after division of the sinus nerves and vagi, the blood pressure fell very rapidly, the withdrawal of blood was continued more slowly until the required volume had been removed. A period of 1 hr was always allowed between the completion of any surgical procedure and a haemorrhage and at least 1½ hr between successive haemorrhages.

Two additional experiments were performed in cats under chloralose anaesthesia to examine the possibility of a selective block of oxytocin release by pentobarbitone sodium. One haemorrhage only was performed in each cat.

Throughout each experiment the general condition of the animals remained satisfactory, and restoration of blood volume was always followed by an immediate return of blood pressure to control levels.

Extraction and assay of blood samples. Blood samples were extracted with alcohol according to the method of Bisset *et al.* (1967), and were assayed for antidiuretic and milk-ejecting activity.

Antidiuretic activity (ADA) was assayed by intravenous injection into the water loaded rat under alcohol anaesthesia according to the method of Bisset (1962) which is based on that of Dicker (1953). Two further modifications have been introduced. Intragastric replacement of the water load has been abandoned in favour of an intravenous infusion of a solution containing glucose, 0·11 M; NaCl, 0·03 M and ethanol, 3 % (v/v). Fluid balance was maintained automatically, but instead of using the intermittent motor operated syringe described by Bisset *et al.* (1967) the infusion fluid was delivered continuously by a servo-controlled system (J. E. Lewin, unpublished). Not only is there no danger of gastric regurgitation using the intravenous route, but diuresis is established more rapidly and maintained for longer periods. Urine flow was measured using a phototransistor drop detector with an integrating circuit which displayed the flow for each minute on a potentiometric recorder (Goerz type RE 511). Simultaneously a printing counter (Sodeco) progressively recorded the total number of drops each minute to facilitate calculations (J. E. Lewin, unpublished) The calculation of ADA 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.

Milk-ejecting activity (MEA) was assayed by a new method recently developed in our laboratory (G. W. Bisset, B. J. Clark, J. Haldar, M. C. Harris, J. P. Lewis & M. Rocha e Silva, unpublished experiments). Lactating rats, separated from their litters 3–21 days after parturition, were anaesthetized with pentobarbitone sodium (45 mg/kg) given intraperitoneally. A fifth of the initial dose of anaesthetic was given intravenously whenever necessary. Milk-ejection pressure was recorded from a cannulated teat of an abdominal mammary gland by means of a strain gauge transducer (Statham, type P 23 A) and potentiometric recorder (Leeds & Northrup, Speedomax, type H). Standard and blood extracts (0·2–0·4 ml.) were given by rapid retrograde injection through a cannula introduced into

the saphenous artery to a point just distal to the origin of the superficial epigastric artery. In our experience this preparation is at least 10 times more sensitive than the lactating guinea-pig, giving a measurable response usually to 5 μ-u. and occasionally to 1 μ-u. of posterior pituitary standard.

Calculation of vasopressin and oxytocin in blood extracts. The concentrations of vasopressin and oxytocin were calculated from the assays of ADA and MEA respectively. The ADA in a blood sample is equivalent to the concentration of vasopressin, but the MEA does not necessarily represent the concentration of oxytocin, because vasopressin itself has MEA. In a series of eight experiments (G. W. Bisset *et al.* unpublished) synthetic arginine vasopressin was assayed against pituitary (posterior lobe) extract (PPLE) for ADA, using the water loaded rat preparation, and for MEA by retrograde arterial injection into the lactating rat. It was found that an amount of arginine vasopressin with ADA equivalent to 100 μ-u. PPLE had MEA equivalent to $13{\cdot}6 \pm 0{\cdot}59$ μ-u. PPLE (mean $\pm$ S.E.). In calculating the level of oxytocin in blood samples an allowance was made for the intrinsic MEA of vasopressin. This is illustrated in the following example. If 1 ml. of a sample tested against standard PPLE was found to contain 200 μ-u. ADA and 40 μ-u. MEA, 27·2 μ-u. of the MEA could be attributed to vasopressin (i.e. 13·6 % of the ADA), and the oxytocin content of the sample would therefore be 12·8 μ-u.

Posterior pituitary hormones were identified by incubating the blood extracts with 0·01 M sodium thioglycollate for 30 min at pH 7·5 (Van Dyke, Chow, Greep & Rothen, 1942).

Statistical treatment. The results were analysed by taking the differences between logarithms of hormone levels obtained from the same cat in the same experiment. Student's t-test was applied to determine whether or not the mean differences were significantly greater than zero.

Materials. All polythene cannulae, glassware and needles which were to be in contact with blood samples were siliconed (Silicone Repelcote, Hopkin & Williams) to prevent kinin formation.

Extracts were assayed against pituitary (posterior lobe) extract (PPLE). A laboratory standard containing 2 u./ml. was prepared from a sample of Third International Standard for Oxytocic, Vasopressor and Antidiuretic Substances (Bangham & Mussett, 1958) according to the specifications of the British Pharmacopoeia.

The dextran solution was Dextran Injection B.P. (Intradex, Glaxo).

RESULTS

Carotid occlusion. Bilateral occlusion of the carotid arteries in the vagotomized animal resulted in a selective release of vasopressin which was abolished by division of the sinus nerves. This result was obtained in six experiments in which blood samples were collected before and during occlusion with the sinus nerves intact and again after they had been divided (Table 1). With intact sinus nerves, the concentration of vasopressin rose from a control level of $87 \pm 24{\cdot}3$ μ-u./ml. (mean $\pm$ S.E.) to $302 \pm 60{\cdot}9$ μ-u./ml. (mean $\pm$ S.E.) during occlusion (mean differences of log responses significantly greater than zero $P < 0{\cdot}001$). In four experiments the vasopressin concentration was estimated in blood samples taken 40 min after the carotid clamps had been released. In each case the concentration had fallen to a level approaching the control value. In Expts. 1 and 5 the carotid arteries were occluded twice before division of the sinus nerves; the level of hormone rose each time showing that the response to

occlusion is reproducible in the same animal. After division of the nerves the control level of hormone was redetermined ($103 \pm 18{\cdot}9$ μ-u./ml. mean $\pm$ S.E.). But when the carotid arteries were again occluded, the vasopressin level ($91 \pm 23{\cdot}8$ μ-u./ml.) was not significantly different from the control (mean difference greater than zero $P > 0{\cdot}6$).

TABLE 1. Release of posterior pituitary hormones by carotid occlusion in cats with vago-sympathetic trunks divided (pentobarbitone sodium anaesthesia)

	B.P. rise during carotid occlusion	μ-u./ml. blood before, during and after carotid occlusion						
		Vasopressin			MEA		Oxytocin*	
Expt.	(mm Hg)	Before	During	After	Before	During	Before	During
				Sinus nerves intact				
1	60	34	255	62	< 20	< 17	< 15	0
1	70	62	250	—	< 6	< 7	0	0
2†	80	82	304	—	< 5	< 15	0	0
3	80	61	147	101	7	20	0	0
4	50	250	530	274	15	25	0	0
5	80	92	275	38	9	15	0	0
5	70	38	80	—	6	7	1	0
6†	70	79	579	—	< 10	< 15	0	0
Mean	70	87	302	—	—	—	—	—
S.E.	—	24·3	60·9	—	—	—	—	—
		$P < 0{\cdot}001$						
				Sinus nerves divided				
1	20	185	125	95	< 7	< 7	0	0
1	0	95	82	—	< 15	< 15	< 3	< 5
2†	30	53	30	—	5	2·5	0	0
3	10	150	125	—	18	12·5	0	0
4	20	117	188	112	12·5	12·5	0	0
5	30	62	88	62	6	6	0	0
5	20	62	62	—	12	12	4	4
6†	—	—	—	—	—	—	—	—
Mean	19	103	91	—	—	—	—	—
S.E.	—	18·9	23·8	—	—	—	—	—
		$P > 0{\cdot}6$						

* After correction for the milk-ejecting activity of vasopressin (see text).

† External jugular blood.

Oxytocin was never released during occlusion either before or after division of the sinus nerves. Although measurable amounts of MEA were detected in eighteen samples, in only three of them (two controls and one test) was it greater than the intrinsic MEA of the vasopressin in the sample. The remaining twelve values indicate the smallest amount of MEA which could have been detected at the limit of sensitivity of the assay.

That the active substance in the blood extracts was vasopressin was evident from the typical antidiuretic response in the rat urine flow preparation (Beleslin *et al.* 1967). In addition, the MEA of the extracts, which was considered to be due to vasopressin, was reduced by 90% in two of them following incubation with sodium thioglycollate. It is possible that

the absence of MEA in many of the samples was due to substances inhibiting the response of the mammary gland. To investigate this, PPLE was added to ten blood extracts in amounts sufficient to give a sub-maximal milk-ejection response, and in no case was the effect of the mixture less than that of the same amount of PPLE in 0·9 % NaCl solution.

Haemorrhage. In intact animals, the concentration of vasopressin in blood greatly increased following haemorrhage and only returned to control levels after the blood volume had been restored. The response was reduced

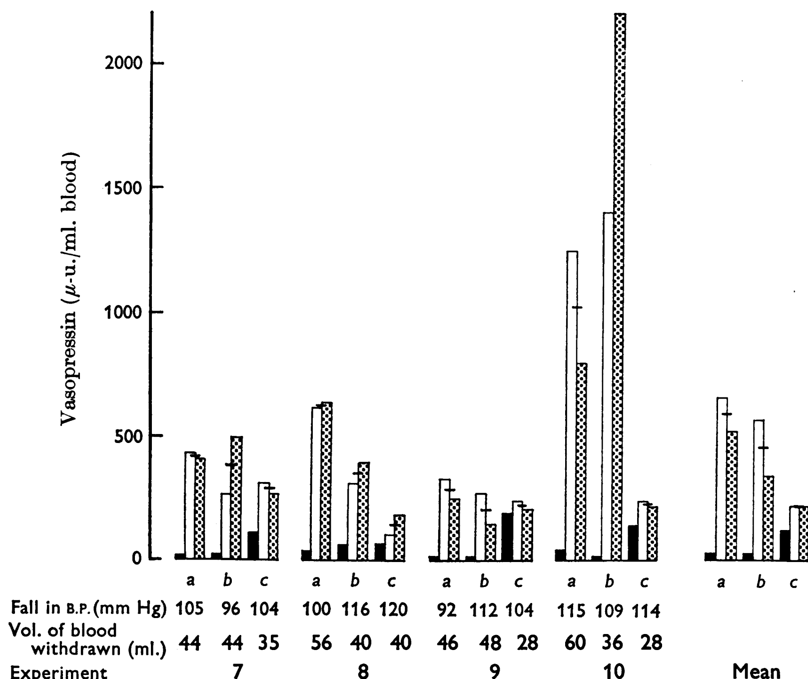

Fig. 1. Release of vasopressin in response to haemorrhage (sinus nerves divided first). ■ 5 min before haemorrhage. □ 5 min after haemorrhage. ▩ 20 min after haemorrhage. — Mean of 5 and 20 min samples. (*a*) Nerves intact. (*b*) Sinus nerves divided. (*c*) Sinus nerves and vagi divided.

when the sinus nerves and the vagi were divided. However, the levels of vasopressin in the two blood samples taken following a haemorrhage were often widely different. Because of this, when describing the results shown in Figs. 1–3, comparisons have been made between the means of the hormone levels in the 5 and 20 min samples.

Division of the sinus nerves with the vagi still intact (Fig. 1) resulted in a small reduction in the mean vasopressin release following haemorrhage. However, the effect in Expt. 7 was minimal and in Expt. 10 the 5 min

sample contained a higher level of hormone than either sample taken after haemorrhage in the intact animal, and the level of hormone in the 20 min sample was even higher. This latter result is not included in the mean values shown, since during collection of the sample the blood pressure fell again to a level 80 mm Hg below the pressure before haemorrhage and the high hormone content of the sample was thought to be due to this additional stimulus. When the vagi were subsequently divided, the control level of vasopressin in three of the experiments rose and the increases in hormone concentration above control level in response to haemorrhage in all four experiments diminished.

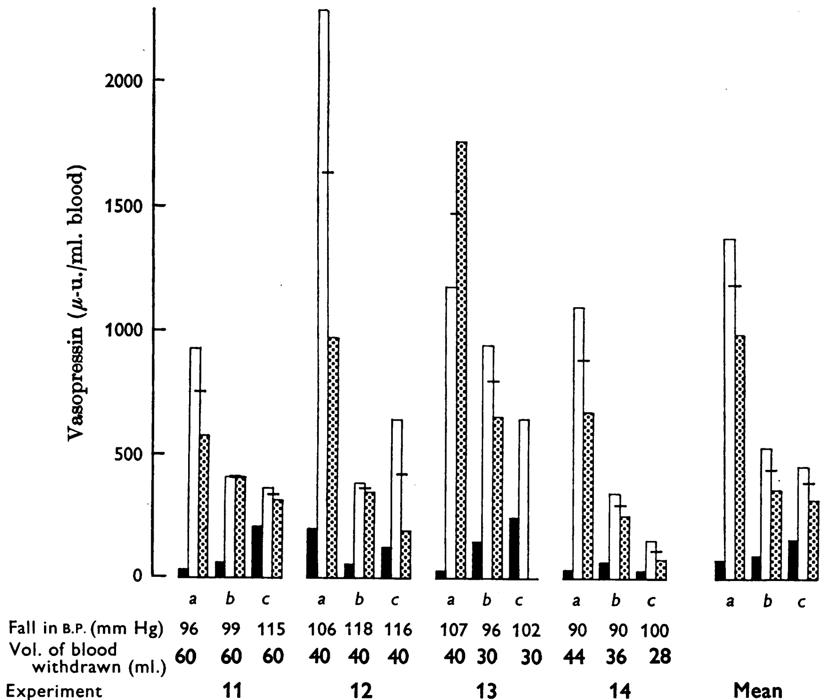

Fig. 2. Release of vasopressin in response to haemorrhage (vagus nerves divided first). ■ 5 min before haemorrhage. □ 5 min after haemorrhage. ▩ 20 min after haemorrhage. — Mean of 5 and 20 min samples. (*a*) Nerves intact. (*b*) Vagus nerves divided. (*c*) Sinus nerves and vagi divided.

When the procedure was reversed, more consistent results were obtained (Fig. 2). Division of the vagi alone substantially reduced the response to haemorrhage in all four experiments. Subsequent division of the sinus nerves resulted in an increase in the control level of hormone in three experiments and a further reduction in response in all four experiments.

Table 2 combines the results of these eight experiments and shows the

responses to haemorrhage in the intact animal and those following bilateral division of both the sinus nerves and the vagi (i.e. haemorrhages *a* and *c* in Figs. 1 and 2).

In the intact animal the level of vasopressin rose from a control value of 47 ± 22 μ-u./ml. (mean + S.E.) to 1020 ± 220 μ-u./ml. (mean ± S.E.) 5 min after haemorrhage, and remained high at 763 ± 169 μ-u./ml. (mean ± S.E.) 20 min after haemorrhage (mean differences greater than zero $P < 0{\cdot}001$). However, when the nerves had been divided the new control level of hormone (139 ± 26 μ-u./ml. mean ± S.E.) was higher than in the intact animal

TABLE 2. Release of vasopressin by haemorrhage in cats, before and after division of the sinus nerves and vagi (pentobarbitone sodium anaesthesia)

	Vasopressin μ-u./ml. blood before and after haemorrhage					
	Nerves intact			Sinus nerves and vagi divided		
Expt.	(*a*) 5 min before	(*b*) 5 min after	(*c*) 20 min after	(*d*) 5 min before	(*e*) 5 min after	(*f*) 20 min after
7†	32	935	575	208	365	320
8	200	2300	975	134	650	117
9†	26	1189	1799	250	650	—
10	31	1100	670	33	150	75
11	9	425	410	105	315	270
12	30	625	630	64	105	180
13	10	330	250	188	236	212
14	39	1260	795	132	232	220
Mean	47	1020	763	139	338	199
S.E.	22	220	169	26	74	32

† External jugular blood.

(*a*) v (*b*), $P < 0{\cdot}001$; (*a*) v (*c*), $P < 0{\cdot}001$; (*a*) v (*d*), P 0·02; (*d*) v (*e*), $P < 0{\cdot}01$; (*d*) v (*f*), $P < 0{\cdot}02$.

Student's *t*-test was applied to the mean difference of logged responses.

(mean difference greater than zero, $P < 0{\cdot}02$) and the subsequent response to haemorrhage much smaller. Five minutes after haemorrhage the blood level had risen to only 338 ± 74 μ-u./ml. (mean ± S.E., mean difference greater than zero $P < 0{\cdot}01$) and had fallen in the 20 min sample to 199 ± 32 μ-u./ml. (mean ± S.E., mean difference greater than zero $P < 0{\cdot}02$). This diminished response cannot be attributed to the high control levels of hormone since a control level of 200 μ-u./ml. in the animal with nerves intact does not preclude a big response (Expt. 8). In the haemorrhages performed after division of the nerves, the blood pressure not only fell more abruptly during the withdrawal of blood than in the intact animal, but remained at a lower level until the blood was replaced. If it is hypotension which is the major stimulus for vasopressin release, then the stimulus to the neurohypophysis in such haemorrhages would be greater than in animals with nerves intact, and one would expect the response also to be greater.

The question which then arose was whether this decrease in the release of vasopressin might be due to repeated haemorrhages rather than to division of the nerves. The results of four experiments in which each cat was subjected to three successive haemorrhages are shown in Fig. 3. In Expt. 16 each haemorrhage resulted in the release of similar amounts of vasopressin. In Expts. 15 and 18 the second haemorrhage caused a smaller

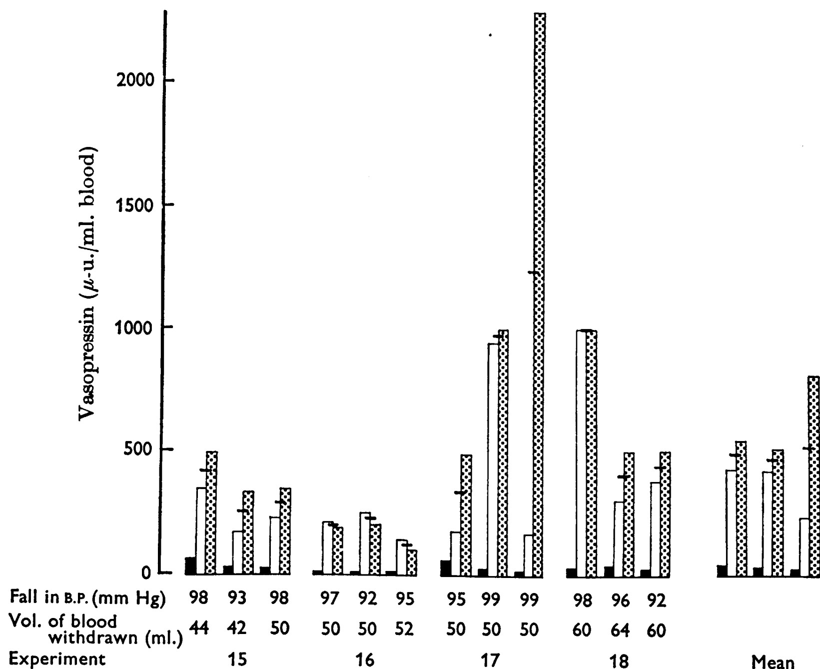

Fig. 3. Release of vasopressin in response to haemorrhage (repeated haemorrhages). ■ 5 min before haemorrhage. □ 5 min after haemorrhage. ▩ 20 min after haemorrhage. — Mean of 5 and 20 min samples.

release than the first but there was no further reduction following the third haemorrhage, whereas in Expt. 17 the release of vasopressin increased with each successive haemorrhage. The mean values show that cats will respond to haemorrhage at least 3 times within 4 hr with no significant diminution in hormone release.

MEA was measured in eight of these twelve experiments but in none of them was any oxytocin detected. The possibility of a selective block of an oxytocin releasing pathway by pentobarbitone sodium was considered, but can be discounted since in the two cats under chloralose anaesthesia (Table 3) haemorrhage still caused independent release of vasopressin.

TABLE 3. Release of posterior pituitary hormones by haemorrhage in cats (chloralose anaesthesia)

			μ-u./ml. blood before and after haemorrhage								
			Vasopressin			MEA			Oxytocin*		
	Blood with-drawn	Fall in B.P.	Before	After		Before	After		Before	After	
Expt.	(ml.)	(mm Hg)	5 min	5 min	20 min	5 min	5 min	20 min	5 min	5 min	20 min
19†	50	115	31	123	190	—	10	12	—	0	0
20	72	105	8	264	125	—	35	15	—	0	0

* After correction for the milk-ejecting activity of vasopressin (see text).

† External jugular blood.

DISCUSSION

Much of the previous work on the release of the neurohypophysial hormones has involved estimating the effectiveness of a stimulus by matching the response of the animal's own target organ against the effects of intravenous injections of oxytocin or vasopressin. Difficulty is often encountered in interpreting the results, since it is uncertain that the effects observed are due only to these hormones. If they are, then there is doubt as to which of the two is responsible since they overlap in their pharmacological properties. A more direct method is to estimate the concentration of oxytocin and vasopressin in blood. A considerable volume of work has been done in which either one hormone or the other has been estimated in blood following different physiological stimuli (Beleslin *et al.* 1967). In the present experiments an attempt has been made to demonstrate independent release by estimating the levels of both hormones in the same sample of blood.

One major problem in such a study is that the methods used to assay the activities of the two hormones may not be equally sensitive. This problem has now been overcome. With the introduction of the lactating rat preparation, levels of oxytocin as low as 3 μ-u./ml. of blood can be detected, corresponding to the limit of sensitivity for the assay of vasopressin. This has provided conclusive evidence for the independent release of vasopressin in response to carotid occlusion; moreover, it has confirmed the absence of oxytocin in the blood following haemorrhage. The great sensitivity of the lactating rat preparation has permitted the detection of a measurable amount of MEA in sixty-nine out of ninety-five blood samples collected after carotid occlusion or haemorrhage but in only four of them was the MEA greater than the intrinsic MEA of vasopressin in the sample, i.e. 13·6 % of its antidiuretic activity. Thus the presence of oxytocin in these samples could be totally excluded. The MEA/ADA ratio in these sixty-nine samples was calculated as $8{\cdot}7 \pm 0{\cdot}48$ % (mean ± S.E.), but this

level is lower than the expected 13·6 % suggesting that substances might be present in the blood extracts inhibiting the response of the mammary gland. An inhibition test was carried out on ten extracts containing no measurable MEA, by adding PPLE to each and comparing the milk-ejection response with that of PPLE in 0·9 % NaCl solution. No significant inhibition was noted, and no explanation can be offered for the discrepancy. But the MEA detected must be due to a neurohypophysial hormone because the activity was abolished by thioglycollate, and no pharmacologically active substance is known, other than vasopressin, which would show the ratio of MEA to ADA that occurred in these assays.

The present experiments have confirmed the finding that vasopressin is released during carotid occlusion and that this response is abolished when the sinus nerves are divided (Share & Levy, 1962). In addition they have shown that division of both the sinus nerves and the vagi substantially reduces the release of vasopressin in response to haemorrhage. The results also show that afferents in the vagi are of greater importance than those in the sinus nerves adding support to the suggestion of Gauer & Henry (1963) that sinus nerve afferents only take on a dominant role when the vagal afferents are deficient. It should be noted that the response to carotid occlusion can only be elicited after vagotomy.

Beleslin *et al.* (1967) demonstrated that in the cat, release of vasopressin can occur during peripheral vagal stimulation but only if the fall in blood pressure is at least 80 mm Hg. This is in agreement with the concept that the release of hormone in haemorrhage is more closely related to the fall in blood pressure than to alterations in blood volume (Ginsburg & Brown, 1957).

It is known that a fall in arterial blood pressure results in a decrease in the rate of firing of the sino-aortic baroreceptors with a resultant release of the vasomotor efferents from an inhibitory tonus (Chungcharoen, Daly & Schweitzer, 1952). Conversely, chemoreceptor fibres become active during hypotension owing to anoxia of the receptors and exert an excitatory action on vasomotor efferents (Landgren & Neil, 1951).

Hypothalamic structures concerned with the release of vasopressin might also be under an inhibitory tonus exerted by the baroreceptors. This would not only account for the increase in vasopressin release during carotid occlusion and haemorrhage, but would also explain the rise in the resting level of hormone which occurs after division of the sinus nerves and the vagi.

But release from inhibition is not the only mechanism involved. An excitatory pathway connected with the release of vasopressin was suggested by Chang, Chia, Hsu & Lim (1937) and Chang, Chia, Huang & Lim (1939). They found that on electrical stimulation of the afferent axons in

the vagus, vasopressin was secreted in amounts sufficient to cause a pressor in addition to an antidiuretic response in dogs with only vascular connexions between the head and trunk. These effects were abolished by hypophysectomy. More recently Share & Levy (1966) showed that stimulation of carotid body chemoreceptors by perfusing the sinus with deoxygenated blood resulted in vasopressin release in artificially respired, vagotomized dogs, suggesting that chemoreceptors are also concerned with the release of vasopressin through an excitatory reflex in much the same way as they are concerned with the regulation of vasomotor tone. Share & Levy (1966) also showed that during perfusion of one sinus with deoxygenated blood, the other being left intact, vasopressin release was greater when the blood pressure was kept constant than when it was allowed to rise. This lends further support to the suggestion that baroreceptor fibres effectively exert an inhibitory tonus over the release of vasopressin.

However, although hypotension appears to be the major stimulus, the decrease in blood volume in haemorrhage cannot be ignored. There is a considerable body of evidence to indicate that atrial stretch receptors are involved in the regulation of vasopressin release and it is conceivable that they, too, play a part in haemorrhage.

Henry & Pearce (1956) showed that distension of the left atrium increased the activity of atrial stretch receptor fibres and that this was associated with an increase in urine flow, whereas small reductions in blood volume decreased the activity of these neurones and was often associated with antidiuresis. They concluded that these receptors are involved in a reflex regulation of blood volume by controlling urine output. This view was supported by Baïsset & Montastruc (1957) who proposed that the reflex might operate through the vagus by exerting an inhibitory control over the release of vasopressin from the neurohypophysis.

The suggestion that left atrial distension is involved in the regulation of blood volume through urine output was challenged by Ledsome, Linden & O'Connor (1961) and Lydtin & Hamilton (1964) who showed that the diuresis resulting from left atrial distension is transient and variable and cannot be inhibited by the administration of vasopressin. Nevertheless, the fact that vasopressin release is effectively inhibited by left atrial distension was recently confirmed by Share (1965).

We can therefore conclude that in the extreme physiological condition imposed in the present experiments, the inhibition arising from atrial receptors would be removed. This would tend to enhance the effect of excitatory impulses and lead to a greater increase in vasopressin release.

But even after removing both excitatory and inhibitory impulses by cutting the sinus nerves and vagi, haemorrhage still induced a small release of hormone. One might possibly invoke here the peripheral 'volume

receptors' described by Heymans, Bouckaert & Wierzuchowski (1937) and Gammon & Bronk (1935). These receptors have been located in the mesenteric vascular territory and abdominal aorta, and since impulses from them travel in the splanchnic nerve, they would not be affected by vagotomy.

The finding that the release of vasopressin without oxytocin during haemorrhage is largely mediated via the sinus nerves and vagi is of particular interest with respect to the observations of Bisset *et al.* (1967) on the release of neurohypophysial hormones. They have shown that electrical stimulation of discrete regions of the hypothalamus results in the release of these hormones. Whereas stimulation in the tuberal region releases vasopressin with or without oxytocin, stimulation of the supraoptic nucleus or the supraoptico-hypophysial tract releases vasopressin alone.

These experiments suggest the possibility that the two peptides may exist as separate entities in different neurosecretory cells and are available for independent release in response to appropriate physiological stimuli.

It is known that oxytocin is released in response to suckling by the activation of a reflex arc, but although an afferent pathway exists, parts of which have been demonstrated, it has not been established whether it is in the paraventricular or supraoptic nucleus that the efferent fibres originate. It is now possible to propose a complete reflex arc for the release of vasopressin.

We can assume that inhibitory fibres arising from atrial stretch receptors and sino-aortic baroreceptors as well as excitatory fibres arising from chemoreceptors, impinge directly or indirectly on the neurones of the supraoptic nucleus. The selective release of vasopressin in response to changes in blood pressure and volume would thus be regulated by reflex arcs of which the fibres of the sinus nerves and vagi form the afferent component and the neurones of the supraoptic nucleus the efferent component.

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