Stimulation of sodium transport by antidiuretic hormone in bullfrog intestine[1]

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Summary. Antidiuretic hormone (ADH) increased the potential difference (PD) and shortcircuit current (SCC) across the small intestine of the bullfrog. This effect was independent of those produced by amiloride or high calcium but was masked by a theophylline-induced effect. Net active sodium (Na^+) absorption accounted for the observed electrical changes.

In the intestine, Soergel et al.[2], demonstrated that administration of ADH inhibited sodium chloride absorption by human jejunum and ileum in vivo and in some cases resulted in sodium, chloride and water secretion. The reduction of sodium absorption was the result of a decrease in the mucosal-to-serosal unidirectional flux with no significant change in the serosal-to-mucosal flux. Field et al.[3], reported that ADH increased the short-circuit current across a stripped preparation of rabbit ileum; they suggested that ADH brought about active secretion of chloride and bicarbonate. However, the same investigators demonstrated that ADH had no effect on PD or SCC when the intestine was bathed in a sulfate-based Ringer. The present work was therefore undertaken in order to assess: 1. the ionic nature of the ADH-induced increase in electrical characteristics in the gut; and 2. possible mechanisms of action of ADH.

Materials and methods. Adult bullfrogs, *Rana catesbeiana*, of either sex were kept fasting at room temperature of 25 °C prior to experimentation. The PD and SCC were measured across the sheets of bullfrog small intestine using the in vitro preparation described by Quay and Armstrong[4]. The intestine was oxygenated and mounted between identical phosphate-buffered Cl-free Na_2SO_4 Ringer solution made isosmotic with mannitol, of the type described by Adrian[5].

Results. In oxygenated substrate-free Na_2SO_4 Ringer solution, addition of 200 mU/ml ADH to the serosal bathing medium elicited increases in both PD and SCC. The responses to ADH were abolished by rinsing and replacing the serosal compartment with fresh ADH-free Ringer solution (figure). Addition of 200 mU/ml ADH to the mucosal bathing solution had little or no effect on PD and SCC ($N=3$).

The lowest effective concentration of ADH added to the serosal bathing solution to elicit an increase in the measured electrical characteristics; PD and SCC, was 4 mU/ml while 200 mU/ml ADH was the concentration at which a maximal response was gotten ($N=6$).

The presence of high Ca^{2+} and amiloride in the outside bathing medium interferes with Na^+ transport across the frog skin through a mechanism different from that of ADH[6,7]. The possible interaction between ADH and high Ca^{2+} or amiloride was thus studied in the intestine. The increase of Ca^{2+} concentration in the mucosal bathing medium from 1.8 to 10 mM in the presence of 200 mU/ml ADH in the serosal compartment had no effect on the ADH induced effects on PD and SCC ($N=4$). Addition of 10^{-5} M amiloride to the mucosal bathing medium after 200 mU/ml ADH had stimulated PD and SCC caused no change in the measured electrical characteristics.

Addition of 10 mM theophylline to the serosal bathing medium stimulated both PD and SCC. Then, addition of 200 mU/ml ADH to the same compartment just as the theophylline-induced effects reached steady-state caused little or no stimulation of PD or SCC. Similar results were obtained with the addition of 200 mU/ml ADH to the serosal solution after serosal 7.5 mM cyclic adenosine monophosphate (cAMP) or serosal 5 mM dibutryl cyclic adenosine monophosphate (DbcAMP) had stimulated PD and SCC.

In order to discern the ionic nature of the ADH-induced SCC, determination of the unidirectional mucosal to serosal (J_{MS}) and serosal to mucosal (J_{SM}) Na^+ fluxes using $^{22}Na^+$ in paired preparations when their respective short-circuit currents matched were performed. Differences between means were analyzed statistically using the Student's t-test.

As shown in the table, the mean J_{MS} of Na^+ before ADH addition is less ($p < 0.005$) than the mean J_{MS} of Na^+ after the addition of ADH. However, there is no significant difference in the mean J_{SM} of Na^+ before and after the addition of ADH. Also, there is no significant difference between the increase in the mean J_{MS}^{NET} and the mean SCC after the addition of ADH.

Discussion. The present results indicate the ADH alters the rate of Na^+ transport across the small intestine of bullfrog. On the basis of the bullfrog intestinal model proposed by Quay and Armstrong[4], some speculations can be made about the mechanism of this effect. Their model proposes that there are 2 main functional barriers to transport in the intestine corresponding to the mucosal and serosal membranes of the epithelial absorptive cells.

It seems that the site of ADH action is localized in or around the serosal membrane of bullfrog gut epithelial cells, for addition of ADH to the mucosal solution caused little or no change in the endogenous PD or SCC.

Theophylline, by itself, stimulates PD and SCC similarly to that of ADH; however, when 200 mU/ml ADH was added to the serosal bathing solution after 10 mM theophylline had stimulated PD and SCC, little or no effect was observed. Similar results were obtained when ADH was added to the serosal bathing solution after cAMP or DbcAMP had stimulated the measured electrical characteristics. These observations suggest that these agents (theophylline, cAMP and DbcAMP) and ADH are acting through the same mechanism in the stimulation of PD and SCC with theophylline, cAMP or DbcAMP saturating the mechanism, thereby masking the ADH effect.

Since the receptor for adenyl cyclase has been deduced to

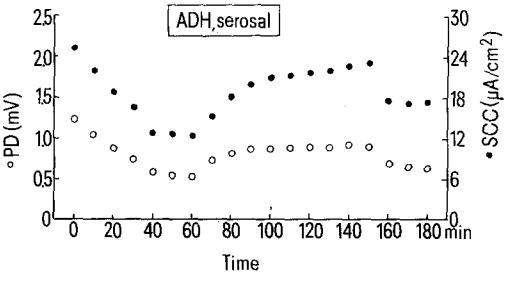
Sodium fluxes in sodium sulfate amphibian ringer

	J_{MS} (nEq/cm²/min)	J_{SM} (nEq/cm²/min)	J_{MS}^{NET} (nEq/cm²/min)	SCC (nEq/cm²/min)
Before ADH addition	43.8 ± 3.3 (6)	36.1 ± 2.8 (6)	7.7 ± 2.6	7.0 ± 0.8
After ADH addition	50.1 ± 3.4 (6)	37.5 ± 3.0 (6)	12.6 ± 3.1	10.8 ± 1.0

Average values ± SEM are given for the number of experiments shown in parentheses.

be localized on the inner or serosal rather than the outer or mucosal membrane of several epithelia[8], it is feasible to presume that ADH would be more accessible to adenyl cyclase when it is placed in the serosal bathing medium. Hence, the reason for stimulation of the electrical characteristics when ADH was placed in the serosal bathing medium and not in the mucosal bathing medium. This increased enzyme activity would lead to increased intracellular concentrations of cyclic AMP which would be the final mediator for increasing Na^+ permeability of the mucosal membrane. The increased intracellular Na^+ concentrations would then stimulate greater Na^+ pump activity which would be reflected in an increase in the unidirectional J_{MS} of Na^+ and SCC, which is shown in the data (figure and table).

ADH stimulates only the J_{MS} of Na^+, therefore, it is possible that it can effect its stimulation of acting directly on the Na^+ pump located in the serosal membrane. If ADH directly stimulates a Na^+ pump located in the serosal membrane, this could lower intracellular Na^+ concentration and therefore, indirectly could increase the permeability of the mucosal membrane to Na^+ as suggested by Biber[9] and Lewis and Diamond[10]. The data however, does not allow me to distinguish between the alternatives of ADH acting through adenyl cyclase or by its direct action on a Na^+ pump.



A typical experiment showing the stimulation of transmural potential difference and short-circuit current by the addition of ADH to the serosal bathing medium.

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Do free-living songbirds habituate to species-specific alarm-calls?[1]

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Summary. Free-living birds adapt to constantly repeated species-specific alarm-calls, despite the variable environmental situation.

When chaffinches (*Fringilla coelebs* L.) are played back copies of their 'pink' alarm calls in the wild, they respond to these with the 'pink' alarm call, males sometimes with 'huid' or 'rülsch' calls mixed with the 'pink' calls. Occasionally single aerial enemy or flight calls are intermingled with these[3,4]. The birds approach the source of the sound, changing their position frequently, erecting the head feathers and jerking their tails. It has also been observed that some birds answer but remain perfectly still; others show a high degree of locomotory activity but do not call[5]. These and other behaviour patterns associated with alarm can also be released in the laboratory. If chaffinches are played back the 'pink' alarm call repeatedly over a long period of time under almost constant laboratory conditions, however, after some time an almost complete reduction of response can be recorded[6]. This behaviour is based on a learning process termed 'long-term habituation'[7-9].

These findings raise the question whether such habituation processes are also possible under the unfore seen and continuous changes of the natural environment. In order to test this, 15 breeding chaffinch pairs were played the 'pink' alarm call every morning in the center of their territories. The acoustical response of the birds was recorded on a tape recorder, the non-acoustical responses for 5 min following stimulus presentation by written notes. If the responsiveness of a pair, measured by the number of alarm calls per day, is compared from day to day, it emerges that the animal respond less and less strongly to the stimulus on succeeding days (figures 1 and 2).

This reduction of response, however, is not continual but can be reversed in correlation to occurrences taking place during the experimental period. The first of these are associated with breeding biology: nest building, the start of

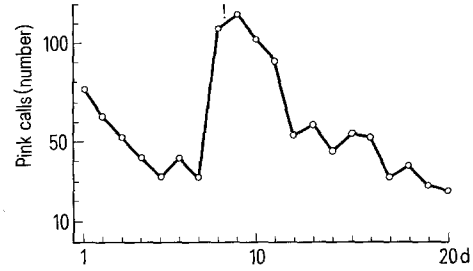

Fig. 1. Example for habituation to species-specific alarm calls in the chaffinch *Fringilla coelebs* in the wild: the 'pink' alarm call model which is presented once daily results in a gradual reduction of response. Ordinate: number of alarm calls given by a pair in the 5 min following stimulation. Abscissa: sequential days (d). The rapid increase in response on days 8 and 9 is synchronous with the hatching of the young (!).