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EFFECTS OF FRUSEMIDE ON SODIUM AND WATER TRANSPORT BY THE ISOLATED TOAD BLADDER

BY

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Frusemide is a potent diuretic which is thought to produce its effects by reducing sodium reabsorption in the renal tubules (Buchborn & Anastasakis, 1964). In amphibia the skin and urinary bladder fulfil an analogous function (Heller & Bentley, 1963) and offer convenient membranes for studying transepithelial active sodium transport and water movement. The following experiments were all performed on the isolated urinary bladder of the toad *Bufo marinus*.

METHODS

Leaf, Anderson & Page (1958) demonstrated that the current required to cancel out the difference in electrical potential between the mucosal and serosal surfaces of the bladder, the short-circuit current, is a measure of the net sodium movement by the bladder cells. Whereas the transport of sodium will take place against an electrochemical gradient, and is therefore an active transport, water movement will occur through the bladder wall only along an osmotic gradient (Bentley, 1958). The methods used for measuring the short-circuit current and osmotic water loss from the bladder were those of Bentley (1958, 1960). Urinary bladders were dissected from pithed toads; each of the two lobes was tied on to a glass tube, and suspended in a 10 ml. bath of bicarbonate-buffered Ringer's solution. The composition of the solution in mM was sodium chloride, 111; potassium chloride, 3.35; calcium chloride, 2.70; sodium bicarbonate, 2.38; glucose, 5.5; *p*H, 7.3 to 7.4. The temperature was maintained at 25° C. and the baths were aerated with a small aquarium aerator. All drug and hormone solutions were brought to *p*H 7.3 with sodium bicarbonate before use, and were added to the bathing solution only on the serosal side of the bladder.

Measurements of the short-circuit current were made every 5 min for 15 min, the bladder was then restored to normal Ringer's solution for 15 min to re-equilibrate. After this re-equilibration period the resting short-circuit current was measured to give a mean resting level during the experimental period. The response was taken as a mean short-circuit current during the period of observation minus the mean baseline level. In the experiments in which sodium transport was stimulated with arginine vasopressin, cyclic 3′–5′ adenosine monophosphate (cyclic AMP) or caffeine, doses of these substances alone were alternated with doses of the same substance in the presence of frusemide, on the same preparation and a direct comparison of the responses made using Student's t test.

In the experiments on water transfer the bladder lobes contained 1 ml. of frog Ringer's solution diluted 1 : 5 and the weight loss during a 15 min period was taken as the response.

The animals used were Jamaican *Bufo marinus* of either sex varying in weight from 50 to 200 g. Arginine vasopressin was purified from ox pituitaries, cyclic 3′-5′ adenosine monophosphate was batch No. 22783 obtained from the Koch-Light laboratories, caffeine base was obtained from Ferris & Co., Bristol, and the sample of pure frusemide was kindly donated by Dr D. R. Chambers of Hoechst Pharmaceuticals Ltd.

RESULTS

The experiments done were of three types.

1. The effects of three concentrations of frusemide on the resting short-circuit current of the toad bladder were measured.

2. The effects of frusemide on the vasopressin-induced stimulation of osmotic movement of water from the mucosal to the serosal side of the bladder were examined.

3. The degree of stimulation of sodium transport produced by arginine vasopressin, cyclic AMP and caffeine was compared with that produced by the same substances in the presence of frusemide.

The effects of frusemide on the resting short-circuit current. Frusemide was added to the bathing fluid to give concentrations of 0.25 mg/ml., 0.025 mg/ml. and 0.0025 mg/ml. (7.6×10^{-4}M, 7.6×10^{-5}M and 7.6×10^{-6}M respectively). The highest concentration produced a slight, but statistically insignificant, rise in the resting short-circuit current of 3.3 ± 1.1 μA (S.E.); the two lower concentrations had no consistent effect. Measurements were made in some cases for up to 30 min.

The effects of frusemide on the increased osmotic movement of water through the bladder wall caused by arginine vasopressin. Arginine vasopressin was added to give a bath concentration of 5 mu/ml. (1.05×10^{-8}M) the mean water loss in 15 min was 161 ± 26.9 mg (S.E. of eight observations). When the bath contained 0.025 mg/ml. (7.6×10^{-5}M) frusemide in addition to the vasopressin the mean water loss was identical, 161 ± 31.3 mg (S.E. of eight observations). When the experiment was repeated with the same frusemide concentration, but with an arginine vasopressin concentration of 10 mu/ml. (2.1×10^{-8}M), the mean water loss with arginine vasopressin alone was 184 ± 24.1 mg (S.E. of eight observations) and with arginine vasopressin and frusemide 201 ± 33.9 mg (S.E. of eight observations). This difference was not significant.

The effects of frusemide on the stimulation of sodium transport produced by arginine vasopressin, cyclic AMP and caffeine. The results of these experiments are given in Table 1. The concentrations of the stimulants of sodium transport all produced submaximal responses, and the variation in response to the stimulants alone was at least partly due to variation in size of the bladders used in the different experiments. The stimulation of sodium transport produced by these substances was antagonized by frusemide.

TABLE 1

EFFECTS OF ARGININE VASOPRESSIN, CYCLIC 3′-5′-ADENOSINE MONOPHOSPHATE AND CAFFEINE ALONE AND IN THE PRESENCE OF FRUSEMIDE, ON SODIUM TRANSPORT BY THE ISOLATED TOAD BLADDER

Stimulant of sodium transport (moles)	Response to stimulant alone (μA)	Frusemide concentration (moles)	Response to stimulant in presence of frusemide (μA)	P	Degrees of freedom
Arginine vasopressin, $2{\cdot}1 \times 10^{-9}$	24	$7{\cdot}6 \times 10^{-6}$	11	$<0{\cdot}05$	20
$2{\cdot}1 \times 10^{-9}$	12	$7{\cdot}6 \times 10^{-5}$	3	$<0{\cdot}001$	10
$1{\cdot}05 \times 10^{-8}$	10	..	5	$<0{\cdot}01$	18
Cyclic 3′-5′ adenosine monophosphate, 1×10^{-3}	5	..	0	$<0{\cdot}005$	17
Caffeine, 1×10^{-2}	13	..	1	$=0{\cdot}001$	8

DISCUSSION

Measurements of the effects of various diuretics on the resting level of sodium transport by frog skin have been reported by Herms and Hofmann (1965), who found that the diuretics stimulated rather than reduced sodium transport.

Frazier and Hammer (1963) have demonstrated that, although AVP increases the active transport of sodium by the isolated toad bladder only when added to its serosal side, it acts by increasing the permeability of the mucosal cell surface to sodium. This in turn makes more sodium available to the sodium pump at the serosal surface of the cell, which exchanges sodium for potassium ions. The steps by which the changes produced by arginine vasopressin are mediated are unknown, but it has been shown by Orloff and Handler (1962) that both cyclic AMP and xanthines themselves produce an increase in active transport of sodium by the bladder similar to that produced by arginine vasopressin. It is possible, therefore, that cyclic AMP is an intracellular mediator of vasopressin action.

The fact that frusemide has no effect on the resting short-circuit current suggests that it has no direct effect on the sodium pump itself, but prevents the increase in permeability to sodium of the mucosal cell membrane which is caused by vasopressin. That frusemide is not a competitive antagonist of vasopressin receptors is shown by the fact that it inhibits the vasopressin-induced increase in sodium transport, whereas the change in water permeability of the bladder wall is unaffected. If cyclic AMP is one of the intracellular mediators of the action on arginine vasopressin, then frusemide either acts

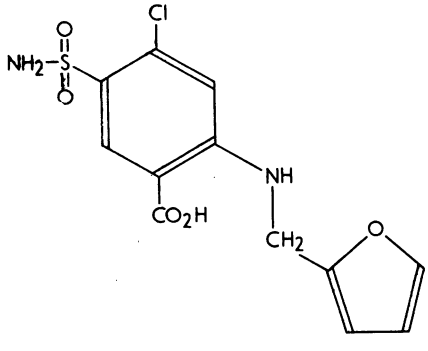

Fig. 1. Molecular structure of frusemide.

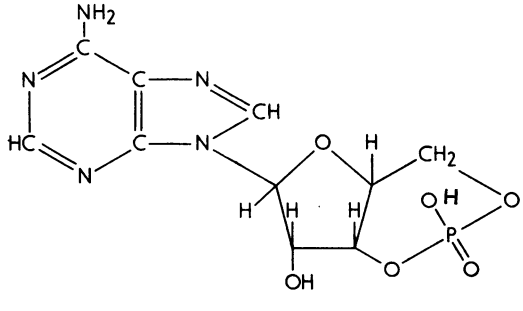

Fig. 2. Molecular structure of cyclic 3′-5′ adenosine monophosphate.

as a competitive antagonist of cyclic AMP or blocks a later step in the pathway. Certain molecular similarities between frusemide and cyclic AMP suggest the possibility that frusemide is a cyclic AMP antagonist (see Figs. 1 and 2). The possibility that the effects of frusemide on the mammalian kidney are due to an antagonism of cyclic AMP remains to be explored.

SUMMARY

1. Frusemide had no effect on the resting short-circuit current of the isolated bladder of *Bufo marinus*.

2. Frusemide antagonized the stimulation of sodium transport produced by arginine vasopressin, cyclic AMP and caffeine.

3. The increased permeability of the bladder to water produced by vasopressin was unaffected by frusemide.

4. It is concluded that frusemide has no direct effect on the sodium pump at the serosal cell surface, but may act as an antagonist of cyclic 3′-5′ AMP.

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