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Transepithelial water movement in response to carbamazepine, chlorpropamide and demeclocycline in toad urinary bladder

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1 Osmotic water movement across toad isolated hemibladders was measured by a gravimetric method.

2 The influence of carbamazepine, chlorpropamide and demeclocycline on the antidiuretic hormone (ADH)-induced water flow rate was examined.

3 No antidiuretic activity due to carbamazepine alone was observed but a slight inhibition due to ADH-induced water flow was observed in the presence of carbamazepine over a selected dose-range. This was unexpected and is inconsistent with data from *in vivo* studies in man.

4 Chlorpropamide potentiated ADH-induced water flow, in keeping with the hypothesis that chlorpropamide sensitizes the renal tubules to ADH-induced water flow.

5 Demeclocycline inhibited ADH-induced water flow. The mechanism of action remains unclear.

Keywords: Toad bladder; hydrosmotic water flow; carbamazepine; chlorpropamide; demeclocycline; antidiuretic hormone

Introduction

Several pharmacological agents are known to influence water handling by increasing or decreasing the effect of antidiuretic hormone (ADH). Their precise site and mechanism of action is fairly certain in some instances but speculative in others. Chlorpropamide (CPM) for example, appears to sensitize the renal tubule to the effect of endogenous ADH (Gilman *et al.*, 1985) and demeclocycline (DMC) blocks reversibly the increase in tubular permeability induced by both ADH and adenosine 3':5'-cyclic monophosphate (cyclic AMP) (Singer & Rotenberg, 1973).

The antidiuretic effect of carbamazepine (CBZ) is well documented (Stephens *et al.*, 1977), but the mode of action remains unclear. Fraham *et al.* (1969) showed an increase in plasma antidiuretic activity after CBZ therapy but Meinders *et al.* (1974) could find no increase in plasma arginine-vasopressin (AVP) concentration in patients receiving CBZ and Hanefield *et al.* (1970) found no evidence of increased AVP production by the hypothalamus in response to CBZ. It has been suggested that carbamazepine increases renal responsiveness to ADH but may also act upon circulatory osmoreceptors (Stephens *et al.*, 1978).

Our understanding of the cellular mechanism of action of neurohypophyseal hormone has been derived extensively from studies on the toad urinary bladder, which serves as an excellent model epithelium for investigation of the role of the mammalian renal nephron in salt and water homeostasis (Leaf *et al.*, 1958) but has not been used widely to investigate pharmacological effects.

The urinary bladder of *Bufo marinus* is a bilobed structure and may contain as much as 100–150 ml of fluid when fully distended. The size of the organ makes it possible to obtain large sheets of tissue for gravimetric determination of water movement where one cannulated lobe serves as an experimental sac with the other as a control (Bentley, 1958). The simpler histological structure compared with mammalian renal tubules or amphibian skin assists interpretation of the results. We have used this preparation to investigate the effects of certain drugs upon water transport across the epithelial membrane.

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Methods

All studies were carried out on the isolated urinary bladder of the toad, *Bufo marinus* (Mexican), obtained from Xenopus Ltd, Surrey. The toads were fed once a week on house crickets and allowed continuous free access to tap water. They were killed by double pithing and their bilobed urinary bladders were resected.

Measurement of osmotic water movement

Osmotic water movement across isolated hemibladders was measured gravimetrically in paired experiments as described by Bentley (1958). Each hemibladder was fixed with surgical thread to the end of a short length (4 cm) of plastic tubing (0.5 cm o.d.) forming a bag, and rinsed inside and out with incubation medium (composition, mM: NaCl 90, KCl 3.5, $NaHCO_3$ 25, Mg_2SO_4 0.5 KH_2PO_4 0.5, $CaCl_2$ 1, glucose 6, gentamicin 5 mg l^{-1}, 1 N-2-hydroxyethylpiperazine-N-2 ethanesulphonic acid (HEPES) 10 mM, 22 mosmol) pH 7.4–7.5 under air at 22–25°C. Each hemibladder was filled with 5 ml quarter strength incubation medium and suspended in a bath containing 50 ml of aerated full strength incubation medium.

Experimental procedure

After an initial 1 h period of equilibration, the hemibladders were emptied, rinsed, refilled with quarter strength incubation medium and resuspended in freshly aerated full strength incubation medium so that their tops were just covered. Periodic weighting was carried out on an analytical balance at 20 min intervals during a 1 h experimental period. The hemibladder was out of the bath for no more than 15 s during each weighing. Any weight lost was taken to indicate the amount of fluid lost from the lumen by transfer across the bladder membrane.

Arginine vasopressin

Once the hemibladder sacs were equilibrated and steady 20 min interval water flow rates obtained, one of the paired bladder sacs was exposed to a fixed dose of AVP for 20 min. The second bladder sac from the same toad served as a control. The rate of transepithelial water movement was estimated in mg min^{-1} and expressed as $\mu l\ min^{-1}$ water flow rate for each of the 6 doses of AVP tested.

Pharmacological agents

After obtaining a steady reading, for each of the two bladder sacs, one bladder sac was exposed to the test agent such as CBZ, CPM or DMC (applied to the serosal side) while the second bladder served as a control. After 20 min of exposure of one bladder sac to the test agent, both the bladder sacs were exposed to 50 nM AVP (applied to the serosal surface). When changing bathing solutions or washing out the test agents, the two hemibladders were treated identically. The weight change of each hemibladder was measured at 20 min intervals after exposure to AVP and the 40 min values used. The rate of transepithelial water movement was thus estimated in mg min^{-1}, and the rates of mean weight change in the paired hemibladders were compared and expressed as $\mu l\ min^{-1}$ water flow rate.

Although the hydration status of the toads may influence the responsiveness of the hemibladder to AVP, the error introduced was minimized by using toads of the same batch within each experiment. Also each toad provided its own internal control. With the test agents, the experimental protocol was designed to examine the difference between the control and the experimental preparation and not the absolute water flow rate values due to AVP.

Results are expressed throughout as mean $\pm$ s.d. ($\pm$s.e.mean on the graphs). Statistical significance was evaluated by Student's *t* test.

Reagents

Fresh solutions of each test compound were made up daily. As CBZ is practically insoluble in incubation medium, the compound was first dissolved in 5% ethanol (Analar) to give a 1 mM stock solution from which serial dilutions were made with incubation medium. Chlorpropamide is insoluble both in water and ethanol and the aqueous compound was prepared in McIlvaine's standard buffer pH 7.4 (McIlvaine, 1921). Demeclocycline and vasopressin are both water-soluble and millimolar concentrated stock solutions of each were prepared in de-ionised water and diluted with normal strength incubation medium; all solutions were kept refrigerated until required.

Results

Effect of arginine vasopressin alone

The mean rate of osmotic water movement across the isolated control hemibladder was $1.44 \pm 0.19\ \mu l\ min^{-1}$. The presence of AVP significantly raised the basal rate. The lowest dose of AVP applied (0.5 nM) produced an almost twenty fold increase in hydrosmotic response (Figure 1). A peak water flow rate of $53.4 \pm 13.63\ \mu l\ min^{-1}$ ($n = 8$; $P < 0.001$) was observed in the presence of 10 nM AVP. Further increase in AVP dose from 10 to 100 nM resulted in a gradual reduction of water movement to $27.01 \pm 17.45\ \mu l\ min^{-1}$ ($n = 8$; $P < 0.001$), identical to the hydrosmotic response observed at 0.5 nM.

Effect of chlorpropamide

CPM alone showed no antidiuretic activity. The test hemibladders that were incubated for 20 min with CPM, before addition of AVP (50 nM), showed a significant potentiation of the response due to AVP (Figure 2) for CPM doses above 50 μM ($n = 6$; $P < 0.001$). The increased ADH response in the presence of CPM was dose-dependent and exceeded the control value by $59 \pm 9.3\%$ ($n = 6$; $P < 0.001$) at a CPM concentration of 500 μM.

Effect of demeclocycline

DMC alone showed no change in the basal water flow rate across the hemibladders. There was a significant inhibition in

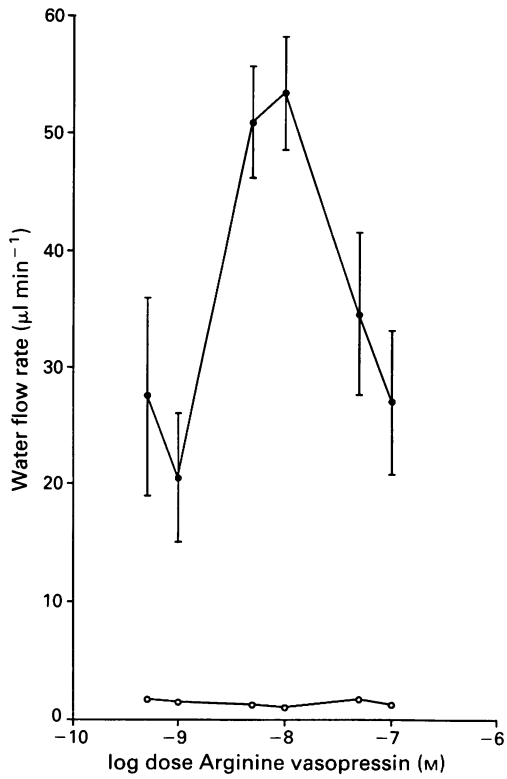

Figure 1 Effect of increasing doses of arginine vasopressin on the hydrosmotic response of the toad bladder. Experimental (●), control (○); mean of eight paired experiments with s.e.mean shown by vertical bars.

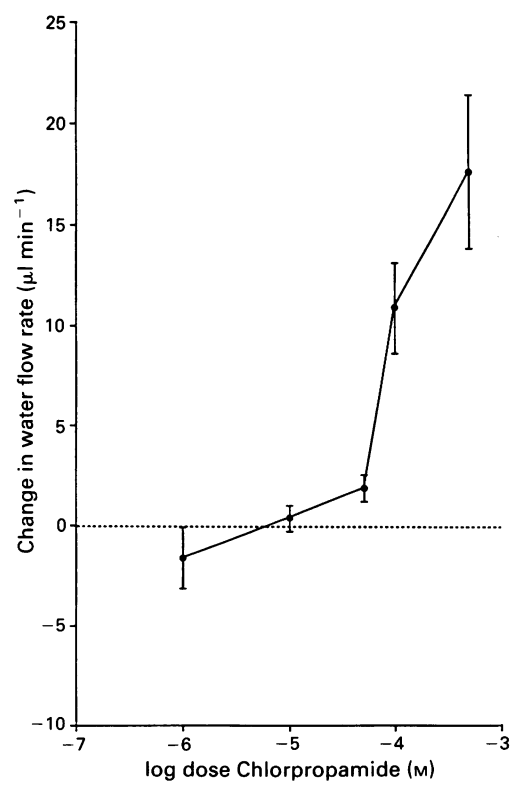

Figure 2 Effect of increasing doses of chlorpropamide on the hydrosmotic response to a single 50 nM dose of arginine vasopressin. The difference between the control and the experimental preparations at each dose is shown as the mean of six paired experiments; s.e.mean shown by vertical bars.

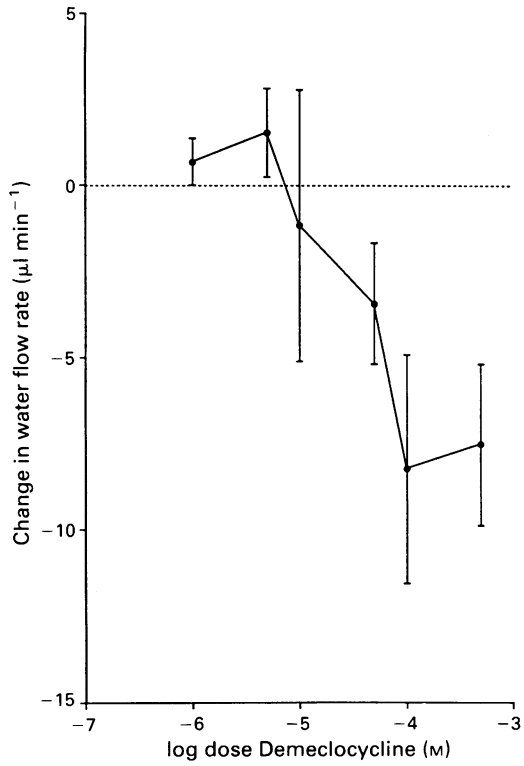

Figure 3 Effect of increasing doses of demeclocyline on the hydrosmotic response to a single 50 nM dose of arginine vasopressin. The difference between the control and the experimental preparations at each dose is shown as the mean of six paired experiments; s.e.mean shown by vertical bars.

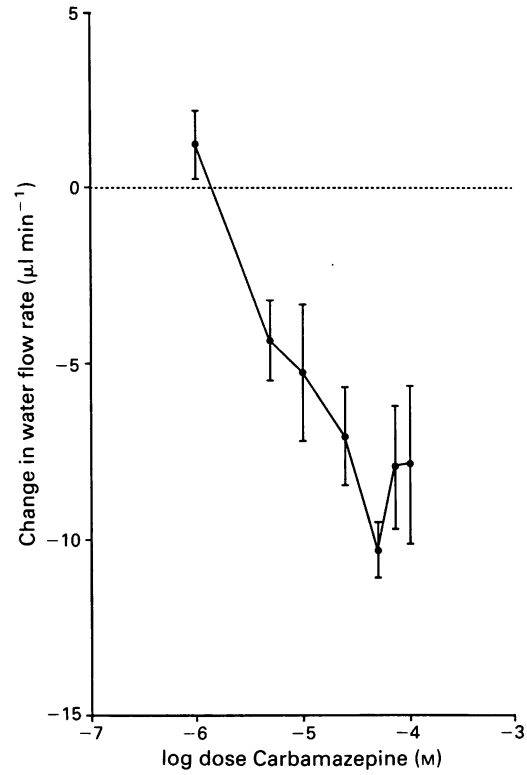

Figure 4 Effect of increasing doses of carbamazepine on the hydrosmotic response to a single 50 nM dose of arginine vasopressin. The difference between the control and the experimental preparations at each dose is shown as the mean of six paired experiments; s.e.mean shown by vertical bars.

the response to AVP (50 nM) when the test hemibladders were incubated for 20 min with DMC. The inhibitory influence of DMC on hydrosmotic responses in the paired hemibladder is shown in Figure 3. The differences between treated and untreated bladders become apparent at doses of 50 μM to 500 μM ($n = 6$; $P < 0.001$). The inhibition was dose-dependent, test and the control values differing by $37 \pm 5.7\%$ ($n = 6$; $P < 0.001$) at a DMC concentration of 500 μM.

Effect of carbamazepine

The basal rate of osmotic water movement across isolated hemibladders in the absence of AVP was not influenced by exposure of tissue to CBZ, 1 to 100 μM. The mean basal weight loss was 0.71 ± 4.78 l min^{-1} in the control bladder and 1.08 ± 3.19 μl min^{-1} in the test bladder ($n = 6$) with no significant differences between test and control. The increased rate of water movement elicited by AVP 50 nM was consistently reduced in hemibladders previously exposed for 20 min on their serosal surface to CBZ 5 μM to 500 μM (Figure 4). The inhibition was dose-dependent and reached $42 \pm 3.4\%$ ($n = 6$, $P < 0.001$) after 20 min before exposure to CBZ, 25 μM. Further increase in CBZ doses resulted in lesser rather than greater inhibition. Three preparations failed to respond to CBZ treatment over the entire dose-range.

Discussion

Effect of arginine vasopressin

Mammalian antidiuretic hormone is acknowledged to be the primary hormonal regulator of water excretion and stimulator of active sodium transport. In amphibian urinary bladder, its mechanism of action is thought to be similar to its action on the mammalian renal collecting ducts (Eggena & Ma, 1986). Vasopressin binds initially to V_2 receptors located in the basolateral cell membrane and activates adenylate cyclase, resulting in the generation of cyclic AMP which serves as an intracellular messenger for both the hydrosmotic and natriferic action of the hormone. The mechanism of the events that follow cyclic AMP generation is not clear but cytoskeletal organelles, such as microtubules and actin-containing microfilaments, are thought to be involved in producing specific changes in the surface architecture (Taylor *et al.*, 1987).

We have confirmed that toad hemibladder tissue is highly sensitive to the hydrosmotic effect of AVP and that the preparation can be used to relate the antidiuretic activity of other pharmacological agents to AVP.

Effect of chlorpropamide

Inappropriate secretion of antidiuretic hormone and hyponatraemia due to CPM has been reported (Moses *et al.*, 1973) but others have suggested that there is no direct action on the pituitary or hypothalamus resulting in release of ADH (Meinders *et al.*, 1975). The most favoured explanation for the effect of CPM is that the drug increases the sensitivity of target epithelium to ADH (Beck *et al.*, 1974).

We have shown that CPM enhances the effect of AVP on hydrosmotic water flow across the hemibladder and the data support the hypothesis that CPM increases renal responsiveness to ADH. The mechanism of action at cellular level is unclear and reports are contradictory. It has been suggested that CPM decreases the inhibitory effect of prostaglandins on ADH-dependent generation of cyclic AMP (Ozer & Sharp, 1973) but Omachi *et al.* (1974) have shown that CPM lowers cyclic AMP levels in toad urinary bladder in response to ADH. Ozer & Sharp, (1973) have interpreted the potentiating effect of CPM upon ADH in toad urinary bladder as the consequence of raising levels of intracellular cyclic AMP; while Kusano *et al.* (1983) proposed that CPM enhances the effect of ADH by a mechanism that increases the osmotic driving force for water reabsorption in collecting tubules.

Effect of demeclocycline

DMC has been shown to inhibit the hydrosmotic effect of ADH in man (Singer & Rotenberg, 1973; Feldman & Singer, 1974). Our findings indicate that the presence of DMC inhibits the increase in hydrosmotic water flow due to ADH and confirms the work of Feldman & Singer, (1974), who used Dominican Republic toad bladder incubated with DMC in Ringer solution. Although the mechanism is not clear, DMC probably impairs ADH-induced cyclic AMP generation and also impairs cyclic AMP action (Singer & Rotenberg, 1973). However, other possibilities, such as the physicochemical properties of DMC and its ability to bind to cell membrane proteins involved in water transport, need to be explored.

Effect of carbamazepine

The antidiuretic effect of CBZ was first described in 1966 (Braunhofer & Eroffnet, 1966) and further reports of hyponatraemia and water intoxication have since appeared (Lahr, 1985). Suggested mechanisms have included enhancement of renal sensitivity to AVP (Smith *et al.*, 1977; Stephens *et al.*, 1978), direct stimulation of AVP release (Kimura *et al.*, 1974; Smith *et al.*, 1977) and impaired AVP degradation (Smith *et al.*, 1977). The reports on the mechanism of action of CBZ are contradictory and unclear (Meinders *et al.*, 1974; Wales, 1975).

Our results have failed to show any enhancing effect, direct or AVP-mediated, upon hydrosmotic movement of water across toad isolated hemibladder. It is not clear from our investigations why CBZ should inhibit the effect of ADH as observed between the CBZ doses 10 μM to 75 μM and why 3 toads failed to respond to CBZ.

CBZ might be exerting its influence indirectly on renal tubular performance in man by acting at sites other than AVP receptors, such as on the adrenal gland to alter circulating aldosterone levels or the release of adrenocorticotrophic hormone (ACTH). There is a possibility that a metabolite of CBZ, such as 10, 11 epoxy-carbamazepine (CBZ-E) or 10, 11 dihydroxy-carbamazepine could possess antidiuretic activity or sensitize renal tubules to AVP.

The water flow rates observed in Figure 1 in response to 50 nM AVP were considerably higher than those observed in later experiments involving the three pharmacological agents. The explanation for this is likely to be due in part to the fact that different batches of toads were used during the course of the overall study; moreover, the dose-response curve for AVP was obtained at a different time of the year (May-June) from the testing of the three pharmacological agents (August-December). Bentley (1966) also reported variations in the toad bladder membrane performance at different times of the year. Nevertheless, the use of matched hemibladders permitted controlled observations at every dose.

The experiments described in this paper demonstrate that toad bladder is a suitable biological preparation for the study of antidiuretic hormone and other pharmacological agents that affect water transport *in vitro*. The technical simplicity, high sensitivity to antidiuretic hormone and the reproducibility of results enhance the value of the hemibladder preparation in studies of agents that are thought to influence water transport.

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