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THE INFLUENCE OF ANAESTHETICS ON THE INCREASE IN THE WATER PERMEABILITY OF THE TOAD BLADDER INDUCED BY VASOPRESSIN

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1 Single lobes of the bladder of *Bufo marinus* were isolated and filled with, and suspended in, oxygenated Ringer solution. The fluid in contact with the outside (serosa) of the lobes had a total osmolarity of 225 m-osmol/litre, and that bathing the inside (mucosa) of 45 m-osmol/litre.

2 Osmotic water flow from mucosa to serosa was measured by weighing the lobes every 30 minutes. It was negligible unless vasopressin was added to the serosal bath. Standard concentrations of either 1.25 or 6.25 mu/ml were used to render the bladder lobes permeable to water.

3 The presence in the serosal medium of pentobarbitone or thiopentone in concentrations ranging from 0.25 to 2.5 mM, or of chloralose in concentrations ranging from 0.65 to 6.5 mM, diminished the increase in water permeability induced by vasopressin.

4 The three anaesthetics exerted similar inhibitory effects on the action of vasopressin from the serosal and from the mucosal surface of the bladder.

5 In the presence of a constant high concentration of anaesthetic, increasing the concentration of vasopressin over three orders of magnitude led to stepwise increases of osmotic water flow out of the lobes, although at every dose level the effect of vasopressin was depressed by the anaesthetic. However, it was not completely abolished even if the concentration of vasopressin was close to threshold.

6 The increase in water permeability of the bladder induced by 3′,5′-adenosine monophosphate (cyclic AMP) was also depressed by the three anaesthetics.

7 Possible explanations of the findings are discussed.

Introduction

The anti-diuretic activity of the plasma in the rat, cat and dog is elevated during anaesthesia with ether, urethane and with many barbiturates (Dicker, 1953; Ginsberg & Brown, 1957; Beleslin, Bisset, Haldar & Polak, 1966; Bonjour & Malvin, 1970). Chloralose has been reported not to increase anti-diuretic activity of plasma (Ginsberg & Brown, 1957). The marked anti-diuresis which follows administration of morphine has been ascribed to excess release of anti-diuretic hormone (ADH) by the drug (de Bodo, 1944; Duke, Pickford & Watt, 1951). In man and dog, the renal elimination of an oral water load may be impaired or suppressed during anaesthesia, including chloralose anaesthesia, although water is absorbed from the gut (Verney, 1929; Bonsman, 1930; de Bodo & Prescott, 1945; Aprahamian, Vanderveen, Bunker, Murphy & Crawford, 1958). Yet exogenous ADH in a dosage which produces antidiuresis in a conscious water-loaded dog fails to reduce urine flow in a water-loaded anaesthetized animal, an anomaly discussed at length by Ledsome, Linden & O'Connor (1961).

These facts suggest that during anaesthesia the function of the kidney is affected not only by the varying power of different anaesthetics to release vasopressin from the neuro-hypophysis, but also by other systemic effects of these drugs, including possible direct actions on cellular function in the kidney and its sensitivity to circulating vasopressin. It was therefore of interest to see whether the presence of anaesthetics altered the effect of vasopressin on the isolated bladder of *Bufo marinus*, since the mechanism by which vasopressin increases the permeability of a cell barrier to water is probably analogous in the mammalian kidney and in the toad bladder, despite profound differences of structure and of vasopressin-sensitivity between these tissues (Bentley, 1958; Leaf, 1960).

The present paper records the results of this study. Some of the findings were briefly reported previously (Grey & Ullmann, 1969).

Methods

Single lobes of the bi-lobed bladder of the South-American Giant Toad *Bufo marinus* were used. As described by Bentley (1958), they were filled with, and suspended in, oxygenated Ringer solution which contained (mM) NaCl, 111; KCl, 3.35; $CaCl_2$, 2.7; $NaHCO_3$, 2.4; and dextrose, 5.5. Its total osmolality was 225 m-osmol/kg, and its pH was adjusted to 7.4-7.8 with NaOH. Oxygen was bubbled through the solution continuously before and during use.

After an initial resting period the inside of the lobe was rinsed several times with a 1 : 5 dilution of the Ringer and was then refilled with 2-5 ml of this dilute fluid (total osmolality 45 m-osmol/kg). The filled lobe with its supporting structures was weighed on a sensitive, highly damped balance and re-suspended in a bath of undiluted Ringer so that a 1 : 5 osmotic gradient across the bladder wall was now present. Thirty minutes later the lobe was weighed again, and any weight loss during the interval was taken to equal osmotic water flow from the mucosa to the serosa of the bladder. In the absence of mechanical leaks, functionally intact lobes are almost completely impermeable to water despite the osmotic gradient, as shown by Bentley (1958). If a lobe lost more than 0.01 g in 30 min it was discarded. Next, vasopressin was added to the undiluted Ringer bathing the serosa of the lobe; this rendered the bladder wall permeable to water. The weight loss during the next 30 min was taken to represent the osmotic water flow out of the lobe in control conditions; subsequent changes were compared with this value. Each complete assessment consisted of three parts: the control period just described, during which vasopressin was present in the Ringer bathing the outside of the lobe; a 30 min test period with the same concentration of vasopressin as before in the outer bath together with an anaesthetic in selected concentration; and lastly, a recovery period of 30 min during which the serosal bath again contained vasopressin only. Between control, test and recovery periods the inside and outside of the lobe were thoroughly washed several times with plain undiluted Ringer fluid and the lobe allowed to rest for at least 30 minutes. The usual experimental procedure is illustrated in Figure 1. Deviations from this routine are described in the text.

The drugs used were: arginine-vasopressin (Pitressin; Parke, Davis & Co.); 3′,5′-adenosine monophosphate (cyclic AMP; Sigma Chemicals Ltd.); sodium pentobarbitone (Abbott Laboratories); sodium thiopentone (May & Baker); and chloralose (α-chloralose; Kuhlmann, Paris). The four last-named materials were freshly dissolved from the solid without adjuvant on each occasion immediately before use.

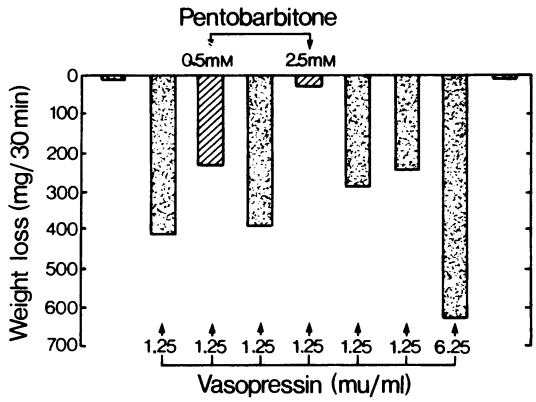

Fig. 1 The course of a typical experiment. The columns represent weight losses, i.e. the osmotic water flow due to a 1 : 5 osmotic gradient out of a single bladder lobe in nine consecutive experimental periods each of 30 min duration. Between every experimental period and the next the lobe was thoroughly washed in plain Ringer and rested for 30 minutes.

The first and ninth column show the osmotic water movement when the lobe was kept incubated in plain Ringer without vasopressin. Columns 2 to 7 show the osmotic flows when the serosal medium contained 1.25 mu/ml of vasopressin. The third and fifth columns (diagonal hatching) illustrate the depression of the response to vasopressin caused by the simultaneous presence in the serosal bath of 0.5 or 2.5 mM pentobarbitone. Recovery from exposure to the higher pentobarbitone concentration was incomplete, but the bladder was still capable of responding to a higher dose of vasopressin (column 8).

Results

The sensitivity of isolated bladder lobes to vasopressin

Different batches of toads varied in their sensitivity to vasopressin. In the presence of a 1 : 5 osmotic gradient 10 lobes lost 460 ± 66 mg (mean and s.e.) in 30 min under the influence of 1.25 mu/ml of the hormone. Another group of 29 lobes required 6.25 mu/ml vasopressin for a similar weight loss, i.e. 438 ± 20 mg. The magnitude of the response was not systematically related either

to the size of the lobe or to the initial fluid content.

On repeatedly exposing a lobe to the same vasopressin concentration without addition of any other drug, the increase in water permeability caused by vasopressin sometimes diminished after the first 2 or 3 hours. Therefore, in comparing results obtained from different lobes at different times after the start of observations, the weight losses in the test and recovery periods were expressed as percentage of the weight lost in the first initial control period of each triad of experimental exposure.

The effect of vasopressin in the presence of pentobarbitone, thiopentone or chloralose

The influence of the three anaesthetics was studied at three dose levels related as 1 : 2 : 10. The findings are set out in Table 1. All three depressed the influence of a standard concentration of vasopressin on osmotic water movement across the bladder wall. Except at the lowest dose level, the depression was statistically significant (Student's *t* test) in every case. Of the two baribarates, applied in equi-molecular concentrations, pentobarbitone was found to produce a significantly greater inhibition of vasopressin activity at the highest and lowest dose levels ($P < 0.01$ and $P < 0.05$, respectively); but there was no significant difference between the potencies of pentobarbitone and thiopentone at the intermediate dose level.

Only at the lowest dose levels did the bladders recover completely from exposure to any one of the three anaesthetics, but the residual depression was not statistically significant.

None of the anaesthetics used had a discernible effect on the permeability of the bladder wall to water if present alone without vasopressin in the Ringer bathing the serosa.

The effect of vasopressin in the serosal fluid in the presence of anaesthetics at the mucosal surface

Vasopressin does not change the water permeability of the isolated toad bladder if it is introduced into the mucosal (inner) instead of the serosal bathing medium. In several experiments, we introduced the anaesthetics into the dilute Ringer contained inside the lobes. They reduced the effect of vasopressin acting on the lobes from the serosal side. Thiopentone and chloralose at the highest dose level were nearly as effective as inhibitors from the mucosal as from the serosal surface, pentobarbitone somewhat less so; but too few experiments were performed to establish definitively differences between the three drugs in this respect. After exposure of the mucosa to anaesthetics a much more marked residual depression persisted during the recovery period than when the drugs had been applied to the serosal side during the test period (Table 2).

The effect of increasing the concentration of vasopressin in the presence of a constant concentration of anaesthetic

In one series of experiments, the twin lobes derived from single bladders were set up separately

Table 1 Effect of anaesthetics in serosal medium on weight loss of bladder lobes under the influence of a fixed concentration of vasopressin.

Anaesthetic		*n*	*Test*	*P*	*Recovery*
None; repeated exposure to vasopressin		9	108.3 ± 6.4	>0.1	104.2 ± 6.8
Pentobarbitone	0.25 mM	8	63.2 ± 9.5	<0.01	106.0 ± 13.1
	0.50 mM	7	66.4 ± 10.5	<0.02	85.4 ± 24.3
	2.50 mM	5	18.5 ± 7.2	<0.001	89.0 ± 15.3
Thiopentone	0.24 mM	13	86.0 ± 8.0	>0.1	81.0 ± 6.0
	0.48 mM	7	70.4 ± 10.1	<0.05	98.4 ± 14.7
	2.48 mM	6	55.2 ± 11.0	<0.01	96.1 ± 12.3
Chloralose	0.65 mM	5	85.6 ± 16.7	>0.1	102.0 ± 17.8
	1.30 mM	7	78.1 ± 5.0	<0.01	94.0 ± 6.9
	6.50 mM	7	39.4 ± 9.2	<0.001	85.0 ± 11.4

Results for 'test' and 'recovery' periods expressed as percent of weight loss during initial control period (means and s.e. mean).
P values refer to the difference of the mean for the test period from 100%.

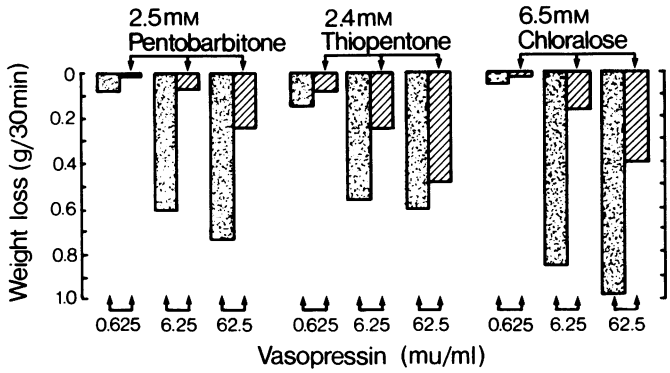

Fig. 2 Comparison of the effects of increasing concentrations of vasopressin in the serosal medium on weight loss, i.e. the osmotic water flow during 30 min in twin lobes of the same bladder. One of the pair was exposed to vasopressin only (stippled columns); the other to the same concentrations of vasopressin together with a constant concentration of one of the anaesthetics (diagonally hatched columns). For this batch of toads a vasopressin concentration of 0.625 mu/ml was just above threshold, and the response to 62.5 mu/ml was nearly maximal.

and compared as follows: one of the pair, the control lobe, was bathed in Ringer solution containing vasopressin alone. In successive 30 min test periods, separated as usual by thorough washing and rest periods in plain Ringer, vasopressin concentration was raised stepwise from 0.625 to 6.25 and finally 62.5 mu/ml. The twin lobe was simultaneously exposed to the same rising vasopressin concentrations, but one of the anaesthetics was present in the serosal bath at the same time. Its concentration was kept constant in each test period, and usually the highest dose employed in the present work was chosen. Figure 2 illustrates the results of one such set of comparisons for each of the three anaesthetics. Osmotic water flow from the bladder lobes increased with the concentration of vasopressin in the bath, but at every concentration the efficacy of the hormone was diminished if one of the anaesthetics was also present. However, the effect of vasopressin was never totally abolished by the depressant action of the drugs. Conversely, despite the inhibition produced by a given concentration of anaesthetic, the volume of water actually passing across the bladder wall under a fixed osmotic gradient increased when the concentration of vasopressin was raised.

The effect of 3′,5′-adenosine monophosphate in the presence of pentobarbitone, thiopentone or chloralose

Orloff & Handler (1962) showed that 3′,5′-adenosine monophosphate (cyclic AMP) introduced into the medium surrounding the isolated toad bladder renders the wall permeable to water. The effect of anaesthetics on this action was investigated in the same manner as their effect on the action of vasopressin. The concentration of cyclic AMP in the Ringer surrounding the lobes was 5.0 mM. This induced an outflow of water from the lobes (245 ± 36 mg/30 min; $n = 12$) smaller than that

Table 2 Effect of anaesthetics in mucosal medium on weight loss of bladder lobes under the influence of a fixed concentration of vasopressin in serosal medium.

Anaesthetic		*n*	*Test*	*P*	*Recovery*
Pentobarbitone	2.50 mM	4	76.7 ± 19.1	>0.1	79.1 ± 3.5
Thiopentone	2.48 mM	4	69.1 ± 23.6	>0.1	41.2 ± 11.2
Chloralose	6.50 mM	2	44.7 ± 7.0	<0.01	58.8 ± 3.1

Results (means and s.e. mean) expressed as in Table 1.

caused by 1.25 or 6.25 mu/ml vasopressin, but no attempt to find a matching dose level was made. As shown in Table 3, the action of cyclic AMP was depressed by all three anaesthetics. The degree of depression appeared to be more severe than in the case of vasopressin, but this may have been related to the smaller control responses elicited by the limited quantities of cyclic AMP used. However, after washing out the anaesthetic the recovery in Ringer containing cyclic AMP only was found to be poor.

Discussion

Although, in the absence of vasopressin from the bathing medium pentobarbitone, thiopentone and chloralose in the highest concentrations tested had no detectable direct effect on the water permeability of the isolated toad bladder, this only means that they did not make the bladder leaky. If, when acting by themselves, they had enhanced the normal impermeability of the tissue to water this would not have been made apparent by the method used, since bladders kept in plain Ringer solution are already virtually impermeable to water. All three anaesthetics were later found to interfere with sodium transport when added to the Ringer solution on the inside of isolated frog skin (Ng & Ullmann, unpublished observations); but neither the existence nor the nature of direct action was investigated in the experiments under discussion.

What the experiments showed was that the power of vasopressin to make the bladder wall permeable to water was consistently diminished if any one of the three anaesthetics was present in a concentration exceeding 0.25 mM barbiturate or 0.65 mM chloralose. Depression of the effect of vasopressin could arise from at least three fundamentally different causes, alone or in combination. The first is that the anaesthetics may have raised the threshold for vasopressin action by enhancing further the normal impermeability of the membrane; the second, that—being enzyme inhibitors—the drugs interfered with the intracellular actions of vasopressin; the third, that they changed the structural configuration of the cell membranes in such a manner that vasopressin, or rather cyclic AMP, could no longer from inside the cells induce the molecular re-arrangement within the permeability barrier which 'opens pores' and so facilitates water flow across the bladder wall. None of these possibilities is definitely supported or excluded by the limited experimental evidence available.

High dosages of the anaesthetics failed to abolish completely an increase in water flow due to vasopressin administered in very low concentrations which were close to threshold level for bladders kept in plain Ringer fluid (Figure 2). This puzzling finding argues against enhancement of impermeability and a simple upward shift of the vasopressin threshold being the mechanism of the inhibition.

The anaesthetics depressed the effects of exogenous cyclic AMP as well as of vasopressin. This showed that the inhibition was not due to suppression of adenyl-cyclase activity, and probably not to reduction of intra-cellular ATP synthesis. There are other links in the chain of intra-cellular chemical events influenced by vasopressin which might have been affected by the anaesthetics used (Trevor, in Bunker & Vandam, 1965). We have no information about these.

It is still unknown how an increase of cellular cyclic AMP content induced by vasopressin is finally translated at the molecular level into an increase of water permeability of the bladder wall. It is possible that all the unknown intermediate steps proceeded normally in the presence of anaesthetics, but that the cell membrane could not respond normally to a normal stimulus, owing to molecular distortion caused by solution of the drugs in the membrane lipid. Lipid solubility is a property common to a large variety of substances producing anaesthesia, and is supposedly responsible for a disorganization of membrane structure

Table 3 Effect of anaesthetics in serosal medium on weight loss of bladder lobes under the influence of 5.0 mM cyclic AMP on serosal side.

Anaesthetic		*n*	*Test*	*P*	*Recovery*
Pentobarbitone	2.50 mM	4	7.7 ± 1.6	<0.001	60.6 ± 2.2
Thiopentone	2.48 mM	4	15.7 ± 2.9	<0.001	21.9 ± 5.1
Chloralose	6.50 mM	4	14.0 ± 3.1	<0.001	33.1 ± 18.7

Results (means and s.e. mean) expressed as in Table 1.

resulting in functional impairment, such as loss of impulse conduction in nerve. Another such impairment may well be that a critical permeability barrier in the membrane can no longer be opened under the influence of vasopressin and permit passage of water.

The doses of anaesthetics used in the present study were within the range of concentrations which are needed, at least temporarily, for induction and maintenance of surgical anaesthesia in mammals. However, the sensitivity of the mammalian kidney to arginine vasopressin is at least a thousand times greater than that of the isolated toad bladder. For this and many other reasons the validity of inferences based on observations made on isolated amphibian tissues for mammals is questionable. If in the intact mammal anaesthetics inhibited the renal effects of vasopressin, one would expect a diuresis, and not the oliguria commonly present during anaesthesia.

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