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MODE OF ACTION OF VASOPRESSIN ON ISOLATED PROXIMAL COLON OF THE GUINEA-PIG

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Vaughan Williams and Streeten (1952) reported that vasopressin, but not oxytocin, increased the activity of the colon of the dog recorded *in situ*. Botting (1965) found that the isolated proximal portion of the colon of another species, the guinea-pig, was sensitive to vasopressin but was comparatively insensitive to oxytocin. In addition Botting showed that hexamethonium and pentolinum partially inhibited the contractions of the tissue caused by vasopressin. This observation, together with the fact that the colon of the guinea-pig has a high count of ganglion cells (Tafuri, 1957) suggested that vasopressin might be capable of effecting a contraction of this tissue by stimulating nervous elements associated with the smooth muscle. This paper describes experiments which provide evidence in support of this suggestion.

METHODS

Male guinea-pigs (320 to 420 g body weight) were killed and the bulbous proximal portion of the colon was excised. The faeces were flushed out with a pipette and the tissue was suspended in a 5 ml. organ bath containing a modified Tyrode solution vigorously aerated (Botting, 1965). After allowing the tissue to rest for 30 min, regular contractions to vasopressin could be produced every 15 min. The responses were recorded by a frontal writing lever on a smoked drum.

Antagonist drugs

The effects of the following drugs on the contractions produced by vasopressin and reference drugs were investigated: hyoscine hydrobromide; hexamethonium bromide; pentolinium tartrate; tetraethylammonium bromide; mecamylamine hydrochloride; morphine hydrochloride; procaine hydrochloride; cocaine hydrochloride.

Effect of lowered temperature

Standard responses of the tissue to vasopressin and appropriate reference drugs were obtained at 32° C, and were compared with those obtained on the same tissue at temperatures between 18 and 26° C.

Effect of anoxia

Anoxia of the tissue was produced by substituting for the aerated bath solution a solution through which nitrogen gas had been bubbled for at least 20 min. Nitrogen was also bubbled through the solution in the organ bath during the period of anoxia.

Effect of lower calcium concentrations

The reactions of the tissue to vasopressin and to reference drugs were obtained in the normal bath fluid and compared with those obtained in a bath fluid that contained between one-fifth (0.15 m-mole/l.) and one-fiftieth (0.015 m-mole/l.) of the normal calcium concentration (0.75 m-mole/l.). The ratio of the dose required to restore the response in the low calcium solution to the dose that elicited the control response was calculated as an index of the inhibitory effect of the fluids deficient in calcium.

Extraction and assay of acetylcholine-like activity in bath fluid

The method described by Brownlee & Johnson (1965) was followed. In most experiments the colon was incubated with the anticholinesterase N,N-diisopropylphosphorodiamidic fluoride (mipafox) in a concentration of 10 μg/ml. for 1 hr before the experiment. The bath fluid was removed after vasopressin had been in contact with the tissue for 3 min. Between six and 13 such samples were bulked, quick frozen and dried under reduced pressure. The freeze dried samples were extracted with 10 ml. of ethanol and then centrifuged at 3,000 rev/min for 30 min. Eight millilitres of the supernatant were evaporated to dryness under reduced pressure over a desiccant. The residue was dissolved in 2 ml. of distilled water since it was found that sufficient of the salts were extracted with the ethanol to render the extract hypertonic if it were dissolved in Tyrode solution. The extracts were assayed for acetylcholine-like activity on pieces of guinea-pig ileum made sensitive by incubation with mipafox (10 μg/ml.) for 1 hr. The Tyrode solution in which the assay tissue was suspended also contained morphine hydrochloride (5×10^{-6} g/ml.) to reduce spontaneous movement.

In some experiments the activities of the extracts were compared on the isolated heart of *Helix pomatia* perfused at room temperature with Meng's solution (Meng, 1960).

Drugs

The following drugs were used: vasopressin (Pitressin; Parke Davis); nicotine acid tartrate histamine acid phosphate; acetylcholine chloride; carbamoylcholine chloride; methacholine chloride. Bath concentrations stated refer to weight of the salt/ml. of fluid.

RESULTS

Hyoscine

Hyoscine hydrobromide 10^{-9} to 10^{-8} g/ml. had no effect on the contractions of the colon produced by vasopressin; 10^{-7} g/ml. depressed the responses to vasopressin to a greater extent than that of histamine in one out of four experiments, although in two others the responses to vasopressin and histamine were depressed whereas the response to nicotine was unaffected. Increase of the concentration to 10^{-6} g/ml. hyoscine resulted in a non-selective depression of the responses of the tissue to vasopressin and histamine. No evidence for the selective inhibition of the action of vasopressin by hyoscine was therefore obtained.

Ganglion-blocking drugs

Hexamethonium bromide (10^{-5} g/ml.) often, but not always, inhibited the responses of the tissue to vasopressin to 40% of the control, whereas the responses to carbachol or histamine were either unaffected or potentiated. Increasing the concentration of hexamethonium did not increase the inhibition of the vasopressin response—on the contrary, the inhibition was often less, and at the same time the potentiation of the responses to the reference drugs was more pronounced.

Pentolinium tartrate (10^{-6} g/ml.) caused some inhibition of contractions to vasopressin without affecting contractions produced by acetylcholine or histamine in one experiment out of seven. In the remaining experiments responses to acetylcholine were unaffected, yet responses to histamine were depressed as well as those to vasopressin. Increasing the concentration of pentolinium merely depressed the responses of vasopressin and both acetylcholine and histamine in a non-selective manner.

Tetraethylammonium bromide (10^{-6} to 10^{-5} g/ml.) produced variable results. It potentiated responses to histamine and to the cholinesters. The only effect observed on the responses to vasopressin was an occasional potentiation after the tetraethylammonium was replaced by Tyrode solution.

In only one of six experiments with mecamyline (1×10^{-6} g/ml.) was there a selective inhibition of the response to vasopressin compared with those to acetylcholine and histamine.

Morphine

Morphine hydrochloride (5×10^{-6} g/ml.) reduced the contractions to vasopressin without affecting those following histamine or methacholine in only two experiments out of seven.

Local anaesthetic drugs

Neither procaine (1 to 5×10^{-6} g/ml.) nor cocaine (10^{-6} g/ml.) was able to produce a consistent selective inhibition of the effect of vasopressin on the proximal colon. In only three out of eight experiments using cocaine was there a discrimination between the inhibition of vasopressin and that of methacholine. The dose ratios (dose to produce control response in presence of cocaine divided by dose to produce control responses) being 126, 25 and 75 for vasopressin, and 14, 5 and 9 respectively for methacholine. However, histamine was very easily inhibited by cocaine at the concentrations investigated.

Effect of low calcium concentration

The normal calcium content of the Tyrode solution used was 0.75 m-mole/l. The reduction of this concentration to between 0.15 to 0.015 m-mole/l. resulted in a

TABLE 1

EFFECT OF LOWERING CALCIUM CONCENTRATION OF BATH FLUID ON RESPONSE OF PROXIMAL COLON TO VASOPRESSIN, HISTAMINE AND METHACHOLINE

The dose ratio is the ratio of dose necessary to restore the response in the low calcium fluid divided by the dose required to produce the response in normal fluid. In all experiments except experiment 9 there was no response to vasopressin. In experiment 9 600× the control dose produced an effect approximately twenty per cent of the control response. (— indicates that the drug was not tested)

Experiment	Calcium content of bath fluid (mM) (N 0·75)	Dose ratio		
		Vasopressin	Histamine	Methacholine
1	0·15	>80	18	—
2	0·075	>400	—	30
3		>150	—	25
4		>33	—	2
5		>75	—	75
6		>200	—	200
7		>200	—	25
8		>250	250	160
9		>600	8	—
10	0·015	>230	—	7

selective inhibition of the responses to vasopressin when compared with those to methacholine and histamine as seen in Table 1.

A complete loss of the response to vasopressin occurred when the calcium concentration was reduced, and after approximately 1 hr no increase in dose was ever able to restore the response to more than about 20% of the control value and usually no response was obtained at all. Histamine and methacholine responses were also reduced, but these responses could be restored by an increase of the dose by a factor of between 2 and 250. Figure 1 illustrates the effect of lowering the calcium content of the bath fluid on the responses to vasopressin, histamine and 5-hydroxytryptamine. The response to vasopressin is reduced to about the same extent as that to 5-hydroxytryptamine.

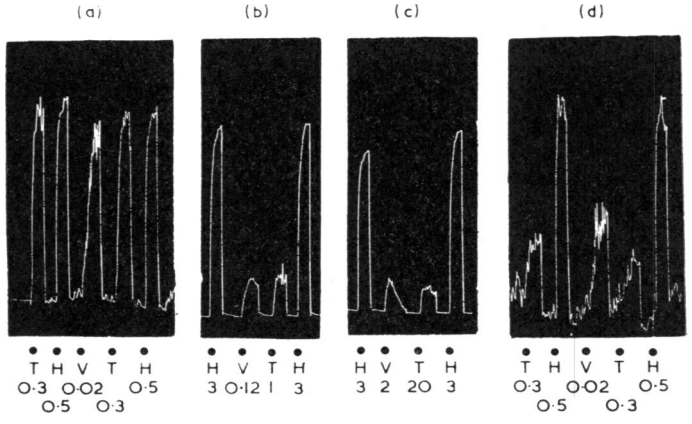

Fig. 1. Effect of lowering calcium concentration on responses of proximal colon to 5-hydroxytryptamine (T), histamine (H) and vasopressin (V); a=responses in normal Tyrode solution (0.75 mM Ca); b=40 min and c=80 min after reduction of calcium content to 0.075 mM Ca; d=15 min after restoration of normal calcium content. 5 ml. organ bath, 32° C. Figures refer to μg added to bath.

Effect of lowered temperature

Reduction of the temperature of the bath fluid to between 18 to 26° C always reduced the contractions produced by vasopressin (Fig. 2(B)) but affected contractions to the reference drugs in a variable manner. Responses to 5-hydroxytryptamine were reduced but not abolished, whereas those to the cholinesters were only rarely depressed. When reduced, the responses to cholinesters could be restored to the control height by a two-fold increase of the dose.

A 100-fold increase in the dose of vasopressin was never sufficient to restore the response.

Raising the bath temperature to 32° C once more restored the response to vasopressin. When the temperature was lowered the inherent tone of the tissue was often raised slightly.

Effect of anoxia

The production of anoxic conditions in the tissue always resulted in a complete abolition of the response to vasopressin (Fig. 2(A)). Responses to histamine were

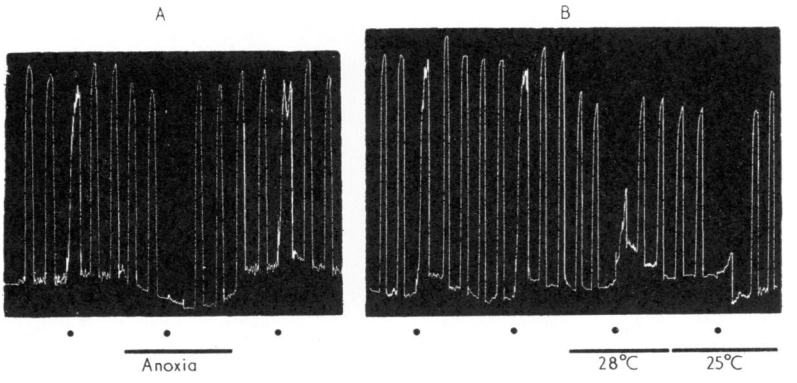

Fig. 2. Effect of anoxia (A) and cooling (B) on responses of colon to methacholine (40 ng/ml. unmarked contractions) and vasopressin (20 ng/ml. at •). In A, for period marked by bar, bath fluid was oxygen free. In B, for two periods indicated, temperature was dropped to 28° C then to 25° C.

usually unaffected, or when reduced needed a three-fold increase in dose to restore the response. The cholinesters were usually unaffected by anoxia as shown for methacholine in Fig. 2(A). It was often difficult to obtain a return of the normal response to vasopressin when the fluid was oxygenated once more. During anoxia the inherent tone of the tissue was usually reduced.

Release of acetylcholine-like substance from colon during action of vasopressin

Acetylcholine-like activity was estimated in extracts of fluid collected from the organ bath during contraction of the tissue to vasopressin and compared with that present in extracts of bath fluid collected while the colon was at rest for an identical period. To these samples, an equivalent amount of vasopressin was added to the bulk fluids after removal from the organ bath. In every case where mipafox was used as the anticholinesterase, there was an increase in activity released from the tissue when vasopressin was present. These results are listed in Table 2.

TABLE 2

RELEASE OF ACETYLCHOLINE-LIKE SUBSTANCE FROM GUINEA-PIG COLON BY VASOPRESSIN

Mipafox was added to the bath fluid surrounding the colon to act as an anticholinesterase except in experiment 1, when eserine 5×10^{-9} g/ml. was used

Experiment	Concentration of vasopressin in bath fluid (ng/ml.)	Acetylcholine-like activity released (pg Ach/min/mg wet tissue)		
		At rest (A)	With vasopressin (B)	B/A
1	3·0	0·33	0·33	1
2	1·5	0·26	1·00	3·9
3	0·75	0·60	2·0	3·3
4	3·0	0·88	3·30	3·8
5	2·25	0·16	0·44	2·9
6	1·5	2·60	5·0	1·9
7	2·25	0·29	0·70	2·4

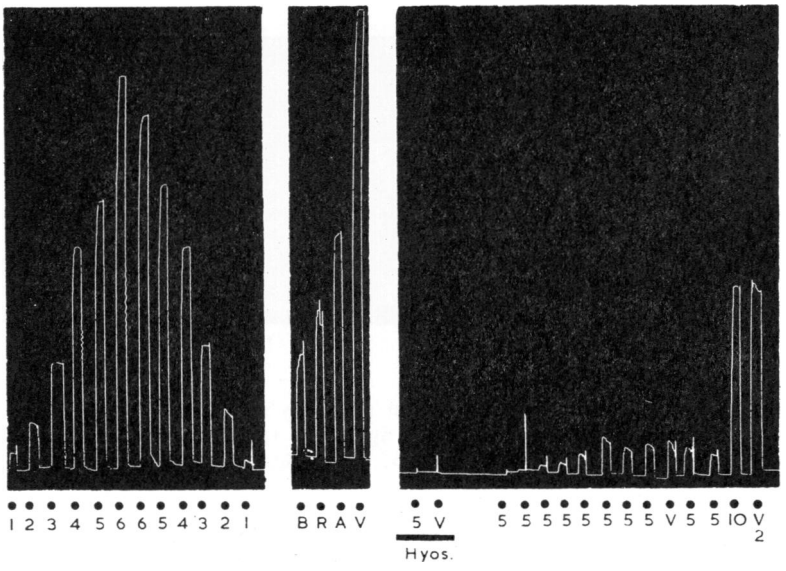

Fig. 3. Effect of hyoscine 10^{-8} g/ml. (Hyos.) on responses of guinea-pig ileum sensitized with mipafox to acetylcholine, 0.1 ml. of extract of bath fluid collected during contraction of colon to vasopressin (V), 0.1 ml. of similar extract collected while colon was at rest (R), 0.1 ml. of extract collected during contraction of colon to submaximal dose of acetylcholine (A), and 0.1 ml. of extract of plain Tyrode solution (B). Figures refer to dose of acetylcholine added to bath in ng; V2=0.2 ml. of extract V. Both responses to acetylcholine and to extract of bath fluid collected during contraction to vasopressin were inhibited by hyoscine, and effects returned in parallel on washing out inhibitor.

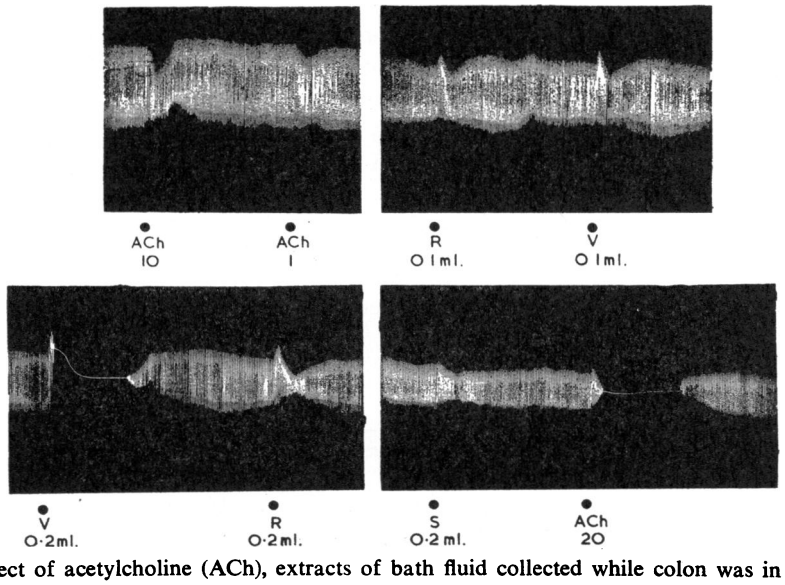

Fig. 4. Effect of acetylcholine (ACh), extracts of bath fluid collected while colon was in contraction to vasopressin (V) or while colon was at rest (R) on perfused heart of *Helix pomatia*. S=saline. Figures refer to ng.

The smooth muscle stimulating substance released was similar to acetylcholine as shown in the following ways. Firstly, Fig. 3 shows that the activity was blocked by hyoscine (10^{-8}), and that the response returned in parallel with acetylcholine. Secondly, extracts exerted an inhibitory activity on the isolated perfused heart of *Helix pomatia* (Fig. 4).

Extracts were also prepared from an equivalent volume of Tyrode solution to establish blank activity, and often from bath fluid collected while the colon was in contraction to a submaximal dose of acetylcholine.

DISCUSSION

The mode of action of the polypeptide hormones of the posterior pituitary on organs containing smooth muscle has always been assumed to be a direct effect on the muscle. This assumption is probably true for the action of oxytocin on the uterus. However, as Ginsburg has emphasized (Ginsburg, 1960), it is obvious that oxytocin and vasopressin, in spite of their similar chemical structure, manifest qualitative differences in their mode of action. For example, variation of the magnesium and calcium content of the bath fluid affects the contractions of the isolated rat uterus produced by oxytocin and vasopressin, but in a non-uniform manner (Lockett & Owen, 1957).

The reaction of the smooth muscle of the gut of different species elicited by these hormones is variously described in the literature. The confusion was probably due to the impurity of the first extracts of the pituitary gland, as well as to their acidity. When the separated principles became available commercially the possiblity of pharmacological activity in the preservatives present in these preparations added to the confusion. Chlorbutol, a preservative present in some commercial preparations of both oxytocin and vasopressin has an inhibitory action on isolated guinea-pig ileum (Botting, unpublished). Nevertheless, a fair conspectus of the many published reports is that vasopressin, but not oxytocin, is a stimulant of the smooth muscle of the gut of many species. However, the obvious differences between the activities of oxytocin and vasopressin on uterine musculature as well as on the smooth muscle of the gut suggests that at least two disinct mechanisms of action are manifested by these polypeptides, and, in the case of certain isolated segments of the gut of some species, a mechanism that involves activation by vasopressin of nervous elements within the gut is considered a possibility.

The unusual sensitivity to vasopressin of the proximal portion of the colon of the guinea-pig (Botting, 1965) is in itself indicative that this polypeptide may act through the nervous tissue associated with the musculature of this organ, since the guinea-pig colon has been shown to contain a significantly greater number of ganglion cells than any part of the ileum (Tafuri, 1957). Guinea-pig ileum is not only insensitive to vasopressin, but is considered by Levy (1963) to be inhibited by this hormone.

Definitive proof of the action of a drug on the nerves associated with the smooth muscle of an isolated tissue is not easy to obtain. Ganglionic blocking drugs will inhibit the effects of some drugs which will stimulate the ganglion cells in the gut plexuses, but other drugs—for example, 5-hydroxytryptamine—may stimulate gut smooth muscle through the activation of nervous tissue yet their effects are not inhibited by drugs that block receptor sites at which nicotine-like drugs act (Brownlee & Johnson, 1963). Other

drugs that have been used to detect an indirect effect of a spasmogen on gut are morphine (Kosterlitz & Robinson, 1958) and local anaesthetics (Feldberg & Lin, 1949 ; Brownlee & Johnson, 1963). Hyoscine also ought to inhibit a drug with an indirect action on gut since, in the absence of evidence for other transmitters, the release of acetylcholine from nerve endings might be expected to be the final path for the stimulation of the smooth muscle.

The effects of these variously acting drugs on the contractions induced by vasopressin on the proximal colon did not provide reliable or consistent evidence for an indirect action of this drug. The colon of the guinea-pig is, however, a much thicker tissue than the ileum, and it is likely that the penetration of the tissue by the potential inhibitors was incomplete. In the case of hexamethonium and tetraethylammonium it is possible that any inhibition of the response was masked by the potentiating effect these compounds have on many reference drugs. For example, Collins (1948) showed that tetraethylammonium can potentiate the action of some drugs on the guinea-pig ileum, and Feldberg (1951) found that acetylcholine was potentiated in some experiments by hexamethonium In addition, it may be that some drugs used as reference compounds in these experiments —for example, histamine—may themselves be exerting part of their effect indirectly on this tissue. Brownlee & Harry (1963) have produced evidence that histamine can act upon strips of circular muscle from guinea-pig ileum by a mechanism that involves mediation by nervous plexuses. If histamine were exerting such an effect on this tissue then it might be difficult to adduce evidence for an indirect action of vasopressin using histamine as a reference drug.

With other conditions used to discriminate between substances with a direct or indirect action on intestinal muscle, such as anoxia (Day & Vane, 1963), reduction of temperature (Ambache, 1946), and reduction of the calcium content of the bath fluid (Johnson, 1963), problems of penetration do not arise. These procedures caused a marked reduction in the sensitivity of the colon to vasopressin, yet only slightly affected the contractions of the tissue caused by the reference drug methacholine. Thus, this evidence can be taken to provide a strong indication that the action of vasopressin is on the nerves associated with the colonic smooth muscle.

The best indication of an indirect action of a drug on an isolated organ is the demonstration of the release of a transmitter from the tissue in the presence of the drug. Although the resting release of acetylcholine-like activity from the proximal colon was variable, the activity was always increased when vasopressin was added to the tissue if mipafox was present. A definitive comparison of this activity with acetylcholine was not carried out due to the limited amounts of the extracts available, but it was inhibited by low concentrations of hyoscine, the most selective muscarinic receptor blocking drug, and it had an inhibitory action on the heart of *Helix pomatia*. Other naturally occurring substances that might be liberated from gut are 5-hydroxytryptamine and histamine ; the former is a powerful stimulant of the heart of *Helix pomatia* whereas the latter has no effect even in a concentration of 1 mg/l. (Gaddum & Paasonen, 1955). The rapid nature of the spasmogenic response of the extracts suggested that it was not due to a polypeptide.

Despite the fact that many drugs that are alleged to discriminate between substances acting directly on smooth muscle and drugs acting through the stimulation of nervous elements within the tissue failed to show a consistent selective depression of the responses

of the guinea-pig proximal colon to vasopressin, the effects of anoxia, cooling, the reduction of the calcium concentration, together with the demonstration of the release of an acetylcholine-like substance during the action of vasopressin, are claimed as evidence that vasopressin is yet another polypeptide that can exert an effect on some organs by a stimulation of nervous tissue. This property has been well established for angiotensin and bradykinin (Feldberg & Lewis, 1964), and suggestive evidence for such an action of gastrin II has been reported by Bennett (1965).

SUMMARY

1. The results suggest an indirect action of vasopressin on the isolated proximal portion of the guinea-pig colon.

2. Ganglionic blocking drugs, morphine, local anaesthetics and hyoscine did not consistently cause a selective inhibition of vasopressin contractions.

3. Cooling the tissue, anoxia and reduction of the calcium content of the bath fluid selectively inhibited vasopressin contractions.

4. An acetylcholine-like substance was shown to be released from the colon when in contraction to vasopressin.

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