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A SENSITIVE METHOD FOR THE ASSAY OF OXYTOCIN IN THE CIRCULATING BLOOD

J.R. VANE[1] & K.I. WILLIAMS[2]

Department of Pharmacology, Institute of Basic Medical Sciences,
Royal College of Surgeons of England, Lincoln's Inn Fields, London WC2A 3PN

1 The sensitivity of a wide range of isolated tissues to oxytocin was investigated. The longitudinal muscle strip of duck pulmonary vein proved the most suitable tissue for use with the superfusion technique, contracting to concentrations of oxytocin as low as 10 μu/ml.

2 The duck pulmonary vein superfused with Krebs solution was contracted by oxytocin, the vasopressins, adrenaline, noradrenaline, 5-hydroxytryptamine, histamine and angiotensin II. Pre-treatment of the preparation with phenoxybenzamine (1-2 μg/ml) abolished the contractions to catecholamines and reduced the effects of histamine and 5-hydroxytryptamine without affecting the sensitivity to oxytocin.

3 The pulmonary vein contracted when superfused with blood from an anaesthetized dog. This contraction was accompanied by a non-specific loss of responsiveness. When the pulmonary vein was superfused with Krebs solution that had been dialysed against blood the initial contraction was greatly reduced or abolished as was the loss in responsiveness.

4 Oxytocin was stable in circulating dog's blood but approximately 50% was bound to plasma proteins. Oxytocin was not destroyed in the pulmonary circulation or the hind limbs. In the doses used oxytocin had a half-life of 60-90 s in the circulation of the dog under steady-state conditions. Disappearance occurred chiefly in the visceral vascular beds.

Introduction

Methods for the bioassay of oxytocin depend upon responses of the physiological target organs. Dale (1909) first showed that uteri from various species were contracted by neurohypophysial hormones; since then, many methods have been developed based on isolated uterine preparations (see Fitzpatrick & Bentley, 1968). Other assays are based on contractions of isolated strips of mammary gland from lactating rabbits (Mendez-Bauer, Cabot & Caldeyro-Barcia, 1960; Moore & Zarrow, 1965), rats (Smith, 1961; Ryden & Sjoholm, 1962) and mice (Fielitz, Roca, Mattei, Melander, Garofolo, Gioia de Coch & Coch, 1970).

Oxytocin has also been assayed on these organs *in situ*, by measuring increases in intraluminal pressure in the rat uterus (Bisset, Halder & Lewin, 1966), in the mammary glands of lactating rabbits (Cross & van Dyke, 1953), guinea-pigs (Tindal & Yokoyama, 1962) or rats (Bisset, Clark, Haldar, Harris, Lewis & Rocha e Silva, 1967). In addition oxytocin has been standardized using its depressor effect on the blood pressure of chickens (Coon, 1939). Of these methods the most sensitive and widely used assays are the contractions of rat or mouse mammary strips *in vitro* or the rise in intra-mammary pressure in lactating rats.

Blood concentrations of neurohypophysial hormones are usually measured by serial removal of blood samples. This has two disadvantages: the extraction procedure is time consuming and the number and size of the samples removed is limited since haemorrhage itself is a potent stimulus for vasopressin release (Ginsburg & Brown, 1956: Beleslin, Bisset, Haldar & Polak, 1967; Rocha e Silva, Jr. & Rosenberg, 1969). The blood-bathed organ technique (Vane, 1964, 1969) allows the continuous assay of circulating hormones *in vivo*. Siddiqui & Walker (1960) used the rat isolated uterus superfused with de Jalon's solution and intermittently with carotid arterial blood from a rabbit, to detect oxytocin-like material in the blood samples. However, the uterus is contracted by many other substances including bradykinin, angiotensin and 5-hydroxytryptamine (Bisset & Lewis, 1962). This lack of specificity makes the preparation unsuitable as a blood-bathed organ so

[1] Present address: Wellcome Research Laboratories, Langley Court, Beckenham, Kent.

[2] Present address: Department of Pharmacology, Medical School, University Walk, Bristol BS8 1TD.

we looked for an assay tissue selectively sensitive to oxytocin. Strips of pulmonary vein from the duck proved suitable. These results have been communicated in part to the British Pharmacological Society (Vane & Williams, 1970).

Methods

Experiments in artificial salt solutions

Smooth muscle preparations from many sources were tested for their sensitivity to oxytocin and other substances. The following tissues were tested: rat stomach strip (Vane, 1957), ascending colon (Regoli & Vane, 1964), uterus and radially cut strips of mammary gland (Smith, 1961); sections of duodenum, jejunum, caecum, uterus, colon (Ambache, Kavanagh & Whiting, 1965) and rectum from the desert rat (*Meriones shawii*); rabbit terminal ileum and sections of colon down to the rectum (Gilmore & Vane, 1970); longitudinal muscle strips of duodenum, jejunum, proximal and terminal ileum and distal colon from the dog; chick oesophagus and rectum (Mann & West, 1950); hen rectal caecum (Barsoum & Gaddum, 1935), oviduct (Munsick, 1960) and spirally-cut strips of pulmonary artery and vein (Somlyo, Somlyo & Woo, 1967); spirally-cut strips of pulmonary artery and vein from the duck and pigeon (longitudinal strips of these vessels were only taken from ducks); and spirally-cut strips of umbilical artery and vein (Somlyo, Woo & Somlyo, 1965) from the human neonate.

The pulmonary vessels of the duck were the most extensively studied. Khaki-Campbell or Aylesbury ducks of either sex and weighing between 1 and 4 kg were killed and bled. The thoracic cavity was opened to expose the heart. The aorta and brachiocephalic arteries were cut between ligatures, thereby exposing the pulmonary arteries. To dissect out the pulmonary veins the apex of the heart was deflected cephalad and the vena cava divided. Lung tissue was cleared completely to expose both primary branches of the veins, which are approximately 1 cm long. Both veins and arteries were cut at their common junction with the heart and distal to the sites of secondary branching to obtain the maximum length of vessel.

All tissues were transferred to pre-gassed Krebs solution. In some experiments preparations were pre-treated with phenoxybenzamine (1-2 μg/ml) in Krebs solution for 30-45 min before use. The initial load on tissues varied from 1-4 g. Some tissues were stored overnight at 4°C.

Preparations were suspended in polypropylene jackets for superfusion (Gaddum, 1953) by Krebs solution or blood. Two banks were used with 3 jackets in series in each. Changes in length of the preparations were detected by Harvard heart/smooth muscle transducers (Cat. No. 356) fitted with an auxotonic lever (Paton, 1957); changes in tension were measured with isometric transducers. The transducer outputs were amplified and displayed on an 8-channel Beckman-Offner dynograph.

The composition of the Krebs solution in g/l distilled water (mM) was as follows: NaCl 6.9 (118), KCl 0.35 (4.7), $CaCl_2$ $6H_2O$ 0.55 (2.5), KH_2PO_4 0.16 (1.2), $MgSO_4$ $7H_2O$ 0.29 (1.17), glucose 1.0 (5.6), $NaHCO_3$ 2.1 (25). The solution was gassed with 95% oxygen and 5% CO_2. Magnesium ion concentration was changed by varying amounts of magnesium chloride. Tonicity of such solutions was not adjusted.

The isolated tissues were superfused with Krebs solution at 37°C and constant flow rates between 5-10 ml/minute.

Blood-bathed organ experiments

Dogs of either sex weighing 6-26 kg were anaesthetized with halothane delivered from a Goldman vaporizer. Anaesthesia was maintained with chloralose (10 mg/ml, 100 mg/kg intravenously). The trachea was cannulated and the animal was mechanically ventilated by a Palmer Ideal respiration pump. Polyethylene cannulae were tied into a femoral artery and a femoral vein for removal and replacement of blood. Other arteries or veins were cannulated as stated in the text. Heparin (1000 i.u./kg) was injected intravenously. In some experiments dextran (molecular weight 110,000; 6% w/v in 0.9% saline) and/or dextrose (5% w/v in water) were injected intravenously. Mean arterial pressure was recorded via a pressure transducer on the pen recorder.

The blood for superfusion of the isolated tissues was pumped either from the femoral arterial cannula or from a catheter in the ascending aorta at a constant rate of 10 ml/min for each bank of tissues. After the blood had superfused the assay tissues it was collected in a reservoir and returned to the dog by gravity through a femoral vein.

Fate in the general circulation

The disappearance of oxytocin during one passage through the general circulation was studied using a double-lumened catheter similar to that described by Ferreira & Vane (1967a).

The blood was obtained from the ascending aorta by a polyethylene catheter (PP 240) which was passed down the right carotid artery until the

pulse pressure recorded through it showed that the tip was in the left ventricle. The catheter was then withdrawn until the change in pulse pressure showed that it was in the ascending aorta. The catheter contained a second much finer polyethylene tube (PP 50) which opened to the periphery 1 cm above the point of blood withdrawal. Drugs infused through this fine catheter mixed with the cardiac output and passed once through the general circulation before being sampled through the main catheter for superfusion of the assay tissues.

Fate in the pulmonary circulation

A catheter was positioned in the ascending aorta as described in the previous section. The effects of infusions through this catheter were compared with those of infusions into the vena cava via a catheter inserted through the left external jugular vein. Femoral blood was sampled for assay.

Incubation with circulating blood

An incubation circuit (Ferreira & Vane, 1967b) was used to assess inactivation of oxytocin in blood. The silicone tubing which carried the blood from the dog to the assay organs was lengthened in order to contain up to 30 ml of blood, thereby increasing the time of passage of the blood to the assay tissues by up to 3 minutes. The blood was maintained at 37°C by immersing the tubing (6 mm diameter, 3 mm bore, Esco Rubber Ltd.) in a water bath.

Dialysis of circulating blood

The technique has been described by Collier (1972). Briefly, instead of being bathed in blood, the assay tissues were bathed in Krebs solution that had been dialysed against blood.

Blood from anaesthetized dogs was pumped by a peristaltic pump (Model 1216; Harvard Apparatus Co.) at 50 ml/min into the inner chamber of a miniature Kiil dialysis machine. The blood was then returned intravenously to the animal. The dialysis sheeting was reconstituted cellulose phosphate (Cuprophan: Bemberg) which allowed passage of substances with molecular weights of less than 1500. Krebs solution at 5 ml/min was pumped in a counter-current direction through the outer chamber of the machine and then used to superfuse the assay tissues.

Drugs

The following drugs were used. Doses of salts are expressed in terms of base: acetylcholine chloride (Sigma), (−)-adrenaline bitartrate (B.D.H.), angiotensin II amide (Hypertensin, Ciba), arginine-8-vasopressin (natural; Parke-Davis), arginine-8-vasotocin (Sandoz), ascorbic acid (B.D.H.), bradykinin (synthetic; Parke-Davis), α-chloralose (Merck, Darmstadt AG), dextran 110 injection B.P. (Dextraven 110; Fisons), dextrose 5% (w/v) (Evans), halothane (Fluothane; I.C.I.), heparin (Boots), histamine acid phosphate (B.D.H.), 5-hydroxytryptamine creatinine sulphate (May and Baker), hyoscine hydrobromide (B.D.H.), lysine-8-vasopressin (Pitressin; Parke-Davis), mepyramine maleate (May and Baker), methysergide bimaleate (Sandoz), (−)-noradrenaline bitartrate (Sigma), oxytocin (Pitocin; Parke-Davis, pentobarbitone sodium (Nembutal veterinary; Abbott), phenoxybenzamine hydrochloride (Dibenyline; Smith, Kline and French), (±)-pronethalol (Alderlin; I.C.I.), (±)-propranolol (Inderal, I.C.I.), prostaglandins E_1, E_2, A_1, $F_{1\alpha}$, and $F_{2\alpha}$ (Upjohn), a crude preparation of slow-reacting substance in anaphylaxis (Parke-Davis), substance P, sodium chloride injection 0.9% (w/v; Boots).

Results

Experiments in Krebs solution

Many different tissues were tested for their sensitivity to oxytocin. They were superfused in cascade so that 6 preparations could be tested at the same time. The uteri of the rat and desert rat, mammary strips from the lactating rat and strips of avian pulmonary vasculature were the only tissues tested which responded consistently to doses of oxytocin below 1 mu/ml (1 mu = 2 ng, Bodanszky & Du Vigneaud, 1959).

Somlyo *et al.* (1967) showed that strips of pulmonary artery and vein from the chicken were contracted by low concentrations of oxytocin. We confirmed this observation and found that concentrations above 100-200 μu/ml contracted longitudinal strips of chicken pulmonary artery. In the hope of finding a still more sensitive preparation, we compared pulmonary arteries taken from chickens with those taken from pigeons and ducks. Those from the pigeon were equisensitive with those from the chicken and were not studied further. However, strips of duck pulmonary vasculature were 4-5 times more sensitive to oxytocin than those from the chicken (Figure 1). Longitudinal muscle strips of vein were preferred to spiral strips of artery for they attained a plateau response faster and maintained a steadier base line (Figure 1).

The uteri had two disadvantages; they were relatively non-specific and contracted to many

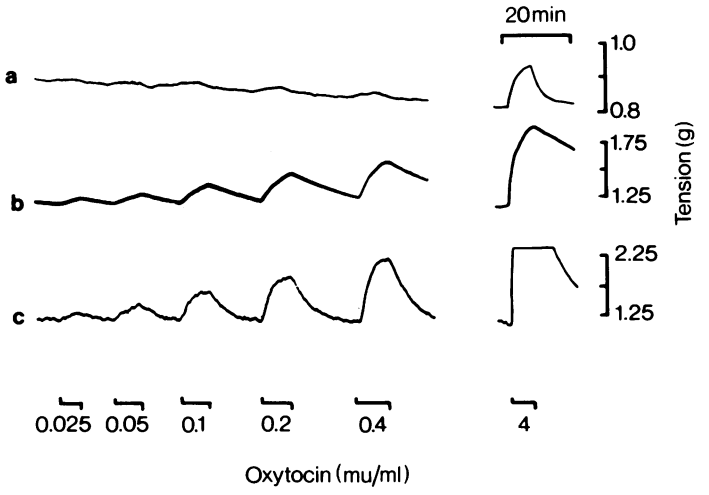

Fig. 1 Comparison of the sensitivities of chicken and duck pulmonary vessels to oxytocin. (a) Spirally-cut strips of chicken pulmonary artery; (b) duck pulmonary artery; and (c) a longitudinal strip of duck pulmonary vein were superfused with Krebs solution at 10 ml/min and 37° C.

other substances as well as to oxytocin. Furthermore, the contractions were not sustained for the duration of an infusion (Figure 2). Rat mammary strips had a flat dose-response curve, showing only small increments in tension over the concentration range of 30-200 μu/ml. The discrimination between oxytocin and vasopressin was also low, as found previously by Fabian, Forsling, Jones & Lee (1969). The longitudinal strips of duck pulmonary vein were, therefore, chosen as the assay tissues.

There was no difference in sensitivity between preparations taken from male or female ducks; nor was there a difference between Khaki-Campbell and Aylesbury ducks. However, the Aylesbury ducks gave a larger preparation and 2-3 strips could be obtained from each of the two pulmonary veins. For this reason and because occasional preparations from the Khaki-Campbell were insensitive to oxytocin, Aylesbury ducks were preferred.

In a few experiments isometric, isotonic and auxotonic recordings were compared. The best results were obtained with isometric transducers, arranged so that the initial tension of the isolated pulmonary vein was 1-2 g. The base line was more stable than with auxotonic or isotonic recording and the rate of contraction and relaxation was faster. Storage of the vessels at 4° C overnight, decreased sensitivity to oxytocin, so fresh preparations were always used.

The sensitivity of the duck pulmonary vein was tested to several substances at concentrations varying from 10-80 ng/ml (Table 1). The vein was contracted by adrenaline, noradrenaline, 5-hydroxytryptamine, histamine and the vasopressins. At higher concentrations (50-100 ng/ml) angiotensin caused a small contraction whereas bradykinin, prostaglandins E_1, E_2 and $F_{2\alpha}$, substance P, slow reacting substance in anaphylaxis (SRS-A) and acetylcholine had little or no effect. When the veins were pre-treated with phenoxybenzamine (1-2 μg/ml for 30-45 min) the contractor actions of adrenaline and noradrenaline were abolished and the effects of 5-hydroxytryptamine and histamine were greatly reduced. The effects of these amines did not return for the duration of the experiments (5-8 hours). Thus, the specificity of the duck pulmonary vein for oxytocin was greatly enhanced by pre-treatment with phenoxybenzamine and this was adopted routinely. The duck pulmonary vein was 10 times as sensitive to oxytocin as it was to arginine-8-vasopressin (activity compared on a weight basis using the conversion factors of Boissonas, Guttman, Berde & Konzett, 1961).

The commercially available preparations of oxytocin and vasopressin contain the preservative, chlorobutanol at a concentration of 5 mg/ml. After dilution to the required strength the chlorobutanol concentration was 2.5 μg/ml in the oxytocin solution and 25 μg/ml in the vasopressin solution. At 25 μg/ml chlorobutanol by itself had no effect on the pulmonary vein preparation; nor did it antagonize or potentiate the effects of oxytocin.

Walker (1960) found that heparin could inhibit the response of the superfused rat uterus to

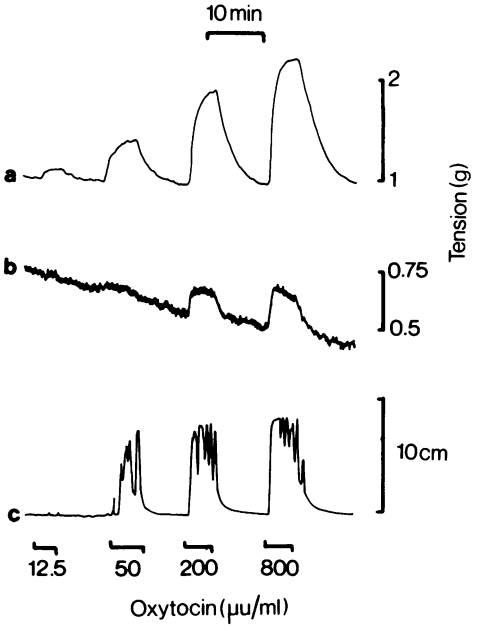

Fig. 2 A comparison of the sensitivities of (a) duck pulmonary vein; (b) rat mammary strip; and (c) rat uterus to infusions of oxytocin. Superfusion with Krebs solution (10 ml/min) at 37°C.

oxytocin. Heparin (10 i.u./ml) did not affect the resting tension or the contractions of the duck pulmonary vein to adrenaline or oxytocin (2 experiments).

That oxytocin and vasopressin act on the same population of receptors was shown by cross tachyphylaxis experiments. In three experiments the pulmonary vein was repeatedly exposed to high concentrations (0.5 u/ml) of oxytocin, until the preparation no longer responded. It was then also insensitive to vasopressin. In two other experiments cross-tachyphylaxis was demonstrated with vasopressin (0.2-0.5 u/ml) as the desensitizing agent. In all of these experiments the contractions induced by adrenaline or 5-hydroxytryptamine were unaffected by the desensitization process.

Somlyo *et al.* (1967) found that the effects of oxytocin on the chicken pulmonary artery were directly related to the concentration of magnesium ions in the bathing fluid. Figure 3 illustrates an experiment in which a strip of duck pulmonary vein was initially superfused with normal Krebs solution which contained 1.2 mM magnesium. Contractions were induced by oxytocin, lysine-8-vasopressin and 5-hydroxytryptamine in the form of cumulative dose-response curves. Krebs solution containing 9.6 mM magnesium was then substituted and the duck pulmonary vein

Table 1 The effect of phenoxybenzamine on the relative potencies of substances which contract the duck pulmonary vein (DPV).

Agonist	*DPV dose (ng/ml)*	*DPV* dose (ng/ml)*
Oxytocin	0.1	0.1
Arginine-8-vasotocin	0.8 (0.7-1.1)	–
Arginine-8-vasopressin	1.2 (0.7-2.1)	1.1 (0.6-1.3)
Lysine-8-vasopressin	1.5 (0.8-1.8)	1.8 (1.2-2.1)
Acetylcholine	over 50	over 50
Adrenaline	10-20	over 50
Noradrenaline	10-40	over 100
5-hydroxytryptamine	5-40	60-100
Histamine	5-40	30-100
Bradykinin	over 50	over 50
Angiotensin II amide	30-80	over 30
Prostaglandin E_1	over 80	
E_2	over 80	
$F_{2\alpha}$	over 80	
A_1	over 40	
Substance P	over 100	
SRS-A	over 100	

Dose of agonist is that needed to produce a similar tension increase (0.2-0.8 g) to 0.1 ng/ml of oxytocin. Figures in parentheses for the vasopressins are the dose ranges in 9 experiments. All other values are from means of 5 observations. Doses of posterior pituitary hormones were converted to weights using the conversion factors: arginine-8-vasopressin, 400 u/mg; lysine-8-vasopressin, 250 u/mg (Boissonas *et al.*, 1961); oxytocin, 500 u/mg (Bodanszky & Du Vigneaud, 1959).
DPV*—pre-treated with phenoxybenzamine 1 μg/ml for 30-45 min before use.

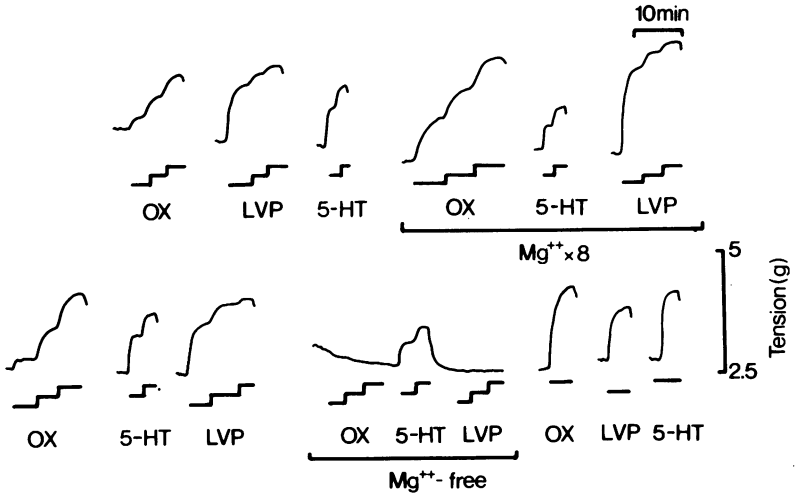

Fig. 3 The effects of altering magnesium ion concentration on contractions of the duck pulmonary vein. A strip of vein was superfused with Krebs solution at 10 ml/minute. Cumulative dose-response curves to oxytocin (OX; 12.5, 50 and 200 μu/ml); lysine-8-vasopressin (LVP; 500, 1000 and 2000 μu/ml) and 5-hydroxytryptamine (5-HT; 10 and 20 ng/ml) were obtained by infusions directly over the assay tissues. The first responses were obtained in normal Krebs solution (1.2 mM magnesium). A subsequent increase to 9.6 mM magnesium (Mg^{++} x 8) potentiated the responses to neurohypophysial hormones but depressed those to 5-hydroxytryptamine. This effect of magnesium was reversible. Omission of magnesium ions from the bathing fluid selectively abolished the responses to oxytocin and vasopressin. The last contractions were induced by oxytocin (200 μu/ml), lysine-8-vasopressin (500 μu/ml) and 5-hydroxytryptamine (20 ng/ml).

again exposed to the agonists. Oxytocin- and lysine-8-vasopressin-induced contractions were potentiated whereas contractions to 5-hydroxytryptamine were unaffected. Superfusion of the tissue with magnesium-free Krebs solution abolished contractions to oxytocin and lysine-8-vasopressin but left unaltered those to 5-hydroxytryptamine (3 experiments).

Blood-bathed organ experiments

When the duck pulmonary vein preparation was first superfused with blood instead of Krebs solution it contracted, as do many other isolated tissues (Vane, 1964). The contractions passed through a peak tension of 1-2 g in the first few minutes and after 10 min the pulmonary vein had usually adopted a new and higher resting tension (Figure 4). In only a few experiments did the resting tension return to its previous level; in most it remained 0.5-1 g higher. Associated with this increase in resting tension was a reduction in responsiveness to the effects of oxytocin. The loss of responsiveness was such that 11 times (range 4-30) the concentration of oxytocin had to be used when the preparation was bathed in blood rather than in Krebs solution (17 preparations compared in 7 experiments). This loss in sensitivity was not specific for oxytocin. In preparations which had not been pre-treated with phenoxybenzamine, a similar loss was observed towards adrenaline, noradrenaline, 5-hydroxytryptamine or histamine. Because the preparation has no spontaneous activity, the loss in sensitivity could be compensated for by a suitable increase in electronic amplification. Thus, concentrations of oxytocin of 25-50 μu/ml were still detectable by blood-bathed preparations.

The duck pulmonary vein showed a similar spectrum of sensitivity when bathed in blood as it did when bathed in Krebs solution. Phenoxybenzamine-treated preparations did not respond to catecholamines, 5-hydroxytryptamine or histamine but still contracted to oxytocin. The potency of vasopressin relative to oxytocin was similar to that found in Krebs solution.

Fate of oxytocin in the circulation of the dog

Incubation with circulating blood. Oxytocin (100-400 μu/ml) was infused into the incubating circuit so that it was in contact with the blood for up to 3 min before being assayed (3 experiments with 3 trials at each dose level). There was no loss of activity of oxytocin.

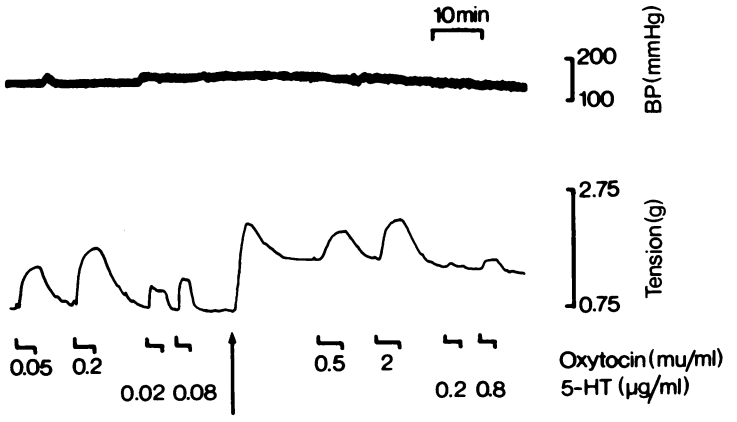

Fig. 4 Desensitization of the duck pulmonary vein by superfusion with blood. A strip of duck pulmonary vein (DPV) was superfused with Krebs solution at 10 ml/min, 37° C and was sensitive to oxytocin (0.05 and 0.2 mu/ml) and 5-hydroxytryptamine (5-HT; 0.02 and 0.08 µg/ml). When superfused with femoral arterial blood (at arrow) from a 16 kg male dog there was a strong contraction of the DPV, and much larger concentrations of drugs were needed to induce similar responses to those obtained during superfusion with Krebs solution.

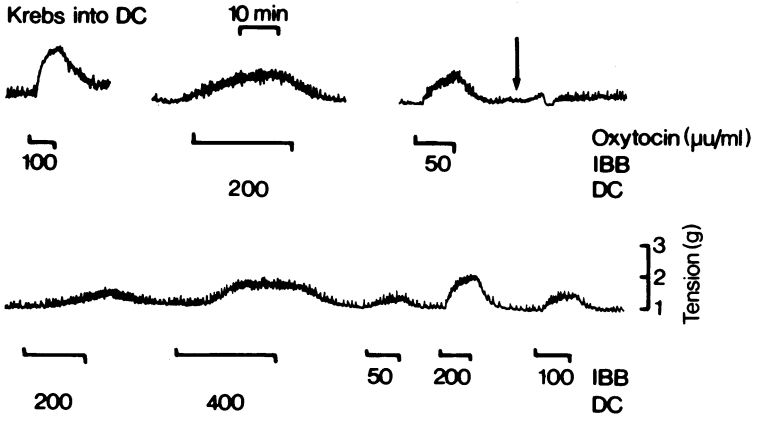

Fig. 5 Dialysis of oxytocin from circulating blood. A strip of duck pulmonary vein was superfused at 5 ml/min with the Krebs solution leaving a dialysis machine. Krebs solution also passed through the other side of the dialysis circuit (DC) at 50 ml/minute. The response to an infusion of oxytocin into the dialysis circuit was bracketed by doses given directly over the tissue (IBB); the oxytocin which dialysed was equivalent to 26% passage across the membrane. Blood was then passed into the dialysis circuit (indicated by the arrow) and the equivalent of 30% of the same dose was found to dialyse. At a higher dose (400 µu/ml) there was an equivalent of 35% dialysis.

Dialysis against circulating blood. Figure 5 shows an experiment in which the dialysis machine was used. Initially, Krebs solution filled both sides of the dialysis machine and oxytocin was infused into one side to give a final concentration of 200 µu/ml. The assay tissues indicated a passage of oxytocin equivalent to 26%. When the stream of Krebs solution at 50 |ml/min was replaced by blood, the initial contraction of the tissues was negligible (compare with Figure 4). Loss of responsiveness was also reduced by dialysis, oxytocin being 2.8 times less potent (range 1.6-3.8, 5 preparations in three experiments). From blood, 30% of an infusion of 200 µu/ml of oxytocin dialysed and 35% at a higher concentration (400 µu/ml).

Fate in the pulmonary circulation

Duck pulmonary veins were superfused with femoral arterial blood, and oxytocin (2.8-5.6 mu kg^{-1} min^{-1}) was infused either into the

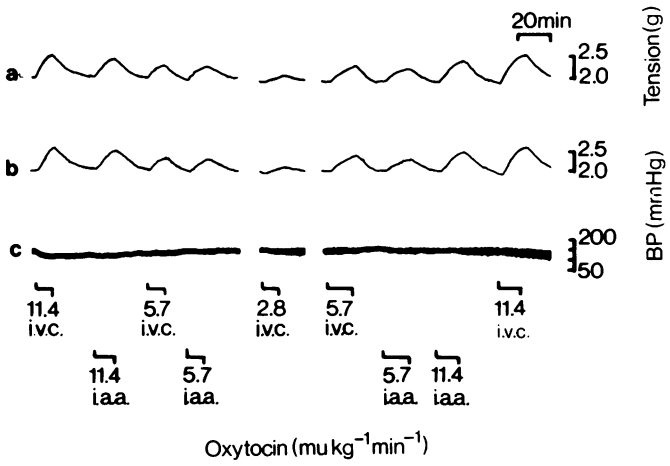

Fig. 6 Dog (6.5 kg; male). The disappearance of oxytocin (Oxy) in one passage through the general circulation. Two strips (a and b) of duck pulmonary vein, pre-treated with phenoxybenzamine were superfused with aortic blood at 10 ml/minute. Responses were obtained by infusing oxytocin into the vena cava (i.v.c.) or into the ascending aorta (i.a.a.). Between 14-25% of the oxytocin infused i.a.a. disappeared during one passage through the circulation.

ascending aorta or into the vena cava. There was no inactivation of oxytocin in the pulmonary circulation (4 experiments).

Disappearance of oxytocin in the general circulation

Duck pulmonary veins were bathed in blood from the ascending aorta and infusions of oxytocin into the total cardiac output were compared with similar infusions into the vena cava. One of these experiments is shown in Figure 6. Steady-state conditions were obtained after 15-20 min of infusion, as shown by the plateau response of the assay tissues. Intra-arterial infusions of oxytocin (2.8-11.4 mu kg^{-1}min^{-1}) produced smaller contractions of the duck pulmonary veins than did similar infusions into the vena cava. Calculation from the dose-response curves showed that 14-25% of the oxytocin disappeared in one passage through

Table 2 Half-life of oxytocin in the dog under steady-state conditions.

Expt.	*Dog weight*	*Oxytocin infusion rate (mu kg^{-1} min^{-1})*					
		2.8		*5.7*		*11.4*	
	(kg)	*% Loss*	*T½(s)*	*% Loss*	*T½(s)*	*% Loss*	*T½(s)*
1	6.5			26	58	14	114
2	7	23	68	20	78	16	100
3	7.5			15	110	11	155
4	8.5	26	58	41	33		
5	12			15	110	18	88
6	14.5	39	35	24	63		
7	15	25 (27)	60 (55)	33	44		
8	15	14 (15)	115 (110)			32	45
9	17.5	44	30	17	94		
10	20	43	31	46	28		
11	25	15 (14)	110 (115)	34	42	22	70
Mean ± s.e. mean		29 ± 4	63 ± 12	27 ± 4	66 ± 10	19 ± 3	95 ± 16

The disappearance of oxytocin from the circulation was assumed to be exponential and the half-life ($T_{½}$) was determined by a semi-logarithmic plot. Circulation time was calculated from the data of Spector (1956) and was taken to be 25 s in these experiments. Figures in parentheses were obtained when the infusion was made at the level of the diaphragm.

peripheral vascular beds. The results of 11 experiments are summarized in Table 2. A mean of 29% of the infused oxytocin was lost in one circulation from the lowest infusion rate of 2.8 mu kg^{-1} min^{-1}, 27% from the 5.7 mu kg^{-1} min^{-1} rate and 19% from the 11.5 mu kg^{-1} min^{-1} rate. The half-life of oxytocin in the circulation of the dog, calculated by assuming that the activity in the blood declined exponentially with time, varied from 28 to 155 s with mean values of 63, 66 and 95 s for the three infusion rates used. In 3 experiments, infusions of oxytocin at the lowest rate were also made through a catheter inserted retrogradely into a femoral artery so that the tip lay at the level of the diaphragm. Similar losses of oxytocin occurred from these infusions as occurred from infusions into the total cardiac output.

Hind Limbs

In 2 experiments (7 and 8, Table 2), the lowest dose of oxytocin was infused close-arterially to the hind-limbs; no loss of oxytocin could be detected.

Discussion

The isolated longitudinal muscle strip of the duck pulmonary vein is sensitive and specific enough to be used for the bioassay of oxytocin. The sensitivity was far higher than that of segments of chicken pulmonary artery and vein (Somlyo *et al.*, 1967).

As well as being sensitive to oxytocin the duck pulmonary vein was reasonably specific. Arginine-8-vasopressin had one eleventh and lysine-8-vasopressin one fifteenth of the activity of oxytocin. Arginine-8-vasotocin, which has a structure intermediate between that of oxytocin and arginine-8-vasopressin was one eighth as active as oxytocin. Experiments in which cross-tachyphylaxis was demonstrated showed that oxytocin and vasopressin contracted the duck pulmonary vein by acting on the same receptors. Of the other vaso-active substances likely to be found in blood only the catecholamines, 5-hydroxytryptamine and histamine were likely to interfere with the assay and their effects were abolished by pre-treatment of the pulmonary vein with phenoxybenzamine. Further treatment with phenoxybenzamine during the experiment was unnecessary for the effects of this substance on isolated tissues are irreversible (Nickerson, 1957).

The duck pulmonary vein compared well as an assay tissue with the established bioassay organs, the rat isolated uterus and mammary strip. The rat uterus was too unspecific and the contraction induced by oxytocin was not maintained.

The sensitivity of the rat mammary strip was similar to that observed by Smith (1961). However there was a low ratio of discrimination between oxytocin and vasopressin, oxytocin being only 5 times more potent. The main disadvantage of the duck pulmonary vein was that the dose-response curve was shallow, a feature shared by many preparations of vascular smooth muscle.

Contractions of the duck pulmonary vein induced by neurohypophysial hormones were dependent upon the magnesium ion concentration in the bathing fluid, a characteristic shared by many tissues including rat uterus (Bentley, 1965), canine vascular strips and strips of rabbit myoepithelium (Somlyo, Woo & Somlyo, 1966). Relaxations of rabbit colon and rectum are also influenced by changes in magnesium ion concentration (Woo & Somlyo, 1967; Gilmore & Vane, 1970). The fact that an 8-fold increase in magnesium ion concentration potentiated the contractions of the duck pulmonary vein induced by the neurohypophysial hormones but depressed those to other agonists suggests that a modified salt solution may increase the specificity of the assay. There was no interference with the assay of oxytocin due to chlorobutanol or heparin.

Superfusion of the vein with blood, rather than Krebs solution caused a contraction which was associated with a decreased responsiveness to oxytocin. This effect was not due to circulating oxytocin because concentrations of oxytocin which matched the contraction caused by the blood were high (about 100 μu/ml) whereas assay of extracted control samples for milk-ejecting activity proved negative (Williams, unpublished observation). The finding that dialysis of the blood reduced both these effects on the pulmonary vein suggests that the factor(s) involved has a molecular weight greater than 1500 and therefore dialyses poorly. The presence of two factors is suggested by the finding that even when an initial contraction of the pulmonary vein to dialysed blood did not occur, some loss of responsiveness was still encountered.

The blood-bathed organ technique is a dynamic assay which allows a more physiological study of the fate of oxytocin in the bloodstream than other methods. There was no loss of activity of oxytocin incubated with circulating blood for 3 minutes. It was impossible to deduce from these experiments whether oxytocin was bound to plasma proteins, for rapid binding could have occurred equally for infusions given directly over the assay tissue or into the incubating circuit. The dialysis experiments showed that similar amounts of infused oxytocin dialysed from Krebs solution to

Krebs solution as did from blood to Krebs solution. Oxytocin does not penetrate into erythrocytes (Ginsburg & Smith, 1959; Fitzpatrick, 1961); thus, assuming a haematocrit of 50% for dog's blood, the effective plasma concentration of infused oxytocin in these experiments was double the estimated blood concentration. Taking this into account, the dialysis experiments indicate that approximately 50% of the infused oxytocin was bound to plasma proteins. This conclusion in the dog supports the ultrafiltration experiments of Thorn (1959) and Fabian *et al.* (1969) who found that 40% of exogenously added oxytocin was bound to rat plasma proteins. Thus, we can conclude that plasma protein binding of oxytocin occurs *in vivo* and is a rapid process.

Oxytocin was not inactivated in the pulmonary circulation of the dog, a finding similar to that of Biron & Boileau (1969) in the rat. Oxytocin can thus be classed as a circulating hormone (Vane, 1969), a conclusion which is consistent with the physiological functions attributable to this hormone.

The half-life of 60-90 s for oxytocin, obtained using the coaxial catheter is the first estimate available in the dog. This is a shorter time than would be expected from the results of other workers (see Ginsburg, 1968). The half-life measured may depend on the doses of oxytocin used, for the longest times were obtained after giving large amounts of hormone (2 u/kg or more) whereas the shorter times were estimated with much smaller doses. The longer half-lives could be due to saturation of the oxytocin-inactivating mechanism, as suggested for vasopressin (Ginsburg, 1957). Our doses were small (<300 mu/kg) and would not be likely to saturate the removal mechanism. However, Errington & Rocha e Silva Jnr. (1972), using conventional methods for estimation of vasopressin, found a half-life of 5 min in the dog. They also used much longer infusions (20 min) and suggested that the infusions of 3-5 min used by Gilmore & Vane (1970) were too short to allow steady-state conditions to develop. Experience with many assays of different hormones by the blood-bathed organ technique (Vane, 1969) suggests that steady-state conditions are reached when the assay tissue achieves a plateau response which is maintained and this usually occurs within 2-4 min of the start of an infusion. The pulmonary vein reached a plateau contraction rather more slowly, even when bathed in Krebs solution and presented with a sudden and stable increase in oxytocin concentration. For this reason, in the dog, we used infusions lasting 15-25 min and assumed that a plateau contraction of the tissue reflected a steady-state plasma concentration. We conclude that the mechanism by which oxytocin is inactivated in the dog is highly efficient at low hormone concentrations.

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