THE ASSAY OF OXYTOCIN IN THE PRESENCE OF VASOPRESSIN ON THE DIOESTRUS UTERUS OF THE RAT

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EXTRACTS of whole posterior pituitary lobes of cattle are known to contain oxytocin and vasopressin in nearly equal proportions. Since oxytocin is assayed in these extracts, the circumstances under which, and the extent to which, vasopressin may interfere with these estimates should be known. Oxytocin is now assayed either by its depressor action on the fowl's blood pressure[1] or by the graded contractions which it induces in the rat's dioestrus uterus[2]. Whereas the possible interference by vasopressin in the assay of oxytocin by the fowl depressor method has been investigated[3], no such study has been made of the rat uterus method. The purpose of this investigation has therefore been to define, if possible, the conditions under which the dioestrus uterus of the rat can be used reliably to assay oxytocin contained in extracts of powders made from whole posterior lobes of the pituitary body.

Previous work on the uterus of the virgin guinea pig by Fraser[4] and by Stewart[5] showed that vasopressin exerted increasing oxytocic action on the uterus of this species as the magnesium content of the Ringer's solution was increased. Stewart[5] found, over all, that increase in magnesium concentration sensitised the guinea pig uterus more to vasopressin than to oxytocin, and that these uteri were almost equally sensitive to the two hormones at a concentration of 0·1 per cent (w/v) of magnesium chloride. Varying concentrations of calcium were also found to influence the sensitivity of the guinea pig uterus differently to oxytocin and to vasopressin. Concentrations of calcium chloride ranging from 0·029 to 0·034 per cent (w/v) predominantly potentiated the action of oxytocin on the guinea pig uterus, whereas higher concentrations of calcium chloride, ranging from 0·034 to 0·049 per cent (w/v), increased the responses of these uteri more to vasopressin than to oxytocin.

This earlier work on the guinea pig uterus has largely directed our study of the effect of variation in the concentration of individual ions on the responses of the dioestrus uterus of the rat to oxytocin and to vasopressin.

METHODS

Single horns from the dioestrus uteri of adult white rats were suspended from frontal writing levers in 20 ml. baths of aerated Ringer's solution at 28°. Contractions of uteri were magnified from three to five times in different experiments.

Dose-effect curves. Selected doses of neurohypophyseal hormone, measured in milliunits (mU.), were given in a random order at 5 minute intervals. Each dose was repeated three times. The fluid was then

changed. Regular doses were continued in the new fluid until the responses of the tissue had become constant at a single dose level; then the dose-effect curve was re-established. The fluid was again changed. The whole process was repeated several times.

Assays of oxytocic activity were made according to the method described by Holton[2], except that the Ringer was modified, and the temperature used was 28°, not 32°.

Hormones. The preparations of oxytocin and vasopressin used were Pitocin (batch number LT803F) and Pitressin (batch number LT507F). Each of these commercially prepared hormones may be expected to contain 4 per cent of the other.

TABLE I

THE COMPOSITION OF RINGER SOLUTIONS. MEASURED VOLUMES OF PER CENT (W/V) SOLUTIONS OF A SINGLE SALT IN DISTILLED WATER WERE ADDED TO 800 ML. OF A BASIC SOLUTION (SEE TEXT) AND THE WHOLE WAS DILUTED TO 1 LITRE

Ringer Code No.	Basic solution	Additional compound	
		Added per litre	Final concentration
		magnesium chloride	mg./litre
I	A	0·5 ml. of 0·01 per cent	0·05
II	A	10·0 ml. of 10·0 ,,	1000
III	A	5·0 ml. of 1·0 ,,	50
IV	A	5·0 ml. of 10·0 ,,	500
		calcium chloride	g./litre
V	B	none	0·03
VI	B	7·5 ml. of 1·2 per cent	0·12
VII	B	2·5 ml. of 1·2 ,,	0·06
		potassium chloride	g./litre
VIII	C	none	none
IX	C	2·0 ml. of 10·0 per cent	0·2
X	C	4·2 ml. of 10·0 ,,	0·42
XI	C	0·7 ml. of 10·0 ,,	0·07

Ringer's solutions. Ringer's solution used for the study of the effect of change in magnesium concentration. A basic solution A, made freshly each day, contained the following substances in g./800 ml. of glass distilled water: NaCl, 9·0; KCl, 0·42; $CaCl_2$, 0·12; $NaHCO_3$, 0·5; glucose, 0·5. Ringer solutions of varied magnesium content were made by adding appropriate amounts of $MgCl_2$ in solution to 800 ml. of basic solution A, then diluting to 1 litre with distilled water (Table I).

Ringer's solution used for the study of the effect of change in calcium concentration. A basic solution B was freshly prepared each day to contain the following compounds in g./800 ml. of glass distilled water: NaCl, 9·0; KCl, 0·42; $MgCl_2$, 0·001; $CaCl_2$, 0·03; $NaHCO_3$, 0·5; glucose, 0·5. Ringer's solutions of varied calcium content were made by adding appropriate amounts of calcium chloride in solution to 800 ml. of the basic solution B, then diluting to 1 litre with distilled water (Table I).

Ringer's solution used for the study of the effect of change in potassium concentration. A basic solution C was prepared freshly each day, and contained the following compounds in g./800 ml. glass distilled water: NaCl, 9·0; $CaCl_2$, 0·12; $MgCl_2$, 0·001; $NaHCO_3$, 0·5; glucose, 0·5.

Ringer solutions of varied potassium content were made by adding potassium chloride solutions of known strength to 800 ml. of basic solution C, then diluting to 1 litre with glass distilled water (Table I).

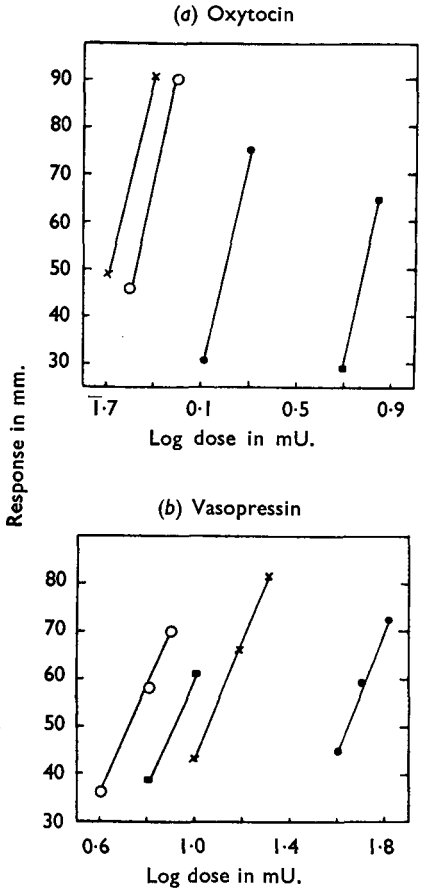

FIG. 1. The effect of variation in the magnesium content of the Ringer on the dose-response curves for the actions of oxytocin and vasopressin on horns from the dioestrus uterus of the rat.
Concentration of $MgCl_2$ mg./l.:—closed circles, 0·05; crosses, 50; open circles, 500; black rectangles, 1000.

RESULTS

Firstly, the changes in the sensitivity of the rat's dioestrus uterus to oxytocin and to vasopressin with alteration in the concentration of single ions have been measured separately for the two hormones. Secondly, a solution which contained both oxytocin and vasopressin in equal proportion has been assayed. Oxytocin was used as a standard. A series of Ringer's solutions was employed in which either the magnesium or the calcium concentration was varied.

The effect of variation in the concentration of magnesium chloride in the Ringer on the dose-response curve for the action of oxytocin on the dioestrus uterus of the rat was studied in five experiments. Four different concentrations of magnesium chloride were used (Table I). Typical results are shown in Figure 1*a*. In each of these five experiments maximum sensitivity of the uterus to oxytocin was found at a concentration of 50 mg. $MgCl_2$/l., and minimum sensitivity at a concentration of 1 g. $MgCl_2$/l. Uteri were only slightly less sensitive to oxytocin at a concentration of 500 mg. $MgCl_2$/l. than they were at 50 mg. $MgCl_2$/l. Figure 1*a* shows that these changes in sensitivity were brought about by shifts in the position of the dose-effect curves, without alteration in slope.

The changes induced in the dose-effect curve for vasopressin by variation in the magnesium concentration of the Ringer's solution were similarly examined in three experiments. Typical results are shown in Figure 1*b*. In each experiment, maximum sensitivity of the uterus to vasopressin was found with a concentration of 500 mg. $MgCl_2$/l., and minimum sensitivity

with a concentration of 0·05 mg. $MgCl_2$/l. A concentration of 1·0 g. $MgCl_2$/l. caused only slight depression in the responses of uteri to vasopressin. Again the changes in sensitivity were occasioned by alteration in the position of the dose-response curves, without change in the slopes of the curves.

The effects of these changes of the responses of the uteri to oxytocin and to vasopressin were reversible and reproducible.

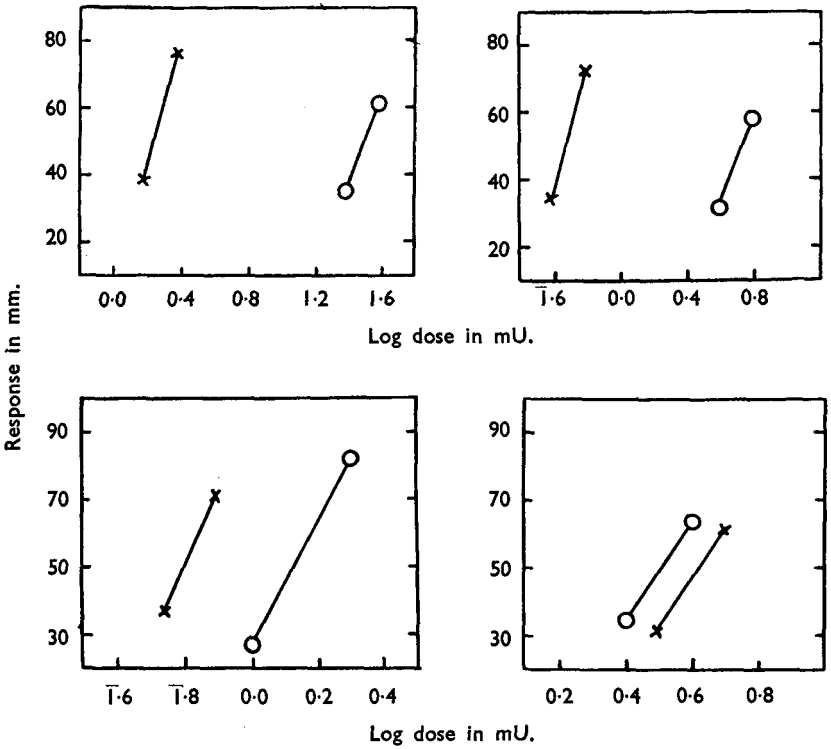

FIG. 2. The effect of variation in the magnesium concentration in the Ringer on the responses of a single rat uterine horn to oxytocin and to vasopressin.

Crosses, oxytocin; open circles, vasopressin.

The relative slopes of the dose-effect curves for the actions of vasopressin and oxytocin, and the effect of change in the magnesium concentrations of the Ringer's solution on the relative potency of these two hormones, were examined in two experiments. The results were in good agreement and are illustrated in Figure 2. First, there was no significant difference in the slopes of the dose-response curves for the actions of the two hormones. Secondly, the ratio of equally active doses, oxytocin to vasopressin decreased as the magnesium concentration in the Ringer's solution was raised from 0·05 mg./l. to 1·0 g./l. At the lower magnesium concentration the commercial oxytocin proved twenty times as effective

as commercial vasopressin; at the higher, the vasopressin was slightly more active than the oxytocin.

The effect of variation in the concentration of calcium chloride in the Ringer's solution on the dose-response curve for the action of oxytocin was examined in four experiments. Three different concentrations of calcium chloride were employed (Table I). Concentrations greater than 0·12 g. $CaCl_2$/l. were avoided, lest the uteri should develop spontaneous activity. Rat uteri did respond to oxytocin in the absence of calcium, but the contractions steadily weakened. By contrast, regular responses to a constant dose of oxytocin were obtained when the concentration of

TABLE II

THE RESULTS OF ASSAYS OF A MIXTURE OF 10 MU. OXYTOCIN AND 10 MU. VASOPRESSIN/ML. AGAINST 10 MU. OXYTOCIN/ML. ON THE RAT'S UTERUS IN RINGER OF VARIED MAGNESIUM CONTENT

Ringer		Oxytocic activity as mU. oxytocin per ml.			*b* (slope)	Assay No.
			Found			
Code	$MgCl_2$ mg./l.	Actual	Mean	Limits (P = 0·95)		
I	0·05	10	10·1	9·3–11·0	196	11
		10	10·7	8·4–13·6	174	12
		10	11·3	9·4–13·5	70	13
		10	10·8	8·6–13·5	182	14
III	50·00	10	10·2	9·3–11·0	747	1
		10	12·2	12·1–12·3	580	5
		10	12·6	10·4–15·3	101	10
IV	500·00	10	12·5	12·4–12·6	2170	4
		10	15·5	15·3–15·7	944	7
		10	19·2	15·7–23·4	126	15
		10	18·3	13·8–24·3	112	16
		10	16·9	14·4–19·8	129	17
		10	14·6	13·0–16·3	1052	18
		10	12·9	11·6–14·4	353	19
II	1000·00	10	20·6	18·6–22·8	319	6
		10	28·5	26·4–31·5	350	9
		10	18·4	16·1–21·7	327	8

calcium chloride in the Ringer was only 0·03 g./l. Uteri became progressively more sensitive to oxytocin as the concentration of calcium chloride in the Ringer was increased from 0·03 to 0·12 g./l. Change in sensitivity took place without alteration in the slope of the dose effect curve, and was reversible and reproducible in each preparation (Fig. 3). Change in calcium concentration from 0·03 to 0·12 g. $CaCl_2$/l. had little effect on the sensitivity of the uterus to vasopressin.

The effects of variation in the potassium concentration of the Ringer's solution on the dose-response curve for the action of oxytocin were also examined. Four different concentrations of potassium chloride were used (Table I). Typical results are shown in Figure 4. Uteri proved insensitive to oxytocin in the absence of potassium. The addition of 0·07 g. KCl/l. increased the sensitivity approximately ten times. Further increase in potassium concentration, up to 0·42 g./l., caused a progressive small gain in sensitivity (Fig. 4). The changes in sensitivity with alteration in potassium concentration occurred without alteration in the slope of the dose effect curve and were reversible and reproducible.

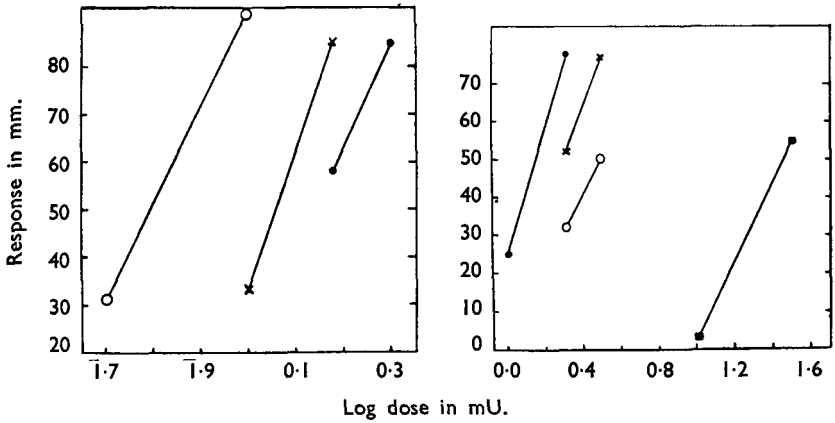

FIG. 3. The effect of variation in the calcium concentration of the Ringer on the dose-response curve for oxytocin on a horn of rat uterus.

Concentration of $CaCl_2$ g./l.:—closed circles, 0·03; crosses, 0·06; open circles, 0·12.

FIG. 4. The effect of variation in the potassium concentration of the Ringer on the dose-response curve for oxytocin on a horn of dioestrus uterus of the rat.

Concentration of KCl g./l.:—black rectangles, nil; open circles, 0·07; crosses, 0·2; closed circles, 0·42.

The assay of a mixture containing both oxytocin (10 *mU./ml.*) *and vasopressin* (10 *mU./ml.*) *against oxytocin* (10 *mU./ml.*)

The effect of variation in magnesium concentration. Assays were made in four Ringer's solutions which differed only in their content of magnesium chloride (Table I). The results of nineteen assays are given in Table II, and the information obtained from them has been summarised in Table III. These Tables show that oxytocin in a 1:1 mixture with

TABLE III

FURTHER ANALYSIS OF THE ASSAYS (TABLE II) OF THE MIXTURE OF OXYTOCIN AND VASOPRESSIN ON THE RAT'S UTERUS IN RINGER OF VARIED MAGNESIUM CONTENT

Ringer		Sensitivity mean dose oxytocin used mU.	Oxytocic activity in mU./ml.		
Code	$MgCl_2$ mg./l.		Actual	Found	Due to 10 mU. vasopressin/ml.
I	0·05	4·75 ± 0·44 (4)	10	10·73 ± 0·25 (4)	0·73
III	50·00	3·15 ± 1·57 (3)	10	11·63 ± 0·73 (3)	1·63
IV	500·00	3·70 ± 0·81 (7)	10	15·70 ± 1·07 (7)	5·70
II	1000·00	10·50 ± 1·55 (3)	10	22·50 ± 2·22 (3)	12·50

Values are entered as mean ±S.E. of mean (Number of Assays).

vasopressin can be reliably assayed on the dioestrus uterus of the rat in Ringer I. The concentration of magnesium chloride in this Ringer was 0·05 mg./l. Increase in the magnesium concentration to 50 mg. $MgCl_2$/l. (Ringer III) rendered uteri more sensitive to oxytocin (Fig. 1*a* and Table IV) but decreased the relative potency of oxytocin to vasopressin to 6:1 (Fig. 2). At 500 mg. $MgCl_2$/l. the vasopressin in the mixture had one half the activity of oxytocin, and at a concentration of 1 g. $MgCl_2$/l. the oxytocin and vasopressin were equiactive.

TABLE IV

THE RESULTS OF ASSAYS OF A MIXTURE OF 10 MU. OXYTOCIN AND 10 MU. VASOPRESSIN/ML. AGAINST 10 MU. OXYTOCIN/ML., ON THE RAT'S UTERUS, IN RINGER OF VARIED CALCIUM CONTENT

Ringer		Oxytocic activity as mU. oxytocin per ml.			*b* (slope)	Assay No.
			Found			
Code	$CaCl_2$ g./l.	Actual	Mean	Limits (P = 0·95)		
V	0·03	10	11·0	9·8–12·4	35	21*
		10	10·5	9·1–12·2	399	23
		10	10·2	9·3–11·1	389	27
		10	11·6	9·9–13·7	44	28
		10	10·0	9·3–10·8	128	32*
VII	0·06	10	10·8	9·8–11·9	37	22*
		10	11·3	9·8–13·0	241	26
		10	11·0	10·2–11·7	37	30
		10	10·1	9·2–11·0	214	31
VI	0·12	10	8·3	6·4–10·6	35	20
		10	9·5	7·7–11·8	284	24
		10	9·7	8·3–12·1	152	25

* The uterine horns used for these three assays were stored at 7° overnight. The dose levels required for these tissues were above normal: they have therefore been excluded from the estimation of sensitivity to oxytocin in the different Ringers in Table V.

The effect of variation in calcium concentration. Twelve assays were made. The three Ringer's solutions all contained 1 mg. $MgCl_2$/l., but varied in calcium concentration from 0·03 to 0·12 g. $CaCl_2$/l. The results of individual assays are shown in Table IV and the information gained from them is summarised in Table V. Oxytocin in 1 : 1 mixture with vasopressin appeared to have been reliably estimated in each of these three Ringer's solutions. The concentration of 1·0 mg. $MgCl_2$/l. was

TABLE V

FURTHER ANALYSIS OF THE ASSAYS (TABLE IV) OF THE MIXTURE OF OXYTOCIN AND VASOPRESSIN AGAINST OXYTOCIN AS STANDARD, ON THE UTERUS OF THE RAT, IN RINGER OF DIFFERING CALCIUM CONTENT

Ringer		Sensitivity mean dose oxytocin used mU.	Oxytocic activity in mU./ml.		
Code	$CaCl_2$ g./l.		Actual	Found	Due to 10 mU. vasopressin/ml.
V	0·03	0·59 ± 0·08 (3)	10	10·66 ± 0·29 (5)	+0·66
VII	0·06	0·72 ± 0·07 (3)	10	10·80 ± 0·51 (4)	+0·80
VI	0·12	0·53 ± 0·07 (3)	10	9·10 ± 0·44 (3)	−0·90

Values are entered as means ±S.E. of mean (number of assays).

preferred to 0·05 mg. $MgCl_2$/l. because the uteri developed less spontaneous activity and were more sensitive to oxytocin (cf. columns 3, Tables III and V). Of the three calcium concentrations examined, 0·12 g. $CaCl_2$/l. was preferred for assay work. Firstly, uteri gave constant responses to a fixed dose of oxytocin in Ringer VI after but short delay. Secondly, the latent period preceding the contractions was shorter in this Ringer's solution than in others. Thirdly, the uteri relaxed readily after contraction occurred.

No significance should be attached to variations in the slopes of the curves which were obtained in different assays, because neither the lever

weights nor the magnification given by the levers were standardised in these experiments. In none of these assays did the slopes of the curves given by oxytocin alone differ significantly from the slope of the curve given by the 1 : 1 mixture of oxytocin and vasopressin.

Discussion

The purpose of this investigation was to define conditions under which the oxytocin present in extracts of posterior lobes of the pituitary body could be reliably assayed on the rat's dioestrus uterus. This object has been achieved, for correct estimates of the oxytocin in 1 : 1 mixture with vasopressin were given in Ringer's solutions numbers I, V, VI, and VII (Table I). Solution VI was preferred for assay work for reasons given.

The effect of variation in magnesium concentration on the dose-response curves for the actions of oxytocin and vasopressin was similar whether these hormones were examined alone (Fig. 2) or in a mixture (Table III). The actions of the two hormones in 1 : 1 mixture was therefore additive. Moreover, their dose-effect curves were parallel; this has been shown both in Figure 2 and by the fact that there was no significant deviation from parallelism between the curves for oxytocin and for the 1 : 1 mixture of oxytocin and vasopressin in any of the assays recorded in Tables II and IV. The assumption that these two hormones, which are so closely related chemically, have the same mode of action on the rat's uterus therefore seems justifiable. A latent period of 45 seconds before the response may indicate, but does not prove, that the primary action of these hormones is exerted within the cell. Magnesium, potassium, and calcium ions are well known to produce effects by action at surfaces. The fact that these ions all proved capable of changing the position, but not the slope of the dose-effect curve for the action of vasopressin and oxytocin on the uterus demands further study.

Summary

1. The effect of $MgCl_2$ in concentrations of 0·05, 50, 500, and 1000 mg./l. on the dose-response curves given by oxytocin and vasopressin on the dioestrus uterus of the rat has been examined. Maximum sensitivity was found to oxytocin at 50 mg. $MgCl_2$/l., and to vasopressin at 500 mg. $MgCl_2$/l. A concentration of 1 g. $MgCl_2$/l. depressed sensitivity greatly toward oxytocin, much less toward vasopressin; the uterus was almost equally sensitive to the two hormones at this concentration. Changes in sensitivity were occasioned by change in position, but not in slope of dose-effect curves.

2. Increase in $CaCl_2$ concentration from 0·03 to 0·12 g./l. caused slight increase in sensitivity toward oxytocin, but no change toward vasopressin.

3. Conditions under which the rat's dioestrus uterus may be used to give reliable estimates of the oxytocin present in 1 : 1 mixtures with vasopressin have been defined. Concentrations of 1 mg. $MgCl_2$/l. and 0·12 g. $CaCl_2$/l. are recommended.

It is a pleasure to thank those honours students of Chelsea Polytechnic who have contributed assays to this work. The Pitocin and Pitressin used were given by Parke-Davis & Co. Ltd.

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