Br. J. Pharmac. (1969), **37**, 306–313.

Studies on the mode of action of vasopressin on the isolated proximal colon of the guinea-pig

J. H. BOTTING AND A. D. TURMER

Department of Pharmacology, Chelsea College, University of London, London S.W.3

1. Contractions of guinea-pig isolated proximal colon produced by vasopressin are not affected by methyloxytocin (a compound that blocks pressor effects of vasopressin).

2. Vasopressin contractions are inhibited by replacement of sodium with mannitol or sucrose, elevation of potassium or magnesium concentrations, the presence of the metabolic inhibitors sodium azide and triethyl tin or tetrodotoxin in the bathing fluid. Contractions produced by histamine or choline esters are comparatively insensitive to these procedures.

3. Contractions of rat isolated uterus following vasopressin, oxytocin and methacholine are equally affected by replacement of sodium, increase of potassium or magnesium or addition of sodium azide.

4. Neither vasopressin contractions nor contractions caused by transmural stimulation were consistently affected by morphine (10^{-6} g/ml.) or hyoscine (10^{-7} g/ml.) although both were reduced by anoxia or cooling the tissue. Morphine did not reduce the output of acetylcholine from stimulated colon.

5. It is concluded that the action of vasopressin on proximal colon is unlike its action on other smooth muscle and is mediated by nervous tissue.

The isolated proximal colon of the guinea-pig contracts on the addition of vasopressin to the bath, but this effect is unlike that of the hormone on other smooth muscle preparations, in that it appears to be acting indirectly on the muscle (Botting & Turmer, 1966). This conclusion was reached because addition of hexamethonium, anoxia, cooling to 25° C and reduction of the calcium concentration of the bath fluid (treatments which had little or no effect on the responses to substances acting directly on smooth muscle) diminished or abolished the response to vasopressin. Further experiments pointed to the involvement of a cholinergic mechanism, and the appearance of an acetylcholine-like substance in the bath fluid during the vasopressin-induced contractions of the colon was demonstrated. On the other hand, local anaesthetics, morphine and hyoscine, showed little or no selective antagonism towards vasopressin, although the ability of these drugs to inhibit responses to indirectly acting drugs on preparations containing smooth muscle is well established.

Further attempts to resolve the mechanism of action of vasopressin on guinea-pig isolated proximal colon are now described.

Methods

Male guinea-pigs (320–400 g) were killed by a blow on the head and the most proximal portion of the colon was removed and suspended in an organ bath (12 ml.)

containing a fluid of the following composition (g/l.): NaCl 8·0, KCl 0·2, $CaCl_2$ 0·08, $MgCl_2$ 0·04, NaH_2PO_4 0·05, $NaHCO_3$ 1·0, glucose 1·0. The temperature was maintained at 32° C and the fluid was vigorously aerated (Botting, 1965).

A few experiments were performed on the rat isolated uterus. The bathing fluid was of the following composition (g/l.): NaCl 9·0, $NaHCO_3$ 0·5, glucose 0·5, $MgCl_2$ 0·001, KCl 0·42, $CaCl_2$ 0·12.

Responses to vasopressin (0·4–4·0 ng/ml.) and appropriate reference treatments were obtained in the normal fluid. The fluid was then replaced by one of different composition, or one to which an inhibitor drug had been added and the effect on responses elicited by vasopressin and the reference treatments was observed. In some experiments the concentrations of agonist drugs were raised so that during the test period responses equal to those in the control period were obtained. The dose ratio was then calculated as an index of the inhibitory action (Gaddum, Hameed, Hathway & Stephens, 1955).

Alteration of ion concentrations

The concentrations of sodium, potassium and magnesium were changed. Sodium chloride was replaced either by mannitol (47 g/l.) or sucrose (80 g/l.). The residual sodium content of the fluid ($NaHCO_3$ and NaH_2PO_4) was 8% of normal (12 mM). When the concentration of potassium was altered the osmotic pressure of the solution was maintained at 305 mosm by addition or omission of NaCl.

Transmural stimulation

Transmural stimulation of the proximal colon was achieved by the method described by Paton (1955) for the guinea-pig ileum. Square wave pulses of 0·5 msec duration (90–100 V) were given every 10 sec. The techniques used for the production of anoxia and for cooling were as described previously (Botting & Turmer, 1966).

In some experiments eserine (2×10^{-6} g/ml.) was included in the bath fluid and the amount of acetylcholine released from the tissue during 30 min stimulation (1/sec), in the presence and absence of morphine (10^{-6} g/ml.) was determined. Acetylcholine was assayed on a segment of guinea-pig ileum suspended in Krebs fluid containing eserine (5×10^{-9} g/ml.) and morphine (10^{-6} g/ml.).

Drugs

The vasopressin used was Pitressin (Parke Davis). 2-*O*-methyl tyrosine oxytocin (methyloxytocin) was synthesized and donated by Professor J. Rudinger. Triethyl tin was supplied by Dr. J. Cremer and sodium azide was obtained from Hopkin and Williams Ltd. Tetrodotoxin was manufactured by Sankyo Company Limited and obtained from Calbiochem.

Results

Methyloxytocin

This analogue of oxytocin, which can inhibit the actions of vasopressin is some preparations, had no action on the contractions of the proximal colon produced by

vasopressin or acetylcholine in concentrations up to 10^{-7} g/ml. At concentrations of approximately 10^{-6} g/ml., methyloxytocin caused contraction of the tissue and was still without effect on contractions following vasopressin at 2×10^{-6} g/ml. A typical experiment is illustrated in Fig. 1.

Replacement of sodium

When the sodium chloride in the bath fluid was replaced by mannitol or sucrose the tissue gave slightly reduced, normal or potentiated responses to carbachol. Responses to vasopressin or nicotine were abolished (five experiments).

Alteration of potassium concentration

Reduction of potassium concentration from 2·6 mM to 1·3 mM caused a marked potentiation of responses to vasopressin but little or no effect on those to carbachol. When the concentration was increased from 2·6 to 6·5 mM responses to vasopressin were reduced while those to carbachol showed little change. As the concentration

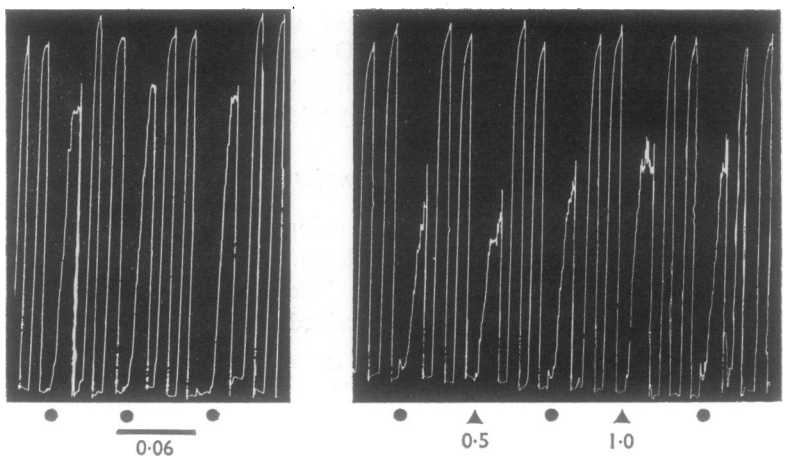

FIG. 1. Action of methyloxytocin (▲) on contractions of the proximal colon elicited by vasopressin (●=2 ng/ml.) and acetylcholine (unmarked contractions, 30 ng/ml.). At the solid bar the bath fluid contained methyloxytocin 60 ng/ml. Figures refer to concentrations in μg/ml. The contractions produced by acetylcholine and vasopressin were not inhibited by methyloxytocin. Drum speed was reduced to one quarter during vasopressin and methyloxytocin contractions.

TABLE 1. *Effect of variation of potassium concentrations on the response of the proximal colon to vasopressin, carbachol and nicotine*

Potassium concentration (mM)	Relative response		
	Carbachol	Vasopressin	Nicotine
1·3	0·85 (3)	2·8 (3)	—
2·6	1·0	1·0	1·0
6·5	1·4 (7)	0·61 (7)	0·7 (1)
13	1·3 (1)	0·2 (1)	—
26	0·55 (2)	0 (2)	—
140	0·32 (2)	0 (2)	0 (1)

—, Not tested. Number of experiments in brackets.

Mean relative response = $\frac{\text{Contraction height in modified fluid.}}{\text{Contraction height in normal fluid}}$

was progressively raised to 13, 26 or 140 mM, responses to vasopressin were abolished but those to carbachol were only reduced. Contractions elicited by nicotine were also abolished by 140 mM of potassium (Table 1) and at this concentration increase of the dose of vasopressin by a factor of 300 and of nicotine by a factor of 200 failed to produce a response.

Increase of magnesium concentration

A clear discrimination between contractions produced by vasopressin or nicotine and those produced by histamine or methacholine was apparent after the increase of the magnesium concentration in the bath fluid from 1·0 to 5·0 or 10·0 mM. The former were rapidly abolished and an increase of the dose by a factor of 300 or 400 failed to produce the control response. In contrast, responses to histamine or methacholine were slightly reduced or potentiated (Fig. 2).

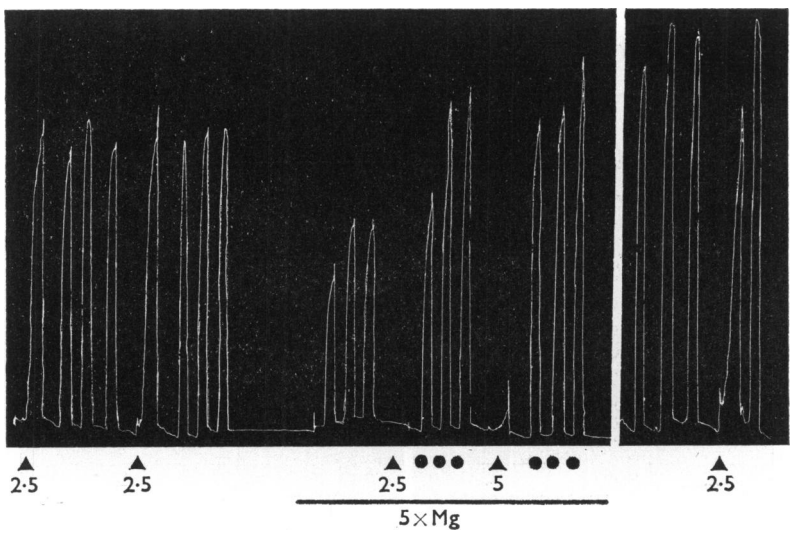

FIG. 2. Effect of increase of the magnesium content of the bath fluid from 1 to 5 mM on contractions of the proximal colon induced by vasopressin (▲, figures indicate concentration in ng/ml.) and methacholine (unmarked contractions, 45 ng/ml., ●, 90 ng/ml.). Drum speed was reduced to one quarter during vasopressin contractions.

TABLE 2. *Effect of sodium azide and triethyl tin on responses of the proximal colon to vasopressin, carbachol and nicotine*

Concentration (mM)	Dose ratios		
Sodium azide	Carbachol	Nicotine	Vasopressin
1	1·5	—	>5
2	4	>100	>250
3	2·5	>30	>7
4	20	>570	>400
Triethyl tin			
0·2	P	—	2
0·6	P	—	2
2·0	P	—	2
5·0	8	—	75

—, Not tested. P, potentiated.

Sodium azide and triethyl tin sulphate

Sodium azide (1–4 mM) caused a blockade of responses to vasopressin and nicotine which increased steadily in its intensity until after 2 hr even very large doses of these drugs failed to produce a contraction (Table 2). Contractions produced by carbachol were relatively insensitive to this metabolic inhibitor. Triethyl tin (0·2–2 mM) potentiated the effect of carbachol but reduced responses to vasopressin by 50%. A 5 mM solution caused a greater blockade of the actions of vasopressin than carbachol (Table 2).

Tetrodotoxin

In two experiments tetrodotoxin (4×10^{-7} g/ml.) markedly reduced contractions following vasopressin but had only a slight inhibitory action on contractions of the colon induced by methacholine (Fig. 3). The tone of the preparation increased slightly when tetrodotoxin was first added to the bath fluid.

Rat uterus

Replacement of NaCl with mannitol, doubling or halving the potassium concen-

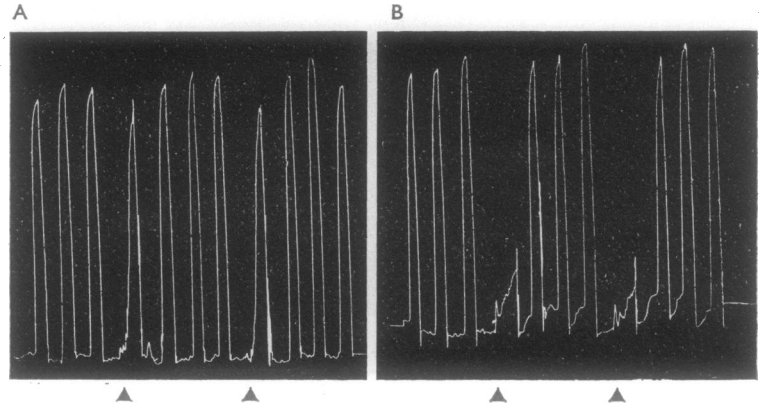

FIG. 3. Inhibition of vasopressin induced contractions by tetrodotoxin. Unmarked contractions induced by methacholine (30 ng/ml.). Vasopressin (▲) 2·5 ng/ml. A, Normal bath fluid. B, Bath fluid contained tetrodotoxin 4×10^{-7} g/ml. Drum speed reduced to one quarter during vasopressin contractions. The baseline of the tissue rose slightly after addition of tetrodotoxin.

TABLE 3. *Summary of the action of anoxia, cooling, morphine and hyoscine on the response of the proximal colon to vasopressin, transmural stimulation, methacholine and histamine*

Procedure	Transmural stimulation	Vasopressin	Methacholine	Histamine
Anoxia	A (6)	A (6)	P (2) O (1)	O (2) P (1)
Cooling to 25° C	A (3) R (1)	A (2) R (2)	O (4)	—
Cooling to 20° C	A (1)	A (1)	—	O
Hyoscine 5×10^{-9} g/ml.	O (2)	O (2)	A (2)	—
Hyoscine 10^{-8} g/ml.	O (1)	O (1)	A (1)	—
Hyoscine 10^{-7} g/ml.	SR (4) R (2)	O (2) P (1) SR (2) R (1)	A (6)	—
Morphine 10^{-7} g/ml.	O (2)	O (2)	O (1)	O (1)
Morphine 10^{-6} g/ml.	O (2) P (1) SR (1)	O (4)	O (4)	

A, Abolished; O, no effect; R, reduced to at least one-third; SR, reduced by approximately one-third; P, potentiated; —, not tested. Number of experiments in brackets.

tration or treatment with sodium azide failed to discriminate between contractions of the uterus elicited by oxytocin, methocholine and vasopressin. A ten-fold increase in the magnesium concentration in the fluid caused a slight potentiation of responses to vasopressin, no effect on those to oxytocin and a slight inhibition of those to methacholine.

Transmural stimulation

Contractions of the colon produced by electrical stimulation were blocked by anoxia in every experiment and were thus regarded as being mediated by nervous tissue because responses to histamine or methacholine were unaffected by this procedure. Reduction of the bath temperature from 32° to 25° or 20° C also reduced or abolished responses to transmural stimulation. Both procedures abolished or substantially reduced contractions elicited by vasopressin.

Morphine (10^{-6} g/ml.) did not alter responses to histamine, transmural stimulation or vasopressin even after contact with the tissues for more than an hour. Similarly contact for 1 hr with hyoscine (10^{-7} g/ml.) often did not block the action of vasopressin or electrical stimulation although responses to methacholine were abolished by hyoscine 5×10^{-9} g/ml. The results of all such experiments are recorded in Table 3.

The amount of acetylcholine-like activity released from the tissue during transmural stimulation was equivalent to 18–72 pg/pulse per g tissue (four experiments). The presence of morphine (10^{-6} g/ml.) did not reduce this output.

Discussion

The contraction of the smooth muscle of the proximal colon produced by vasopressin differs from the reaction of vascular smooth muscle, for it is not reduced by 2-*O*-methyloxytocin, an oxytocin analogue with inhibitory activity against vasopressin (Krejci, Kupkova & Vavra, 1967). As the classification of the mechanisms of action of drugs is only possible by the use of selective blocking drugs, this suggests that the mechanism for the contraction of the proximal colon activated by vasopressin is not similar to that which exists in other tissues. The effects of the metabolic inhibitors and of changing the ionic composition of the bath fluid have discriminated between the contractions produced by vasopressin and those produced by drugs acting directly on smooth muscle. Further, this evidence substantiates the view that the action of vasopressin is on the nervous elements in the colon, since the ion changes produced would be expected initially to affect activity of nerve rather than muscle. Thus, substitution of sodium in a physiological salt solution by an equimolar concentration of another cation, or substitution of sodium chloride by an equimolar solution of a sugar, does not render smooth muscle fibres inexcitable immediately (Bozler, 1960, 1961 ; Bülbring & Kuriyama, 1963). In contrast, the conduction of potentials along frog motor nerve ceases within 0·5 sec of a section being exposed to isotonic solutions of dextrose, sucrose, potassium chloride or choline chloride (Huxley & Stämpfli, 1951). Likewise, while moderate increases in extracellular potassium concentration renders smooth muscle more excitable (Bohr, 1964) the conductivity of nerve is depressed (Huxley & Stämpfli, 1951). However, even when all sodium in the bathing fluid is replaced by potassium, smooth muscle will respond to added drugs (Evans & Schild, 1957 ; Evans, Schild & Thesleff, 1958).

Oxidative metabolism is vital to maintain the excitability of resting nerve, being the source of energy to sustain sufficient potential difference across the membrane (Brink, 1957). Anoxia has been used to discriminate between directly and indirectly acting drugs on smooth muscle, since the muscle can respond anaerobically for several hours (Day & Vane, 1963). Sodium azide and triethyl tin are known to interfere with oxidative metabolism. Azide has been used in the studies of nerve metabolism in view of its ability to inactivate cytochrome c (Brink, 1957 ; Hurlbutt, Asano & Brink, 1956 ; Ritchie & Straub, 1957). Triethyl tin has a complex mode of action but is known to depress the metabolism of nerve, but not of other tissues (Cremer, 1962 ; Stoner, Barnes & Duff, 1955). The experiments using these compounds showed that they selectively blocked responses to nicotine or vasopressin with comparatively little effect against directly acting drugs.

The abolition of responses of the tissue to nicotine and vasopressin, but not methacholine, by an increase in the Mg^{++} concentration substantiates the view that the response of the colon to vasopressin is effected by release of a transmitter from nervous tissue because release of acetylcholine from nerve terminals is known to depend on the ratio of the concentrations of calcium to magnesium ions. This evidence confirms the view that the action of vasopressin on the guinea-pig proximal colon is indirect and unlike that produced in other tissues containing smooth muscle, since Somlyo, Woo & Somlyo (1966) have shown that on most tissues the action of vasopressin is markedly potentiated by magnesium ions. If further evidence is required to consolidate the hypothesis that vasopressin causes contraction of the proximal colon by an action on nervous tissue, then this is supplied by the selective depression of the vasopressin contraction by tetrodotoxin, a compound that blocks the generation of action potentials in nerves by affecting sodium movement (Kao, 1966).

In contrast to the proximal colon, the techniques described could not discriminate between responses to oxytocin, vasopressin and methacholine on isolated rat uterus, except where the magnesium concentration was raised 10-fold. As on other smooth muscle preparations, the sensitivity of the uterus to vasopressin was raised by the extra magnesium (Lockett & Owen, 1957 ; Somlyo *et al.*, 1966 ; Clegg, Hopkinson & Pickles, 1963).

The question of why the response to vasopressin is not blocked by hyoscine or morphine must now be examined. The experiments on the transmurally stimulated proximal colon confirmed that neural stimulation of the preparation is usually resistant to these antagonists but may be blocked by anoxia or cooling. Unlike the stimulated ileum, morphine does not reduce the output of acetylcholine from colon. The fact that morphine does not inhibit responses of the colon to transmural stimulation does not therefore suggest the presence of non-cholinergic motor nerves as some workers have claimed (Bennett & Fleshler, 1969). It must be concluded either that morphine or hyoscine do not always penetrate the tissue in sufficient concentration to cause complete blockade, or that the receptors or mechanisms concerned are insensitive to their action. It is of note that, in the guinea-pig, vagal stimulation of the heart is much less easily blocked by morphine than in other species (Kosterlitz & Taylor, 1959) while the responses of the urinary bladder to parasympathetic and transmural electrical stimulation and to nicotine were not affected by atropine (Chester & Thorp, 1965 ; Chester & James, 1966).

Despite this apparent action of vasopressin on nervous tissue in the proximal colon, preliminary investigations involving arterial injections and infusions of vasopressin to the superior cervical ganglion and adrenal medulla of the anaesthetized cat suggest that these structures are insensitive to the polypeptide.

We thank Miss Susan Lawrence for technical assistance and Miss Jennifer Sykes, who performed the assays for acetylcholine-like activity from stimulated colon. We are grateful to Dr. Jill Cremer for a gift of triethyl tin and to Professor J. Rudinger for supplying the methyloxytocin.

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(*Received November* 20, 1968)