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A sensitive and specific assay for vasopressin in the circulating blood

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Summary

1. A search was made for an assay tissue with selective sensitivity to vasopressin. Of those smooth muscle preparations tested, the longitudinal muscle of the isolated rectum of the rabbit was the most satisfactory.

2. The rabbit isolated rectum, bathed in Krebs solution, was contracted by acetylcholine, angiotensin II amide, bradykinin and 5-hydroxytryptamine. It was relaxed by vasopressin, oxytocin and the catecholamines.

3. Vasopressin was active in concentrations of 4–100 μu./ml (0·01–0·25 ng/ml) and was 20–30 times more active than oxytocin. Bretylium had no effect on the relaxant action of vasopressin ; nor did concentrations of α- and β-adrenoceptor blocking agents sufficient to abolish the actions of catecholamines. Lignocaine reduced the sensitivity of the rabbit rectum to both vasopressin and oxytocin without altering the actions of adrenaline. High concentrations of either vasopressin or oxytocin desensitized the rabbit rectum to the actions of both hormones, without affecting the actions of adrenaline. It was concluded that vasopressin and oxytocin act on a common population of receptors different from those for catecholamines.

4. Phentolamine, unlike other α-adrenoceptor antagonists, reduced the relaxant action of vasopressin on the rectum.

5. When superfused with blood from an anaesthetized dog, the rabbit rectum maintained a higher tone than in Krebs solution ; it retained its sensitivity to vasopressin. Pronethalol, administered intraluminally, reduced spontaneous movement and abolished the actions of low concentrations of catecholamines, thereby increasing the specificity of the assay. No other substance tested relaxed the rectum in concentrations likely to be found in blood.

6. Vasopressin was stable in dog's blood ; it survived passage through the pulmonary vascular bed ; it had a half-life in the circulation of about 1 min.

7. The half-life of vasopressin in the circulation may depend upon the duration of the infusion.

Introduction

Present methods for the bio-assay of vasopressin (Dekanski, 1952 ; Dicker, 1953 ; Botting, 1965) require the removal of blood samples, followed by various purifica-

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tion processes. The blood-bathed organ technique (Vane, 1964) has been used to assay directly and continuously several vaso-active hormones; it has now been adapted for the assay of vasopressin. To test the method, infusions of vasopressin have been used to determine three aspects of the fate of vasopressin in the circulation. These are the activity of vasopressin when incubated with blood; after passage through the pulmonary vascular bed; and after a single circulation through the body.

Methods

Experiments in artificial salt solutions

In a search for a tissue specifically sensitive to vasopressin a wide variety of smooth muscle preparations were studied. From the rat, the stomach strip, cut to preserve either the longitudinal (Vane, 1957) or the circular muscle, the descending colon (Mikos & Vane, 1967) and the ascending colon (Regoli & Vane, 1964). From the desert rat (*Meriones libycus*), the stomach strip, cut to preserve either the longitudinal or the circular muscle, the duodenum, jejunum, ascending colon (Ambache, Kavanagh & Whiting, 1965), caecum, rectum and urinary bladder. From the guinea-pig, the proximal colon (Botting, 1965), the taenia coli, the rectum, the ureter, the urinary bladder and the seminal vesicle. From the chick, the rectum (Mann & West, 1950), the rectal caecum (Cleugh, Gaddum, Holton & Leach, 1961) and other segments of intestine. From the hen, the rectum and the oviduct, cut to preserve either the longitudinal or circular muscle. From the rabbit, the stomach strip, cut to preserve either the longitudinal or the circular muscle, the duodenum, jejunum, ileum, caecum, proximal colon and rectum. From the dog, the stomach strip, cut to preserve either the longitudinal or the circular muscle, the duodenum and the jejunum.

The rabbit isolated rectum was the most extensively studied. New Zealand white rabbits of either sex, weighing between 1·5 to 4 kg, were killed by stunning and bleeding through the carotid arteries. The abdomen and pelvic girdle were opened to expose the distal large intestine. The terminal 3–5 cm of intestine was dissected free of peritoneum and excised. The lumen was washed out with Krebs solution, after which any remaining fascia was carefully stripped away. In some experiments the 3–5 cm of intestine proximal to the rectum was also used; this corresponded to the segment studied by Garry & Gillespie (1954). Segments still further proximal, corresponding to those studied by Woo & Somlyo (1967), were not used.

The rectum was suspended either in a glass isolated organ bath (20 ml volume) or in a polypropylene jacket for superfusion (Gaddum, 1953). The tissue was suspended by threads which occluded the oral end and left the aboral end open. The thread at the oral end was attached to an auxotonic frontal writing lever (Paton, 1957), with a magnification of either $\times 4$ or $\times 16$. The initial load on the tissue was 1–4 g. Changes in the length of the muscle were recorded on a kymograph. The tissue was bathed in an artificial salt solution (usually Krebs solution) at 37° C.

In one experiment a 50 ml organ bath was used. The rabbit rectum was isolated so that the periarterial nerves supplying it remained intact. The nerves were pulled into a coaxial electrode (Paton & Vane, 1963) so that they could be stimulated with a transistorized stimulator (Bell, 1968).

The rabbit rectum was sometimes stored in a refrigerator for up to 48 h in a beaker of Krebs solution at 4° C. After such storage any remaining connective tissue was easier to dissect away.

The composition of the bathing solutions (in g/l. distilled water) were as follows; millimolar concentrations are given in parentheses:

Krebs solution: NaCl, 6·9 (118); KCl, 0·35 (4·7); $CaCl_2 \cdot 6H_2O$, 0·55 (2·5); KH_2PO_4, 0·16 (1·2); $MgSO_4 \cdot 7H_2O$, 0·29 (1·17); glucose, 1·0 (5·6); $NaHCO_3$, 2·1 (25·0); gassed with 95% O_2 and 5% CO_2.

Tyrode solution: NaCl, 8·0 (137·0); KCl, 0·2 (2·7); $CaCl_2 \cdot 6H_2O$, 0·396 (1·8); $MgCl_2 \cdot 6H_2O$, 0·214 (1·5); NaH_2PO_4, 0·05 (0·32); glucose, 1·0 (5·6); $NaHCO_3$, 1·0 (12·0); gassed with oxygen.

Rat uterus Ringer solution: NaCl, 9·0 (154·0); KCl, 0·42 (5·6); $CaCl_2 \cdot 6H_2O$, 0·06 (0·27); glucose, 0·5 (2·8); $NaHCO_3$, 0·5 (6·0) gassed with oxygen (Burn, Finney & Goodwin, 1950).

Botting's solution: NaCl, 8·0 (137·0); KCl, 0·2 (2·7); $CaCl_2$, 0·08 (1·14); $MgCl_2$, 0·04 (0·65); NaH_2PO_4, 0·05 (0·32); glucose, 1·0 (5·6); $NaHCO_3$, 1·0 (12·0); gassed with oxygen (Botting, 1965).

Blood-bathed organ experiments

Dogs of either sex, weighing 5–17 kg, were anaesthetized with halothane, delivered from a Goldman vaporizer. Anaesthesia was maintained with chloralose (10 mg/ml; 100 mg/kg intravenously) and, when necessary, with pentobarbitone (1–5 mg/kg intramuscularly or subcutaneously). The trachea was cannulated and the animal was mechanically ventilated by a Palmer "Ideal" respiration pump. Dextran (mol. wt. 110,000; 6% w/v in 0·9% saline) was injected intravenously (10 ml/kg) as was heparin (1,000 i.u./kg). In some experiments morphine (1 mg/kg intramuscularly) was administered. Polyethylene cannulae were tied into a femoral artery and femoral vein for the removal and replacement of blood. Mean arterial pressure was recorded on the kymograph by a mercury manometer (1 mmHg≡ 1·333 mbar) attached to the side arm of the arterial cannula: when an 8-channel pen recorder was used, mean blood pressure was recorded via a pressure transducer.

The blood for superfusion of the isolated assay organs was pumped either from the femoral arterial cannula or from a catheter in the left ventricle at a constant rate of 8–15 ml/min. The blood from the left ventricle was obtained from the inner tube of a co-axial catheter (Ferreira & Vane, 1967a) passed down the right carotid artery until the pressure pulse recorded through it showed that the tip was in the left ventricle. The outer catheter was 2–3 cm shorter and its tip was in the ascending aorta. Drugs infused through this outer catheter mixed with the cardiac output and passed once through the vascular system before being sampled in the blood taken from the left ventricle.

After the blood had superfused the assay tissues it was collected in a reservoir and returned to the dog through the femoral vein.

In some experiments the lumen of the blood-bathed rabbit rectum was perfused with an antagonist (Hodge, Lowe & Vane, 1966). The aboral end of the rectum was tied onto a piece of polyethylene tubing (PP 260, Portex) inserted through a side-opening in the polypropylene superfusion jacket. This tubing held the tissue firmly in place in the middle of the jacket. A fine polyethylene tubing (PP 30,

Portex) was threaded through the tube into the lumen of the rectum and a constant infusion of antagonist was made (0·05–0·1 ml/min) through the fine tubing. The fluid escaped via the outer tubing. Since the upper end of the rectum was closed, the intraluminal infusions did not contaminate the superfusing blood.

An incubation circuit (Ferreira & Vane, 1967b) was used to measure any inactivation of vasopressin in blood. The tubing which carried the blood from the dog to the assay organs was lengthened in order to contain up to 80 ml blood, thereby increasing the time of passage of the blood to the tissues by up to 8 min. The blood was maintained at 37° C by immersing the tubing (3 mm i.d.; 6 mm o.d.; Esco Rubbers Limited) in a water bath.

Tissue movements were recorded on a kymograph by auxotonic frontal-writing levers (Paton, 1957) with a 16-fold magnification and an initial load on the tissue of 1–4 g. In some experiments tissue movements were transduced by Harvard smooth muscle strain gauges (type 352) and recorded on an 8-channel Offner dynograph.

The following drugs were used. All substances were freshly diluted from stock solutions with 0·9% (w/v) saline. Concentrations of salts are expressed in terms of base. Ascorbic acid was added to all solutions of catecholamines. Acetylcholine perchlorate (British Drug Houses), adenosine triphosphate (Sigma), (−)-adrenaline bitartrate (B.D.H.), corticotrophin (Acthar; Armour), angiotensin II-amide (Hypertensin; Ciba), arginine^{8}vasopressin (Tonephin; Hoechst), ascorbic acid (B.D.H.), bradykinin (synthetic; Parke-Davis), bretylium tosylate (Darenthin; Burroughs Wellcome), carbachol (Savory & Moore), α-chloralose (Merck Darmstadt AG), chlorbutol (Hopkin & Williams), dextran 110 injection, B.P. (Dextraven 110; Fisons), dihydroergotamine methane sulphonate (Sandoz), ergotamine maleate (B.W.), halothane (Fluothane; Imperial Chemical Industries), heparin injection, 5,000 i.u./ml (Boots), hexamethonium bromide (May & Baker), histamine acid phosphate (B.W.), hydergine (Sandoz; dihydroergocornine, dihydroergocrystine and dihydroergokryptine, 0·1 mg/ml each as the methane sulphonate salts), 5-hydroxytryptamine creatinine sulphate (M. & B.), (±)-isopropylnoradrenaline sulphate (B.D.H.), pig pancreatic kallikrein (Glumorin, Bayer AG), lignocaine hydrochloride (Xylocaine; Astra Hewlett), lysine^{8}vasopressin and placebo solution (Sandoz), morphine sulphate (B.D.H.), nicotine hydrogen tartrate (B.D.H.), (−)-noradrenaline bitartrate (B.D.H), oxytocin (natural, Pitocin; P.-D.), oxytocin (synthetic, Syntocinon; Sandoz), pentagastrin (Pentavlon; I.C.I.), pentobarbitone (60 mg/ml, Nembutal Veterinary; Abbott), phentolamine methane sulphonate (Rogitine; Ciba), pronethalol hydrochloride (Alderlin; I.C.I.), propranolol hydrochloride (Inderal; I.C.I.), prostaglandins E_1, E_2, $F_{2\alpha}$ (Upjohn), partially purified slow-reacting substance in anaphylaxis (SRS-A), sodium chloride injection, 0·9% w/v (Boots), substance P (1 mg=13·2 u.), tyramine hydrochloride (B.D.H.), vasopressin (natural, Pitressin; P.-D.).

Results

Experiments in artificial salt solutions

The sensitivities of the tissue segments to various agonists are listed in Table 1. The tissues most sensitive to vasopressin were the proximal colon of the guinea-pig and the rectum of the rabbit. Because the guinea-pig proximal colon exhibited tachyphylaxis and lack of specificity, it was not used further. The rabbit rectum

preparations were relaxed by vasopressin, to a lesser extent by oxytocin, and appeared sensitive enough to be used for bioassay. With the lever system used, a load on the tissue of 1·5–2 g gave optimum results.

An example of the dose-dependent effects of vasopressin on the rabbit rectum is shown in Fig. 1. An after-contraction above the baseline often occurred with higher concentrations of vasopressin and not uncommonly the preparation failed to remain relaxed in the presence of vasopressin. The spontaneous activity varied throughout the day in any one preparation and also from preparation to prepara-

TABLE 1. *Sensitivity of isolated smooth muscles to vasopressin and other substances*

Tissue	Adrenaline (ng/ml)	Acetylcholine (ng/ml)	5-HT (ng/ml)	Histamine (ng/ml)	Angiotensin II (ng/ml)	Oxytocin (mu./ml)	Vasopressin (mu./ml)
Rat							
Stomach strip (1)	7 R	7 C				6 NR	0·5 C*
Ascending colon	7 R	7 C				6 R	0·5 R
Descending colon	7 R	7 C	0·7 C	3·5 C	1 C	7 C	0·7 C
Desert rat							
Stomach strip (1)	7 R	7 C		14 C	7 C		2 C*
Duodenum	7 R	7 C					1·5 R
Jejunum	7 R	7 C					7 NR
Ascending colon	3·5 R	1·4 C					1·4 C
Caecum	7 R	7 C				0·7 NR	0·7 NR
Rectum	3·5 R	1 C	1·7 C		14 C	1·4 C	0·3 R
Urinary bladder	14 NR	7 C					3·5 C
Guinea-pig							
Proximal colon		14 C	7 C	70 C		0·7 C	0·03 C*
Taenia coli	3·5 R		7 C	7 C	7 C	7 NR	1·4 NR
Rectum	7 R	3·5 C	35 C	1·4 C	7 C	1·4 C	0·3 C
Ureter	7 NR	7 C					0·7 NR
Urinary bladder	7 R	7 C	7 C		7 C	7 NR	0·7 NR
Seminal vesicle	7 C	7 C					7 NR
Chick							
Rectum (2)	7 R	7 C				6 C	3 C
Rectal caecum	7 R	7 C					0·7 NR
Hen							
Rectum	7 R	7 R	26 NR			7 NR	3 R*
Oviduct (1)	7 C	14 C	7 C		7 C	1·4 C	2 C
Rabbit							
Stomach strip (1)	0·7 R	7 C				7 NR	1·4 C
Duodenum	7 R	7 C					3·5 NR
Jejunum	7 R						3·5 NR
Ileum	7 R	7 C					3·5 NR
Caecum	7 R		10 C			3 NR	0·7 NR
Proximal colon	7 R	7 C				3 R	0·3 R
Rectum	7 R	3·5 C	2 C	35 C	7 C	3 R	0·03 R
Dog							
Stomach strip (1)	7 R	7 C					0·7 NR
Duodenum	7 R	14 C	9 C	14 C		3·5 NR	0·3 NR
Jejunum		35 C	14 C			7 C	14 C

(1) Responses of tissues cut to preserve the longitudinal muscle were similar to those of tissue cut to preserve the circular muscle.
(2) Other segments of intestine were similar to chick rectum responses to adrenaline, acetylcholine, oxytocin and vasopressin.

*The preparation exhibited tachyphylaxis to a second dose of vasopressin.

Sensitivity is expressed as the concentration necessary to produce either a small contraction (C) or relaxation (R) of a preparation. Responses were not quantitated. At the doses indicated NR, no response of the preparation was obtained. When more than one preparation of the same tissue was studied, the average dose is given.

tion. In some experiments, although the tone of the preparation was high, it progressively diminished over a few hours. These factors made quantitative study of the actions of drugs difficult and various procedures were tried in an attempt to improve the assay. These included storing the tissue for 24 to 48 h in Krebs solution at 4° C; changing the ionic composition of the Krebs solution (calcium or magnesium-free Krebs solution); the use of Tyrode solution, rat uterus Ringer solution or the solution described by Botting (1965); the addition of various agonists to the Krebs solution (acetylcholine, carbachol, 5-hydroxytryptamine, or angiotensin II-amide) and the addition of hexamethonium or lignocaine (5×10^{-5} to 2×10^{-4} g/ml) to the Krebs solution. None of these treatments completely eliminated the drawbacks encountered with the tissue. For instance, although the addition of carbachol (1·25–25 ng/ml) to the Krebs solution elevated the tone of the rectum, it did little to abolish the spontaneous activity and did not increase the relaxation induced by vasopressin.

Use of calcium-free Krebs solution abolished the response of the rectum to vasopressin. Other bathing solutions did not give more stable baselines or reactions. Lowering the temperature of the water bath reduced the spontaneous activity but did not increase the tone and also reduced the response of the rectum to vasopressin. Storage at 4° C for 24 to 48 h diminished the spontaneous activity and the after-contraction but did not raise the tone of the preparation.

Woo & Somlyo (1967) found that magnesium (1·2 mM) potentiated the inhibitory action of vasopressin on the rabbit isolated colon bathed in magnesium-free Krebs solution. Figure 2 is from an experiment in which the magnesium concentration of the Krebs solution was varied from 0 to 2·34 mM. In magnesium-free Krebs solution the spontaneous activity was high and tended to obscure the effects of vasopressin. When normal Krebs solution was used (magnesium concentration 1·17 mM) the effects of vasopressin were much more evident. Doubling the magnesium concentration to 2·34 mM reduced the effects of vasopressin on the tissue.

Lignocaine (5×10^{-5} to $2{\cdot}5 \times 10^{-4}$ g/ml) diminished or abolished spontaneous activity, raised the basal tone of the preparation and stabilized the baseline. The inhibitory response to vasopressin consequently became more evident. In several experiments the relaxations of the rabbit rectum induced by vasopressin, oxytocin

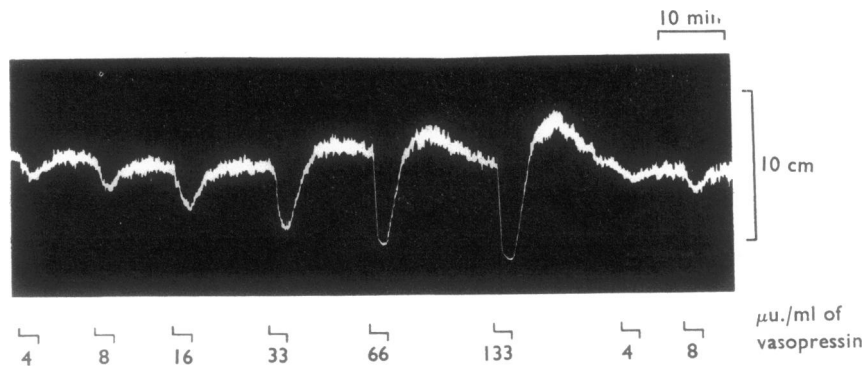

FIG. 1. **Rabbit rectum preparation superfused with Krebs solution at 15 ml/min. Relaxations due to vasopressin (4–133 μu./ml) are shown. The rectum had been stored at 4° C for 48 h before use.** Time scale, 10 min; vertical scale, 10 cm.

and adrenaline were compared in the presence and absence of lignocaine. Table 2 shows that lignocaine significantly increased the concentration of either vasopressin or oxytocin necessary to produce a standard relaxation of the rectum. The effect of lignocaine on the action of vasopressin, however, was relatively less than that on the action of oxytocin. Table 2 also shows the potencies of some other substances

TABLE. 2. *Relative potencies of some substances which relax the rabbit rectum*

	Number of experiments	Mean dose* (ng/ml)	±S.E.M. (ng/ml)	Relative potency† (%)
Arginine^{8}vasopressin	3	0·06	0·002	67
Lysine^{8}vasopressin	5	0·08	0·002	49
Pitressin	38	0·04	0·001	100
Pitressin+lignocaine‡	10	0·08	0·001	49
Syntocinon	3	1·3	0·002	3·0
Pitocin	11	0·9	0·001	4·0
Pitocin+lignocaine§	5	5·1	0·002	0·8
Isopropylnoradrenaline	5	2·6	0·002	1·5
Noradrenaline	8	4·6	0·001	0·9
Adrenaline	5	5·8	0·001	0·7
Adrenaline+lignocaine¶	5	4·8	0·002	0·8
Nicotine	3	491	0·001	0·01

* Concentration necessary to produce a relaxation of the tissue giving a 1·5 cm lever movement (16:1 magnification). In some experiments (+ lignocaine) the effects of the substance were determined in the presence of lignocaine. Doses of pituitary hormones were converted to weights using the following conversion factors (Boissonnnas, Guttmann, Berde & Konzett, 1961): arginine^{8}vasopressin, 400 u./mg; lysine^{8}vasopressin, 250 u./mg; Syntocinon, 450 u./mg. Pitocin was assumed to contain 450 u./mg; Pitressin, 400 u./mg.

† Relative potency is defined as $\left(\frac{\text{mean dose, Pitressin}}{\text{mean dose, X}} \times 100\right)$

‡ Results of the Student t test on the effect of lignocaine: $t=2{\cdot}1355$; $P<0{\cdot}05$.
§ Results of the Student t test on the effect of lignocaine: $t=2{\cdot}7041$; $P<0{\cdot}02$.
¶ Results of the Student t test on the effect of lignocaine: $t=0{\cdot}3347$; N.S.

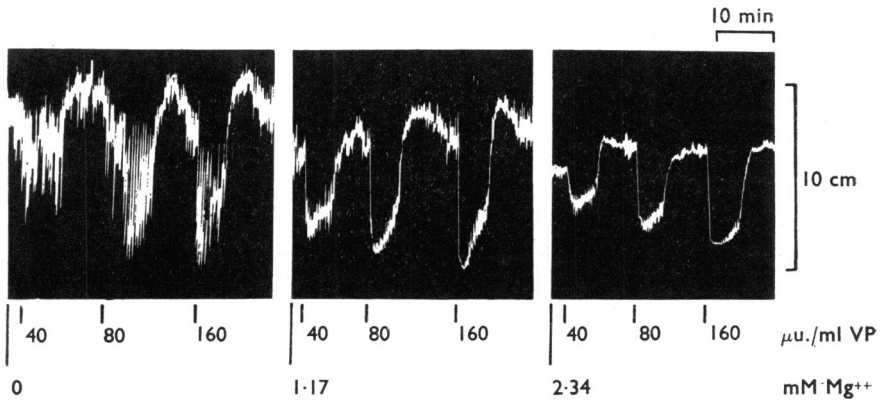

FIG. 2. Effects of vasopressin (VP, 40, 80 and 160 μu./ml) on the rabbit rectum bathed in Krebs solution containing different concentrations of magnesium. Without magnesium (left-hand panel) there was strong spontaneous activity which tended to obscure the relaxations induced by vasopressin. With normal Krebs solution (magnesium concentration 1·17 mM, middle panel) the spontaneous activity was reduced. With double the normal magnesium concentration (2·34 mM, right-hand panel) there was a further reduction in spontaneous activity but the effects of vasopressin were also reduced. Time scale, 10 min; vertical scale, 10 cm.

which produced relaxations of the rabbit rectum. To make comparisons possible, the activity of the pituitary hormones is expressed on a weight basis, using the conversion factors given by Boissonnas, Guttmann, Berde & Konzett (1961).

Chlorbutol, the preservative in commercial preparations of vasopressin and oxytocin, also relaxed the rabbit rectum. However, to obtain a relaxation of the rectum equal to that produced by Pitressin (100 μu./ml), a final concentration of chlorbutol in the bath of $1{\cdot}25 \times 10^{-5}$ g/ml had to be used. This was 50 times the concentration present in the diluted commercial sample of vasopressin. The vehicle for lysine^{8}vasopressin had no activity on the rabbit rectum when diluted the same as the active agent.

Receptor specificity

Propranolol (10^{-7} g/ml) almost completely antagonized the relaxation of the rabbit rectum induced by adrenaline (1–10 ng/ml), but the relaxation reappeared when the adrenaline concentration was increased 10 to 100-fold. Propranolol (up to 10^{-6} g/ml) did not antagonize the action of vasopressin. Although the spontaneous activity of the tissue was not appreciably altered by propranolol, pronethalol ($1–5 \times 10^{-6}$ g/ml) antagonized the relaxant effects of adrenaline and also diminished the spontaneous activity of the rectum.

Since β-adrenoceptor blockade did not completely antagonize the actions of adrenaline, an α-adrenoceptor blocking agent was used in combination with propranolol. Ergotamine ($2{\cdot}5 \times 10^{-6}$ g/ml; one experiment), dihydroergotamine (10^{-5} g/ml; two experiments) and Hydergine (5×10^{-6} g/ml; one experiment) each antagonized the relaxation induced by the higher concentrations of adrenaline in the presence of propranolol, without changing the relaxations induced by vasopressin.

Phentolamine (10^{-6} to 10^{-5} g/ml; six experiments) by itself did not reduce the actions of adrenaline or of noradrenaline on the rectum. However, there was an antagonism of the effects of vasopressin and this was evident even though phentolamine increased the spontaneous activity of the rectum (Fig. 3). The response to vasopressin returned when the phentolamine was removed.

To test whether vasopressin and oxytocin stimulated a common population of receptors, the rabbit rectum was desensitized either to vasopressin or to oxytocin by the addition of a high concentration of one or the other agent (3·3 mu./ml Pitressin, three experiments; 39 mu./ml Pitocin, three experiments) for 20–50 min. The bath was then washed out and doses of vasopressin, oxytocin and adrenaline, which had previously been effective, were added. The effects of adrenaline were unchanged but the effects of both vasopressin and oxytocin were substantially reduced. Thus the peptides appeared to act on the same receptors.

Contractor substances

The rabbit rectum was most sensitive to the contractor actions of bradykinin, acetylcholine, 5-hydroxytryptamine and angiotensin II-amide; less sensitive to the prostaglandins, histamine, tyramine and substance P; and insensitive to the doses of pentagastrin and SRS-A tested (Table 3).

Adrenergic neurone block

In a rabbit rectum with the periarterial nerves intact the effects of nerve stimulation were abolished by bretylium (5×10^{-6} g/ml) but the actions of vasopressin were unaffected (Fig. 4). In a further two rabbit rectum preparations (without periarterial nerves) the addition of bretylium (5×10^{-6} g/ml) did not affect the relaxations produced by vasopressin.

TABLE 3. *Concentrations of various substances needed to produce a contractile response (4 cm lever movement 16:1 magnification) of the rabbit isolated rectum bathed in Krebs solution. NR indicates no response at dose stated*

	Number of experiments	Mean concentration (ng/ml)	
Acetylcholine	9	13	
Angiotensin II-amide	9	21	
Bradykinin	3	5	
Histamine	7	556	
5-Hydroxytryptamine	8	27	
Pentagastrin	3	87	NR
Prostaglandin E_1	2	87	
Prostaglandin E_2	5	570	
Prostaglandin F_2	4	84	
SRS-A	4	140	NR
Substance P	2	0·07	
Tyramine	2	2,000	

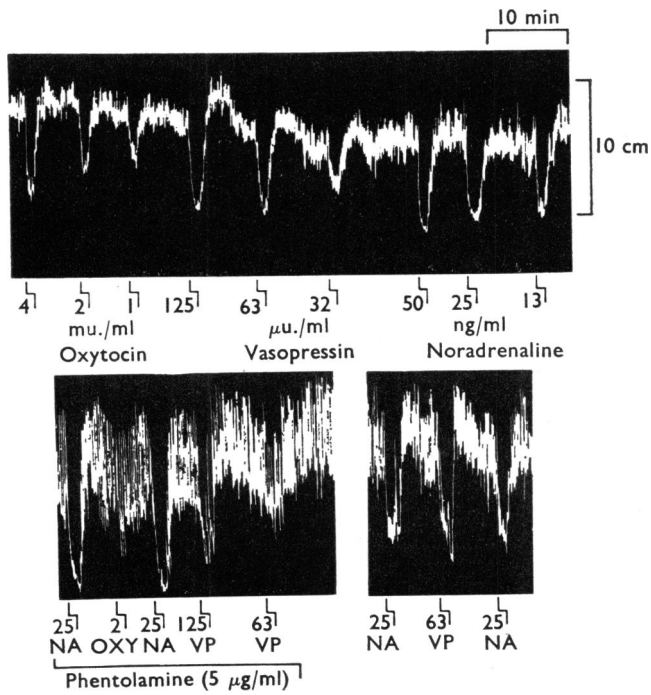

FIG. 3. Effect of phentolamine on the responses of the rabbit rectum. Dose-dependent responses to oxytocin (OXY), vasopressin (VP), and noradrenaline (NA) were obtained (top panel). Addition of phentolamine (5×10^{-6} g/ml) increased the amplitude of the spontaneous activity and raised the tone slightly. Responses to noradrenaline were increased while responses to vasopressin were decreased (left lower panel). Washing out the phentolamine resulted in a restoration of the response to vasopressin (right lower panel). Time scale, 10 min; vertical scale, 10 cm.

Blood-bathed organ experiments

In most of these experiments a rabbit rectum was used together with a rat stomach strip, a chick rectum and sometimes a rat colon. The isolated organs were superfused in series with Krebs solution at 8 to 15 ml/min while the dog was being prepared. When superfusion with blood was started, the rat stomach strip, chick rectum and rabbit rectum all contracted. As with the rat stomach strip (Vane, 1964), the blood-induced contraction did not completely pass off and the rabbit rectum maintained a higher tone. The spontaneous activity was also reduced, although it gradually returned over the next few hours. The isolated tissues took 30–90 min to stabilize at a new baseline; their sensitivity was then measured by infusing vasopressin into the superfusing blood. Neither the rat stomach strip nor the chick rectum were affected by concentrations of vasopressin which relaxed the rabbit rectum. The response of the blood-bathed rabbit rectum to vasopressin was dose-dependent (Fig. 5). The sensitivity of the blood-bathed tissue to vasopressin was usually similar to its sensitivity in Krebs solution, although sometimes the sensitivity was increased.

The specificity of the blood-bathed rabbit rectum was tested by infusions of several substances (Table 4). None of the substances which were active (acetylcholine, adenosine triphosphate, angiotensin II-amide, 5-hydroxytryptamine, the prostaglandins, or substance P) produced a relaxation. Furthermore, those substances which did produce a concentration had far greater effects on the rat stomach strip, chick rectum, or rat colon.

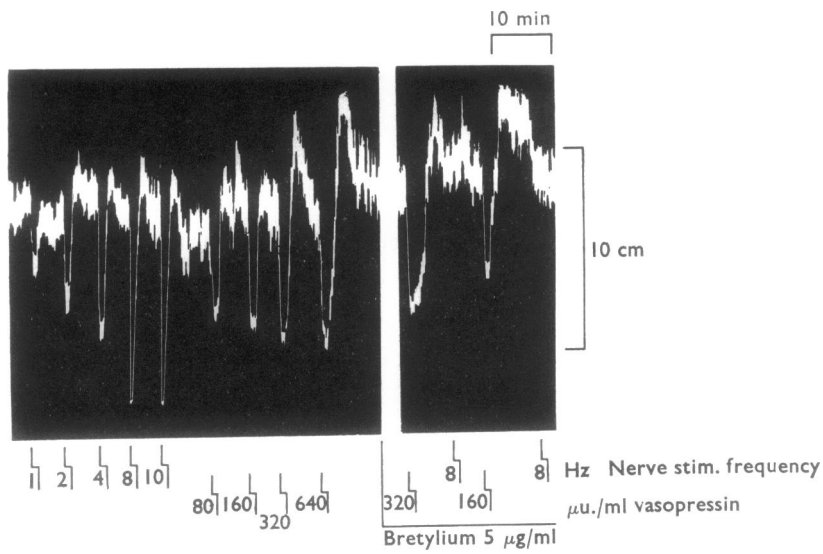

FIG. 4. Effect of bretylium on the responses of the rabbit rectum to nerve stimulation and to vasopressin. The tissue was bathed in Krebs solution in a 50 ml bath. The mesenteric periarterial nerves were stimulated at 1, 2, 4, 8 and 10 Hz (0·5 ms duration; 15 V) for 60 s. Vasopressin was left in the bath for 60 s. Bretylium was added to the Krebs solution and the final concentration was 5 μg/ml. The right-hand panel shows that the tissue continued to respond to vasopressin while the response to nerve stimulation was abolished by bretylium. Time scale, 10 min; vertical scale, 10 cm.

Noradrenaline (1–3 ng/ml) and adrenaline (1–15 ng/ml) relaxed the rabbit rectum but these effects were abolished by the intraluminal infusion of pronethalol (1 mg/ml infused at a rate of 0·05 to 0·1 ml/min).

Oxytocin relaxed the rabbit rectum and, as in Krebs solution, was 1/20 to 1/30 as active as vasopressin. The chick rectum was often more sensitive to oxytocin than was the rabbit rectum. In the experiment of Fig. 6, a chick rectum and a rabbit rectum were superfused with arterial blood from a 5 kg male dog. Infusions of oxytocin scarcely relaxed the rabbit rectum but produced substantial contractions of the chick rectum, whereas vasopressin selectively relaxed the rabbit rectum.

Regoli & Vane (1964) found that the spontaneous activity of the rat colon was diminished by pronethalol. Woo & Somlyo (1967) found that pronethalol prolonged and maintained the inhibitory response of the rabbit isolated colon to vasopressin.

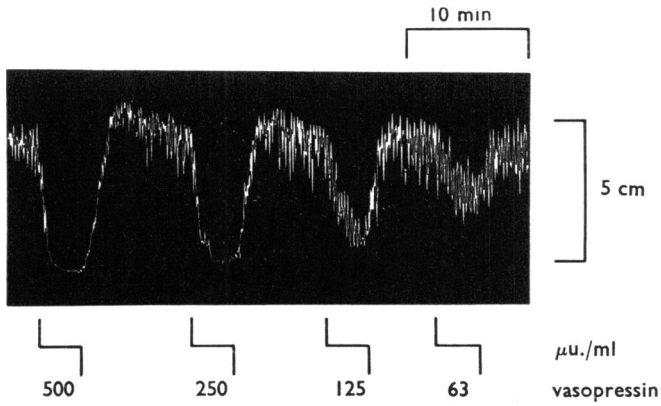

FIG. 5. Dose-dependent responses to vasopressin of the rabbit bathed in dog's blood. Arterial blood from a 16 kg male dog which had received morphine (1 mg/kg, intramuscularly). The rabbit rectum was treated with pronethalol (0·1 mg/min, intraluminally). Infusions of vasopressin were made into the blood superfusing the tissue. Time scale, 10 min; vertical scale, 5 cm.

TABLE 4. *Responses of the blood-bathed rabbit rectum to vaso-active substances*

Substance	Concentration*	Response	No. experiments†	
Acetylcholine	1·25	C	1	(1)
Adenosine triphosphate	4–8,000 (i)	C	1	(1)
Corticotrophin	0·5 u. (i)	N.R.	2	(2)
Angiotensin II	0·5–2·0	C.	4	(2)
Bradykinin	1·0–4·0	N.R.	3	(3)
Histamine	5–500	N.R.	4	(4)
5-Hydroxytryptamine	2·5–5·0	C	5	(2)
Kallikrein	10 mu./ml.	N.R.	1	(1)
Pentagastrin	4,000 (i)	N.R.	1	(1)
Prostaglandin E_1	2·0–4·0	N.R.	3	(3)
Prostaglandin E_2	1·0	C	1	(1)
Prostaglandin $F_{2\alpha}$	1·0–2·0	N.R.	1	(1)
Substance P	0·26–1·31 u./ml (i)	C	2	(1)

* Amounts expressed in ng/ml of blood except for injections (i) which are expressed as the total dose injected (ng). The dose was determined as that which would produce a 4 cm lever movement of one or more of the following tissues, rat stomach strip, rat colon, chick rectum. C=contraction; N.R.=no response obtained at the dose used. None of the substances used (at concentrations indicated) contracted the rabbit rectum sufficiently to produce a 4 cm lever movement.

† Figures in parentheses refer to the number of preparations which showed the indicated response or absence of it.

Infusions of pronethalol (1 mg/ml ; 0·05 to 0·1 ml/min) into the lumen of the blood-bathed rectum diminished the spontaneous activity and prevented the decline in the inhibiting effects of vasopressin (Fig. 7). The tracings are from three assay organs, superfused with arterial blood, a rat stomach strip ; one segment of rabbit rectum which received a constant intraluminal infusion of 0·9% saline (0·1 ml/min) and another segment of the rectum which was treated with pronethalol (1 mg/ml ; 0·1 ml/min). The rat stomach was selectively relaxed by adrenaline whilst the rectum was selectively relaxed by vasopressin. The spontaneous activity of the segment treated with pronethalol was less and the relaxation induced by vasopressin was sustained longer than that of the untreated rectum. In this and in other experiments, the sensitivity to catecholamines of the dog and the untreated assay tissues remained unaltered. Thus, any escape of pronethalol into the circulation was negligible.

Propranolol, a more potent β-adrenoceptor blocking agent than pronethalol (Black, Duncan & Shanks, 1965) neither diminished the spontaneous activity nor prolonged the inhibitory response to vasopressin.

For subsequent experiments, intraluminal infusions of pronethalol were routinely used.

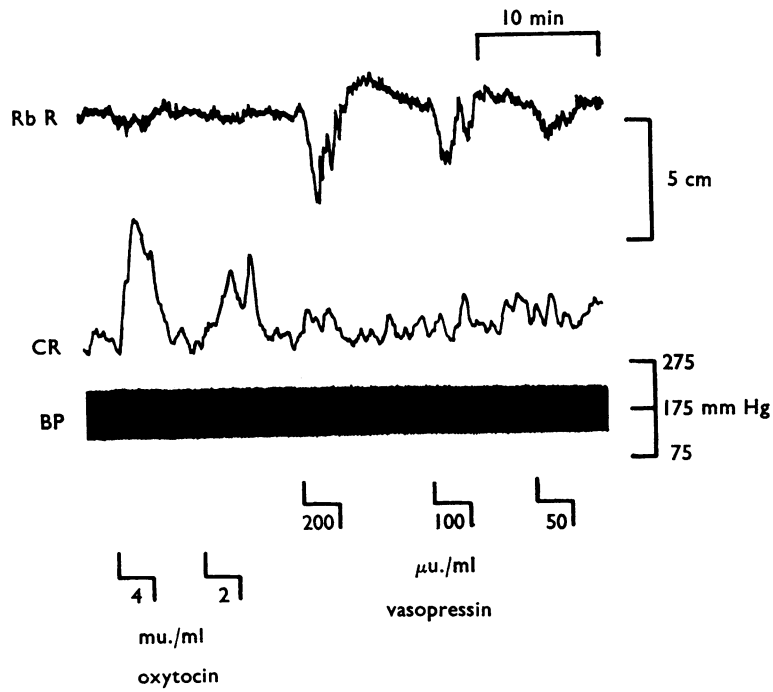

FIG. 6. Responses of a rabbit rectum (RbR) and a chick rectum (CR) to infusions of oxytocin and to vasopressin. The tissues were superfused with arterial blood from a 5 kg male dog. The tissues were not treated with antagonists. Note that in the absence of intraluminal pronethalol, the relaxation of the rabbit rectum induced by vasopressin was not maintained for the duration of the infusion. Infusions of oxytocin scarcely relaxed the rabbit tissue but did contract the chick rectum, whereas infusions of vasopressin relaxed the rabbit rectum without affecting the chick rectum. The blood pressure (BP) is also shown. Time scale, 10 min ; vertical scales, 5 cm, mmHg.

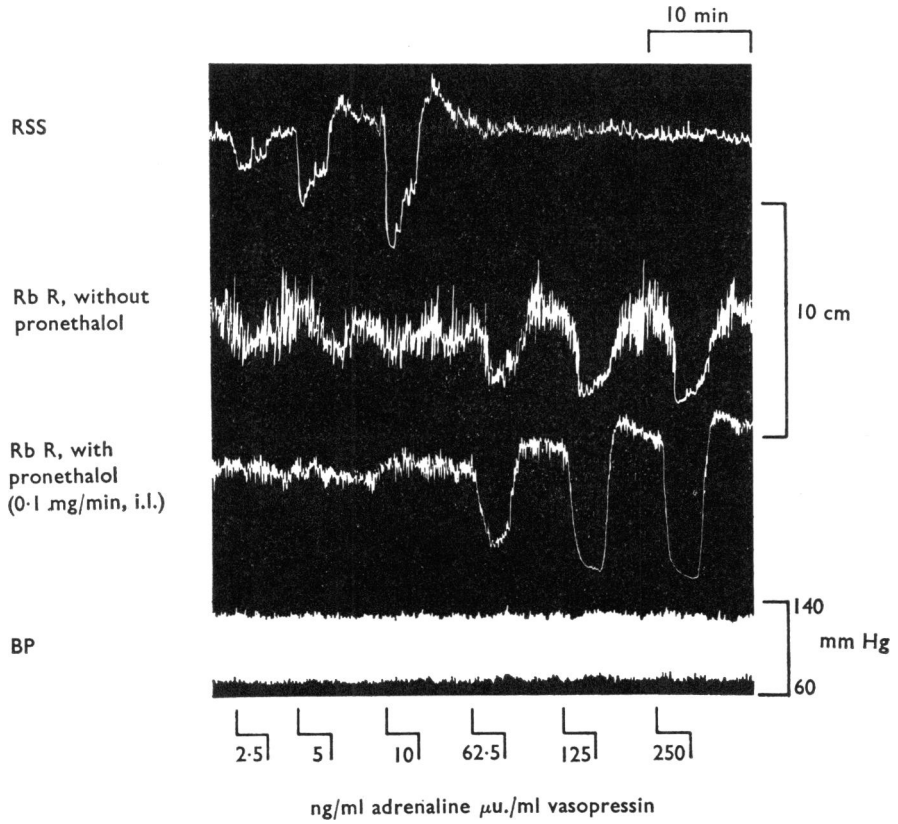

FIG. 7. Responses of some blood-bathed organs to adrenaline and to vasopressin. Arterial blood from a 16 kg female dog which had received morphine (1 mg/kg, intravenously) superfused a rat stomach strip (RSS) and two segments of rabbit rectum (RbR). One tissue received a continuous intraluminal infusion of 0·9% saline while the other received pronethalol (0·1 mg/min). The rabbit tissues were insensitive to concentrations of adrenaline which relaxed the rat stomach strip. Infusions of vasopressin relaxed both rabbit tissues. The rabbit rectum treated with pronethalol showed a maintained relaxation to vasopressin while the untreated rectum did not. Time scale, 10 min ; vertical scales, 10 cm ; mmHg.

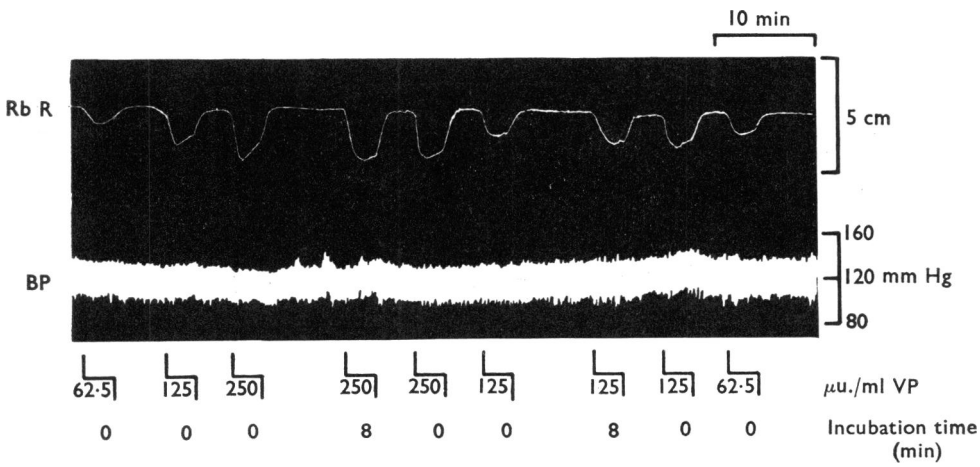

FIG. 8. Incubation of vasopressin with blood from an 11·5 kg male dog which had received morphine (1 mg/kg, intramuscularly). The incubation circuit was filled with arterial blood which subsequently superfused a rabbit rectum (RbR), treated with pronethalol (0·4 mg/min). Arterial blood pressure is also shown. Vasopressin (VP) was incubated at two different concentrations (250, 125 μu./ml) for 8 min. There was no loss of activity. Blood flow through the incubation circuit was 8 ml/min. Time scale, 10 min ; vertical scales, 5 cm ; mmHg.

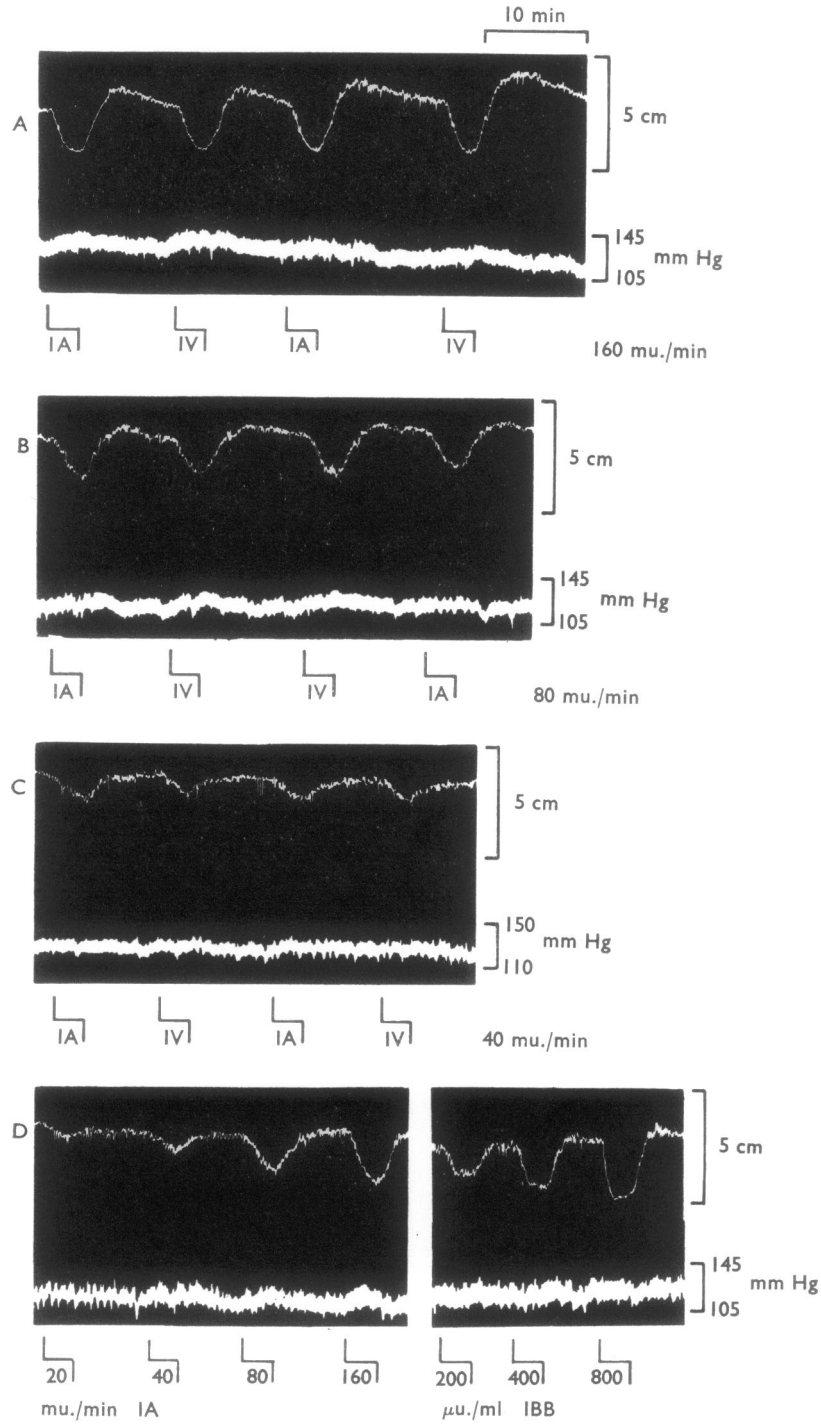

FIG. 9. Survival of vasopressin on passage through the pulmonary circulation. In each panel blood pressure is shown beneath the responses of a rabbit rectum superfused with arterial blood from a 10 kg female dog. Infusions of vasopressin were made into the superior vena cava (IV) or into the aorta near the semilunar valves (IA). Vasopressin was infused at 160 mu./min (A), 80 mu./min (B), 40 mu./min (C), or to produce dose-dependent responses of the rabbit rectum either IA (left-hand panel, D) or into the blood superfusing the tissue (IBB ; right-hand panel, D). Time scale, 10 min ; vertical scales, 5 cm ; mmHg.

Fate of vasopressin in the circulation

Incubation with blood

Vasopressin (13–250 μu./ml) was infused into the incubating circuit so that it mixed with the blood for up to 8 min before being assayed by the rabbit rectum. In four experiments, there was no loss of activity after either 4 min incubation (ten trials) or 8 min incubation (five trials). One of these experiments is illustrated in Fig. 8. Even after an 8 min incubation with blood, no disappearance of vasopressin was detected.

Passage of vasopressin through the pulmonary vascular bed

Vasopressin was infused intravenously and the effects on a rabbit rectum, bathed in femoral arterial blood, were compared with those of an infusion into the ascending aorta. In this way, any change in activity of vasopressin as it passed through the pulmonary vascular bed would have been detected.

In five dogs the rabbit rectum gave similar response to both intra-arterial and intravenous infusions, showing that there was no change in activity of vasopressin in the pulmonary vascular bed. Morphine was given to one dog without affecting the results (Fig. 9).

Disappearance in the peripheral vascular beds

The coaxial cannula was used to determine the loss of vasopressin in one complete circulation through the peripheral vascular beds. The rabbit rectum was bathed in blood sampled from the left ventricle and vasopressin was infused either intravenously or into the ascending aorta. Infusions were made for sufficient time (3–5 min) to give steady-state conditions, as shown by the plateau response of the assay organ. By comparing the responses of the rectum during the intra-arterial infusions with those during the intravenous infusions, the amount of vasopressin disappearing in one circulation was estimated. The results (Table 5) show that

TABLE 5. *Disappearance of vasopressin from the circulation of the dog during steady state conditions*

Dog	Weight (kg)	Sex	Trials No./ dog	Duration of infusion (min)	Infusion rate (mu./kg per min)	Total Vp Dose/trial (mu./kg)	Concentration of vasopressin (μu./ml blood)	% lost (%/circ.)	$t_{\frac{1}{2}}$ (s)
1	12·5	M	2	3	3·2	9·6	45	47	23
2	11·5	F	4	4	5·6	22·3	Not determined	37	30
3	13·0	M	1	4	3·1	12·3	200	10	136
4	16·5	M	2	3	5·5	16·5	189	30	39
5	12·5	M	3	3	4·9	14·9	150	12	112
6	10·5	M	1	4	19·0	76·2	400	20	72
Mean=	12·8	—	2	3·5	7·6	25·3	210	26·0	69·0
S.E.M.=	—	—	-	-	—	—	—	±5·9	±19·0

The half-life ($t_{\frac{1}{2}}$) was determined graphically on semilogarithm paper. It is defined as the time necessary for 50% of a dose of vasopressin to disappear from the circulation during steady state conditions. The time for a complete circulation (C.T.) may be defined (Dow, 1955) as C.T. = 3 × appearance time (A.T.) of an indicator. Data for the A.T. were obtained from the following: Lovenhart, Schlomovitz & Seybold (1922), A.T. = 8·7 s; Moore, Kinsman, Hamilton & Spurling (1929), A.T. = 9·1 s; Heringman, Davis & Rives (1945), A.T. = 8·6 s; Spector (1956), A.T. = 7·8 s (items 67, 68, 69, 75, 79, 83 of Table 275, p. 285). The mean A.T. = 8·6 s., so that C.T. = 26 s. We have assumed the mean circulation time to be 20 s.

$26 \pm 5{\cdot}9\%$ (range 10–47%) of the infused vasopressin disappeared in one transit through the peripheral vasculature. Assuming that the inactivation process is independent of blood concentration of vasopressin, these figures give half-lives in the different dogs of 23–136 s (mean $69 \pm 19{\cdot}0$ s).

A tracing from an experiment (dog 2, Table 5) in which the coaxial cannula was used, is shown in Fig. 10. Two rabbit rectums were superfused with blood taken from the left ventricle. Infusions of vasopressin through the coaxial cannula into the ascending aorta produced smaller responses than did the same rate of vasopressin infusion into the superior vena cava.

In this experiment 30 to 50% of the infused vasopressin was removed from the circulating blood by the peripheral vascular beds.

Discussion

Rabbit isolated intestine is sensitive to vasopressin (Gaddum, 1928 ; Levy, 1963 ; Woo & Somlyo, 1967). In a search for a specific assay organ for vasopressin, we have found the isolated rectum to be more sensitive than any other tissue tested, not only from the rabbit but from several other species.

The longitudinal muscle of the isolated rectum of the rabbit was relaxed by low concentrations of all three vasopressin preparations used but was only about 1/30 as sensitive to Syntocinon and 1/20 as sensitive to Pitocin. The fact that large doses of either peptide desensitized the tissue to both peptides is evidence that they act on the same receptors.

The actions of vasopressin on the rabbit rectum were not mediated by the release of catecholamine, since they were unaffected by an adrenergic neurone blocking

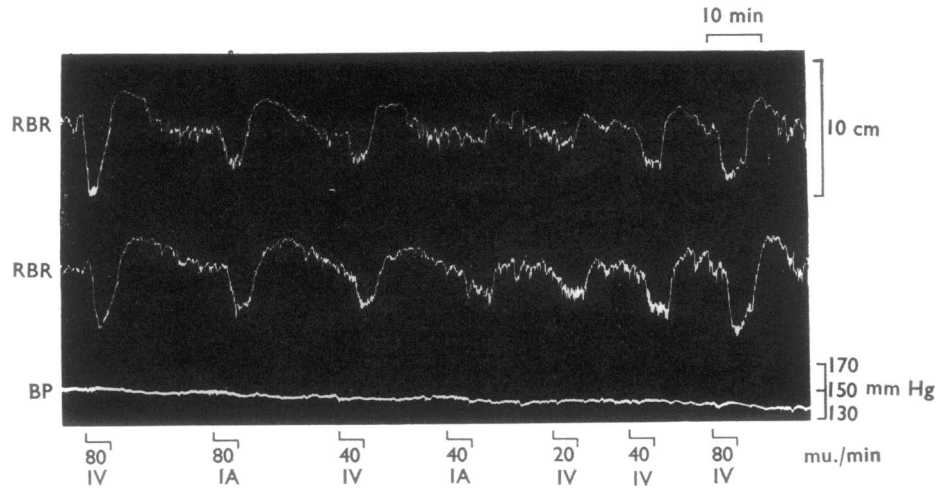

FIG. 10. Disappearance of vasopressin in one circulation through the dog. Infusions of vasopressin were made intra-arterially by means of the coaxial cannula (IA) or intravenously via the superior vena cava (IV) into an 11·5 kg female dog (dog 2, Table 5). No morphine or pronethalol was used. Arterial blood pressure (BP) and the responses of two rabbit rectums (RBR) are shown. Intra-arterial infusions of vasopressin produced smaller responses of the tissues than did the same doses of vasopressin given intravenously, indicating that some of the vasopressin was removed during circulation through the dog. Time scale, 10 min ; vertical scale, 10 cm ; mmHg.

agent (bretylium) or by β-adrenoceptor antagonists, such as propranolol or pronethalol. Lignocaine, in concentrations sufficient to reduce pendular movement and to produce sustained contracture of the rectum, reduced the action of the pituitary hormones on the rabbit rectum without affecting the actions of adrenaline. This reduction in the effects of the pituitary hormones by a local anaesthetic might suggest that they are inducing relaxation of the rectum through a nervous pathway. If this is so, it must be a non-adrenergic pathway, because α- and β-adrenoceptor blockade and adrenergic neurone blockade did not affect the responses of the rectum to vasopressin.

The relaxation of the rabbit rectum induced by catecholamines was mainly through an action on β-adrenoceptors, for it was almost completely abolished by propranolol. The small relaxation still obtained by higher doses of catecholamines in the presence of propranolol, was abolished by addition of an α-adrenoceptor antagonist. These findings are in agreement with those of Woo & Somlyo (1967) and Lawson & Mackenna (1969) on the rabbit colon.

Phentolamine antagonized the relaxant effect of vasopressin on the rectum, a property not possessed by other α-adrenoceptor antagonists. The antagonism was relatively weak, leading to a dose-ratio of about 3, although quantitation was difficult because of the excessive spontaneous activity (see Fig. 3); it is not a general antagonism of the neurohypophysial peptides, since the actions of oxytocin on the mammary gland were unaffected by phentolamine (Bisset, Clark & Lewis, 1967).

Woo & Somlyo (1967) found the inhibitory effects of vasopressin on rabbit intestine required magnesium. We could not confirm this observation on the rabbit rectum, although the spontaneous activity of the preparation was decreased by magnesium, making the effects of vasopressin more apparent.

Many preparations of the rabbit rectum had a high spontaneous activity, which tended to obscure the inhibition induced by low concentrations of vasopressin. Several procedures reduced this spontaneous activity, including storage in Krebs solution at 4° C for 24 to 48 h, lowering the bath temperature to 32° C and adding lignocaine to the bathing fluid. The addition of lignocaine also had the advantage that it increased the specificity of the assay by reducing the relative effects of oxytocin. The assay could be made more specific for vasopressin by addition of α- and β-adrenoceptor blocking agents, thereby eliminating the action of the catecholamines. We have not investigated the use of the rabbit isolated rectum for the assay of processed blood samples, since our interest was to develop the tissue as a blood-bathed organ; however, a rabbit rectum stored in Krebs solution for 24 to 48 h and then bathed at 32° C in Krebs solution containing lignocaine (10^{-4} g/ml), phenoxybenzamine (10^{-8} g/ml) and pronethalol (10^{-5} g/ml) should be both sensitive and specific.

The use of blood instead of Krebs solution to superfuse the rabbit rectum did not substantially change either its sensitivity to or specificity for vasopressin. The maintained contraction induced by blood and the associated decrease in spontaneous activity were both advantageous to the assay. The specificity of the assay was increased by the intraluminal infusion of a β-adrenoceptor blocking agent, which prevented the relaxant effects of catecholamines. For this purpose, pronethalol was preferred, for this substance further reduced the spontaneous activity and prolonged the inhibitory effects of vasopressin. That these additional effects were not due to

antagonism at β-adrenoceptors was shown by the fact that propranolol, a more potent and more specific antagonist (Black *et al.*, 1965) did not produce them.

When superfused with dog blood treated intraluminally with pronethalol, the rabbit rectum was a sensitive and specific bioassay preparation for vasopressin. The addition of a chick rectum in parallel assay (Gaddum, 1959) assured specificity for vasopressin, for oxytocin usually contracted the chick rectum at concentrations which barely relaxed the rabbit rectum. The superfusion of a rat stomach strip in series ensured detection of catecholamines, in concentrations much lower than those which would interfere with the assay of vasopressin.

There was no loss of vasopressin when incubated for up to 8 min with circulating blood ; this agrees with the work of Heller & Urban (1935), who showed that incubation of vasopressin with dog blood did not reduce the antidiuretic activity of vasopressin. Harvey, Jones & Lee (1967) found vasopressin to be bound to plasma protein, but this process would presumably be too rapid to be detected by our technique.

Vasopressin was not inactivated in the pulmonary vascular bed, a result confirming that of Beleslin, Bisset, Haldar, Jaya & Polak (1967). Because of this, either arterial or mixed venous blood can be used for the assay. Since vasopressin is stable in circulating blood and survives transit through the pulmonary vasculature, it fits into the concept of a " circulating hormone " (Vane, 1969) ; this is consistent with a physiological action on the kidney and the blood pressure.

The inactivation of vasopressin must be associated with its passage through peripheral vascular beds. In our experiments, this inactivation varied from 10 to 47%. The calculation of the half-life of vasopressin in the circulation assumes that the process(es) of inactivation reduce the concentration of vasopressin by the same proportion, irrespective of the initial concentration, thus leading to a simple exponential decline. This assumption has been used by other workers in their calculation of half-life values, which vary from 3 to 11 min in individual experiments (Silver, Schwartz, Fong, Debons & Dahl, 1961 ; Lauson & Bocanegra, 1961 ; Share, 1962 ; Czaczkes & Kleeman, 1964 ; Lauson, Bocanegra & Beuzeville, 1965 ; Rocha e Silva & Rosenberg, 1969). It is not immediately apparent why our results give much shorter half-life values (0·25–2·5 min). One possibility is that a metabolite is formed which retains anti-diuretic activity but does not have the smooth muscle relaxant activity. Another possibility is that the inactivation process is saturable. Whereas our infusions were for 3–5 min, those of other workers have been from 20 to 170 min. It may be, therefore, that the longer durations of infusion lead to saturation of an enzymic or binding inactivation mechanism.

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