

## AN ISOLATED PREPARATION WITH A SELECTIVE SENSITIVITY TO VASOPRESSIN

BY

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*(Received May 28, 1964)*

Reports in the literature have shown that extracts of the posterior pituitary gland with pressor activity increase movement of the gut in some, but not all, species. The fraction with oxytocic activity, however, either has no effect on this tissue or, if any, produces relaxation.

In an attempt to find an isolated preparation which would be selectively sensitive to vasopressin, portions of the guinea-pig gut were tested serially, and it was found that the most proximal portion of the colon fulfilled this requirement.

### METHODS

Male guinea-pigs, body weight 320 to 350 g, were starved overnight and killed by stunning and bleeding. The bulbous, proximal portion of the colon, which as an anatomical entity probably corresponds to the sacculus rotundus in other animals (Fig. 1), was excised and suspended in an organ-bath of 5 ml. capacity. A loop was tied through the distal end, and a thread from the rounded proximal end was attached to a

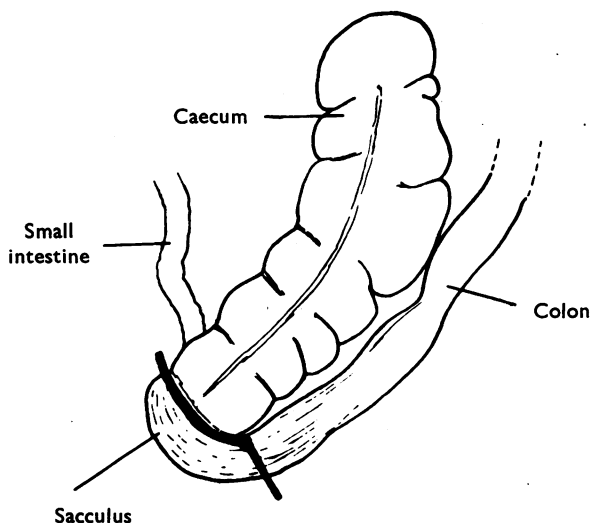


Fig. 1. The junction of the ileum, caecum and colon of the guinea-pig. The heavy line indicates the line of cut made to remove the portion of colon used.

frontal-writing lever, so that contractions of the longitudinal muscle were recorded. The composition of the bath solution was (g/l.): NaCl 8.0, KCl 0.2, CaCl<sub>2</sub> 0.08, MgCl<sub>2</sub> 0.04, NaH<sub>2</sub>PO<sub>4</sub> 0.05, NaHCO<sub>3</sub> 1.0 and glucose 1.0. The temperature of the bath was 32° C and a constant stream of air was bubbled through the bath fluid.

Histological examinations of the tissue were carried out on segments which had been fixed in either 10% formol-saline, Zenker's or Bouin's fluid. Paraffin sections were stained with haematoxylin and eosin, Dominici's stain for mast cells or Fontana-Masson stain for argentaffin granules.

*Drugs.* The following preparations were used: vasopressin [Pitressin, Parke Davis, batch No. T 102 LA which is an extract stated to contain argipressin (arginine<sup>8</sup>-vasopressin)]; a synthetic product, lypressin (lysine<sup>8</sup>-vasopressin, Sandoz); "oxytocin" (Pitocin, Parke Davis); a synthetic oxytocin (Syntocinon, Sandoz); argiprestocin (arginine<sup>8</sup>-vasotocin, an extract of fowl pituitary gland prepared by R. Acher, Paris); lysine<sup>8</sup>-vasotocin; isoleucine<sup>8</sup>-oxytocin; and phenylalanine<sup>8</sup>-lysine<sup>8</sup>-vasopressin (all three synthesized by Sandoz).

### RESULTS

Doses of the polypeptides were added to the bath at intervals of 15 min. The sensitivity of the tissue (threshold concentration) to vasopressin varied from 1  $\mu$ U/ml. to 1 mU/ml. This degree of sensitivity to vasopressin was confined to the proximal portion of the colon. Similar portions of the colon of female guinea-pigs were often insensitive, as were those of male guinea-pigs with a body weight of over 400 g.

After administration of the drug there was a latent period of approximately 1 min, and the contraction was slow to develop so that a contact time of at least 3 min was required. Most preparations exhibited some degree of spontaneous activity, which could usually be suppressed by halving the concentration of calcium in the solution. This, however, reduced the sensitivity of the tissue. Changes of the concentration of magnesium chloride in the bath fluid in either direction also reduced the sensitivity of the tissue. Tachyphylaxis was manifested if the interval between doses was less than 15 min; sensitivity returned, however, after a period of approximately 30 min.

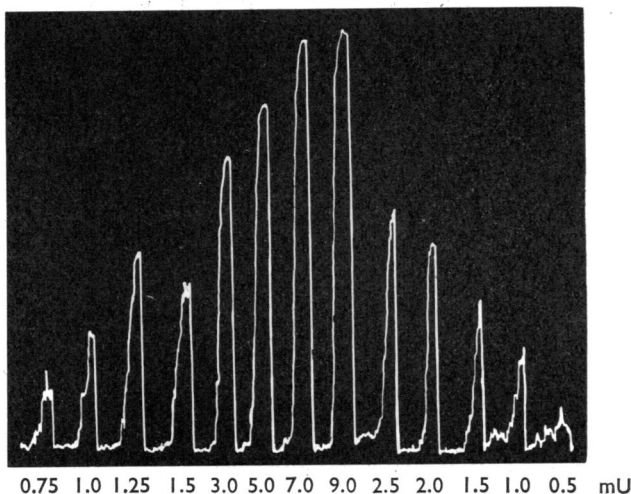
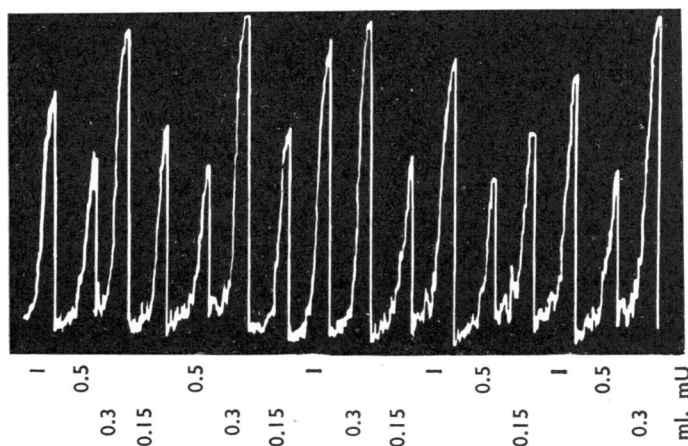
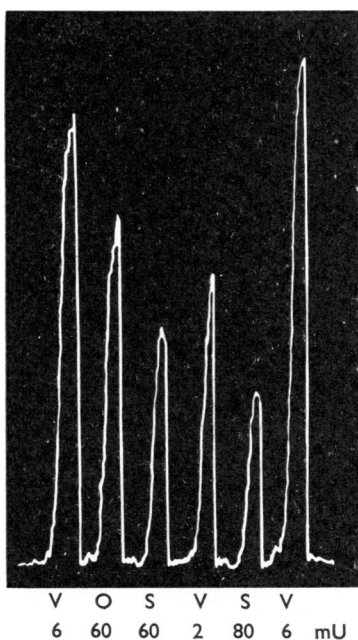


Fig. 2. Response of guinea-pig colon to various doses of vasopressin in a 5-ml. organ-bath at 32° C. Figures represent the number of mU of vasopressin (Pitressin) added to the bath. Dose interval, 15 min. Contact time, 3.5 min. The response to 1.25 mU is anomalous.



**Fig. 3.** A 2+2 dose assay of vasopressin. Contact time, 3 min; other conditions as in Fig. 2. The effects of 1 mU and 0.5 mU of vasopressin are shown, together with those of 0.3 ml. and 0.15 ml. of a solution of unknown potency.



**Fig. 4. Discrimination between vasopressin and oxytocin.** Conditions as for Fig. 2. V=vasopressin (Pitressin); O=commercial extract of oxytocin (Pitocin); S=a synthetic preparation of oxytocin. Figures represent the number of mU added to the bath. The response of the tissue to 80 mU of synthetic oxytocin is less than the response to 60 mU (doses of oxytocin that are just supramaximal strongly depress the response of the tissue, not only to oxytocin itself, but occasionally to vasopressin also).

The relationship between dose and effect is shown in Fig. 2. A 2+2 dose assay is illustrated in Fig. 3. Ratios of the standard deviations of single responses to the slopes of the regression lines varied from 0.03 to 0.08 in six experiments. These ratios are an inverse measure of the precision of an assay and should lie under 0.05 for a satisfactory assay (Holton, 1948).

Fig. 4 demonstrates the effect of oxytocin on the preparation. Comparison of the effects obtained with vasopressin (2 mU) and with synthetic oxytocin (60 mU) show that the tissue is approximately eighteen-times more sensitive to vasopressin than to oxytocin. This calculation is based on a unitage per mg of 450 for oxytocin and 270 for lysine<sup>8</sup>-vasopressin (Boissonas, Guttman, Berde & Konzett, 1961). It is interesting to note that the extract of the pituitary gland, Pitocin, had always a more potent action on the tissue than the synthetic preparation of oxytocin. This suggests that the separation of the posterior pituitary principles in the commercial extracts is incomplete.

*Comparison of the effects of posterior pituitary principles and some of their analogues.* Katsoyannis & du Vigneaud (1959), Dicker & Eggleton (1961) and Sawyer, Chan & van Dyke (1962) have shown that the variations of antidiuretic and pressor potencies of the neurohypophyseal hormones and their analogues can be related to the degree of basicity of the amino acid in position 8, as well as to the specificity of that in position 3. It was, therefore, of interest to see whether a similar relation between molecular configuration and activity would obtain with the present preparation. With the exception of oxytocin and isoleucine<sup>8</sup>-oxytocin, all the compounds used were assayed for pressor activity on the blood pressure of the anaesthetized rat (Dekanski, 1952), and equipressor doses were tested on the isolated colon; oxytocin and isoleucine<sup>8</sup>-oxytocin were administered in equal oxytocic unitage. The results, summarized in Table 1, show that, although the preparation was not able to differentiate well between the lysine or arginine analogues, it was able to differentiate between oxytocin, vasotocins and vasopressins. When the concentration of the analogues were expressed in milligrams instead of pressor units the same relative order

TABLE 1

COMPARISON OF THE ACTIVITIES OF SOME ANALOGUES OF OXYTOCIN AND VASOPRESSIN ON THE PROXIMAL COLON OF THE GUINEA-PIG

All the drugs, except oxytocin and isoleucine<sup>8</sup>-oxytocin, were compared in terms of pressor unitage. The two oxytocins were administered in concentrations of equal oxytocic unitage (rat uterus). The last column indicates the activity on the tissue relative to lysine<sup>8</sup>-vasopressin (=1)

Polypeptide	Structure									Activity
	1	2	3	4	5	6	7	8	9	
Oxytocin	Cys	Tyr	Ileu	Glu(NH <sub>2</sub> )	Asp(NH <sub>2</sub> )	Cys	Pro	Leu	Gly(NH <sub>2</sub> )	0.05
Isoleucine <sup>8</sup> -oxytocin	Cys	Tyr	Ileu	Glu(NH <sub>2</sub> )	Asp(NH <sub>2</sub> )	Cys	Pro	Ileu	Gly(NH <sub>2</sub> )	0.1
Argiprestocin	Cys	Tyr	Ileu	Glu(NH <sub>2</sub> )	Asp(NH <sub>2</sub> )	Cys	Pro	Arg	Gly(NH <sub>2</sub> )	0.5
Lysine <sup>8</sup> -vasotocin	Cys	Tyr	Ileu	Glu(NH <sub>2</sub> )	Asp(NH <sub>2</sub> )	Cys	Pro	Lys	Gly(NH <sub>2</sub> )	0.5
Argipressin	Cys	Tyr	Phe	Glu(NH <sub>2</sub> )	Asp(NH <sub>2</sub> )	Cys	Pro	Arg	Gly(NH <sub>2</sub> )	1.0
Lypressin	Cys	Tyr	Phe	Glu(NH <sub>2</sub> )	Asp(NH <sub>2</sub> )	Cys	Pro	Lys	Gly(NH <sub>2</sub> )	1.0
Phenylalanine <sup>2</sup> -lysine <sup>8</sup> -vasopressin	Cys	Phe	Phe	Glu(NH <sub>2</sub> )	Asp(NH <sub>2</sub> )	Cys	Pro	Lys	Gly(NH <sub>2</sub> )	2.5-3.0

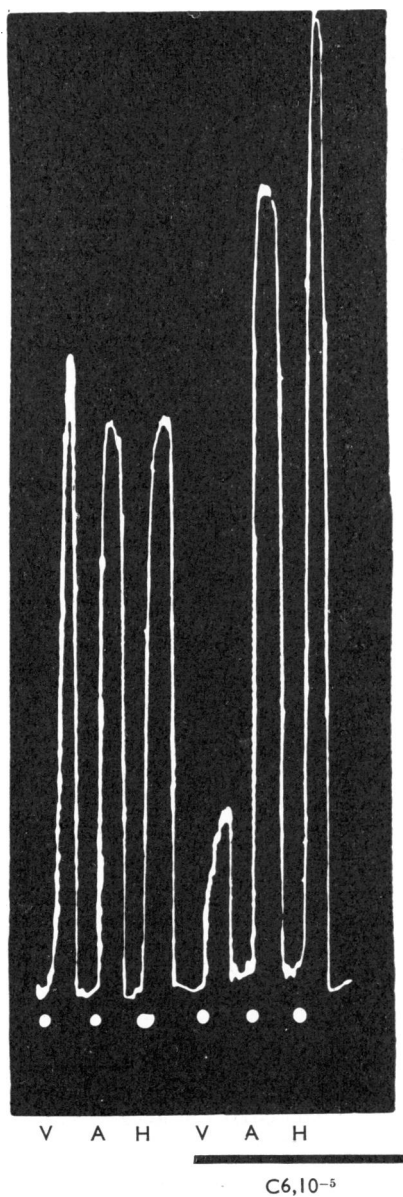


Fig. 5. The inhibition of the response to vasopressin by hexamethonium. V=4 mU of vasopressin; A=0.5  $\mu$ g of acetylcholine; H=0.5  $\mu$ g of histamine acid phosphate. Where indicated (by the black line) the bath fluid was changed to one containing hexamethonium bromide ( $C6, 10^{-5}$  g/ml.). A potentiation of the responses of guinea-pig gut to acetylcholine and histamine is usually observed with this concentration of hexamethonium bromide.

of activities was found, with the exception that phenylalanine<sup>2</sup>-lysine<sup>8</sup>-vasopressin was approximately equipotent with arginine and lysine vasopressin.

*Other drugs.* Histamine, acetylcholine, angiotensin, nicotine, 5-hydroxytryptamine and adrenaline had the same effect on the preparation as they have on guinea-pig ileum.

*The effects of drugs on the response to vasopressin.* Hexamethonium, in concentrations of  $10^{-5}$  g/ml., reduced the size of the contractions produced by vasopressins, but had either no effect on the responses of the tissue to acetylcholine and histamine or slightly potentiated them (Fig. 5). Pentolinium ( $10^{-6}$  g/ml.) also inhibited the responses to vasopressin. Atropine ( $10^{-6}$  g/ml.) caused only slight inhibition of the response to vasopressin, and mepyramine had no effect even in a concentration of  $10^{-5}$  g/ml.

*Histological findings.* Sections made from the portion of the colon sensitive to vasopressin were compared with sections taken from part of the colon insensitive to vasopressin. No obvious difference could be observed in the disposition of longitudinal and circular muscle, in the content of mast cells or in the amounts of chromaffin or argentaffin tissue present.

#### DISCUSSION

The tissue described exhibits a sensitivity to vasopressin which is unusual for the smooth muscle of guinea-pig gut. Ileum or more distal parts of the colon will rarely contract to vasopressin. The reason for this sensitivity is unknown. A cursory histological examination of the tissue has shown no obvious reason for this phenomenon.

The tissue also reacts to oxytocin but its lack of sensitivity to this hormone is such that it can discriminate between oxytocin and vasopressin in a manner similar to that of the uterus but in a reverse fashion.

It is of interest that oxytocin and vasopressin should affect smooth muscle at two different sites, namely the uterus and the proximal colon, and yet exhibit a complete reversal of their relative activities at each site. An obvious difference between the muscle of the gut and that of the uterus is that the former has a complex nerve plexus associated with it. The possibility that vasopressin, but not oxytocin, is capable of exerting an action on the muscle of the gut through the nervous elements is supported by the fact that hexamethonium and pentolinium, both ganglionic blocking agents, inhibit the response to vasopressin. Though Robertson & Rubin (1962) have demonstrated that the action of angiotensin on gut muscle may be mediated by nerve cells, and though an action of substance P on the nervous elements of the reflex arc in intestinal muscle is considered a possibility (Pernow, 1960), such a mechanism has not been considered for the action of neurohypophyseal hormones or their analogues.

#### SUMMARY

1. An isolated preparation of the proximal portion of the guinea-pig colon contracts in the presence of vasopressin in concentrations of  $1 \mu\text{U/ml.}$  to  $1 \text{ mU/ml.}$
2. This tissue will also contract in the presence of oxytocin but is much less sensitive to this hormone.
3. Hexamethonium and pentolinium inhibit the response to vasopressin. The possibility that vasopressin acts through the nervous elements in the gut is discussed.

I wish to acknowledge the help of Dr. S. E. Dicker in the preparation of this manuscript.

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