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α-ADRENERGIC INHIBITION OF Na^+ TRANSPORT: THE INTERACTION OF VASOPRESSIN AND 3′,5′-AMP

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SUMMARY

It has been proposed that α-adrenergic inhibition of Na^+ transport across isolated frog skin is mediated by a decrease in adenyl cyclase activity and a resultant decrease in 3′,5′-AMP synthesis. To test this hypothesis the interaction of phenylephrine, predominantly an α-adrenergic substance, with vasopressin and 3′,5′-AMP was studied in the presence of propranalol, a β-blocker. The short-circuit current (I_s) technique was used. Phenoxybenzamine, an α-adrenergic blocking agent, prevented the phenylephrine-induced decrease in I_s. Dose-response studies indicated the minimum and maximum effective concentrations to be approx. 1 and 100 μM, respectively. A submaximal concentration of phenylephrine (2.5 μM) decreased I_s in the presence of a low vasopressin concentration (1 munit/ml), but the effect was prevented by a higher vasopressin concentration (100 munits/ml). The higher vasopressin concentration did not prevent an I_s decrease by the maximal effective phenylephrine concentration (0.1 mM). These findings suggest that vasopressin and α-adrenergic stimulation affect the same Na^+ transport regulatory system. In the presence of 0.1 mM phenylephrine, vasopressin did not alter I_s. However, a concentration of 3′,5′-AMP which was equipotent or less increased I_s in the presence of the same phenylephrine concentration. The findings are compatible with the hypothesis that α-adrenergic stimulation decreases adenyl cyclase activity and 3′,5′-AMP synthesis in the frog-skin epidermis which in turn mediates a decrease in Na^+ permeability and net Na^+ transport.

INTRODUCTION

Catecholamines and adrenergic-blocking agents produced changes in frog skin Na^+ transport, *in vivo* and *in vitro*[1,2], which indicated response to both α- and β-adrenergic stimulation. In more detailed isolated skin studies, α-adrenergic stimulation was found to decrease net Na^+ flux and short-circuit current (I_s) to an equivalent degree. Kinetic studies during α-adrenergic stimulation demonstrated a decrease in rate coefficient for entry into the skin-transporting compartment but no change in the rate coefficient presumed to be related to active transport. In contrast, β-adrenergic stimulation produced an equal increase in Na^+ influx and outflux with no change in net flux[3,4]. The results suggested opposing effects of α- and β-adrenergic stimulation on Na^+ permeability, although on different pathways for Na^+ movement, *i.e.*, α-

stimulation decreases Na^+ permeability of the epidermal pathways for active transport and β-stimulation increases permeability to Na^+ *via* another pathway. Opposing change in adenyl cyclase activity and resultant change in the tissue levels of 3',5'-AMP were proposed as a possible mechanism of the opposing alterations in Na^+ permeability produced by α- and β-stimulation[3,4].

There is evidence in support of a portion of this hypothesis. Catecholamines increase frog-skin 3',5'-AMP levels and this is probably a β-adrenergic effect[5]. Living-frog studies suggested that mucous gland stimulation by catecholamines is a β-effect[2] and is probably mediated by increased 3',5'-AMP tissue levels[6]. Thus, this mucous gland effect may be the mechanism of the increase in Na^+ permeability noted in the isolated skin studies cited above.

The present study was performed as a partial test of the other portion of the hypothesis, *i.e.*, that the decrease in epidermal Na^+ transport produced by α-stimulation is mediated by a decrease in 3',5'-AMP synthesis. α-Stimulation was produced with phenylephrine in the presence of propranalol, a β-blocking agent. A dose–response relationship was determined with the I_s technique. The capability of α-stimulation to decrease I_s following the administration of vasopressin and 3',5'-AMP was evaluated. The ability of vasopressin and 3',5'-AMP to increase I_s in the presence of α-stimulation was also tested. The results were interpreted according to the schema used by HANDLER *et al.*[7] in the evaluation of possible inhibitors of the vasopressin-responsive adenyl cyclase system in toad bladder. The studies support the hypothesis that α-adrenergic stimulation decreases Na^+ transport by decreasing 3',5'-AMP synthesis.

METHODS

Animals

In most studies, unfed 40–60 g male and female frogs (*Rana pipiens*) were used. They were kept in large bins with running tap water.

Chemicals

All compounds used to prepare electrolyte solutions were of AR grade. The following drugs were used: L-phenylephrine·HCl (Sigma) in modified Ringer's solution (see below) prepared 1–3 min before administration; propranalol (Ayerst) in Ringer's solution; phenoxybenzamine (Smith, Kline and French) in Ringer's solution; vasopressin (Pitressin, Park-Davis); 3',5'-AMP (Sigma) neutralized with $NaHCO_3$. All drugs were placed in the inside bathing medium. All except vasopressin were of a concentration necessary for the administration of the desired quantity in 0.1 ml. Propranalol was always administered 30 min prior to phenylephrine (except as shown in Table IB) to achieve a concentration of 8.5 μM. Phenoxybenzamine, final concentration of 6 μM, was administered 6 min before phenylephrine. These concentrations of adrenergic blockers were the maximum shown not to influence I_s when given alone.

Measurement of Na^+ flux

Isolated abdominal skin studies were conducted by the method of USSING AND ZERAHN[8], using the automatic recording device described by CAMPBELL *et al.*[9]. A pair of conical Lucite hemi-chambers, 23 and 20 ml in volume with a skin area of 7.0 cm^2,

were used for all flux studies. Unidirectional $^{22}Na^+$ flux measurements were performed in the usual manner by removing samples from the two fluid compartments. Simultaneous bidirectional studies were done with $^{22}Na^+$ and $^{24}Na^+$. The electrolyte solution bathing the skin, a modified Ringer's solution, had the following composition: 110 mM NaCl; 10 mM KCl; 1.3 mM NaH_2PO_4; 4 mM $NaHCO_3$. The solution was aerated and adjusted to pH 8.1. During the flux experiments, the solution in each chamber was mixed and oxygenated with moisturized air. Two pieces of abdominal skin from the same animal were used in paired studies as previously described[4].

Counting procedure

$^{22}Na^+$ and $^{24}Na^+$ were counted in a well scintillation counter. In double-labeling experiments, $^{22}Na^+$ was counted after allowing 4 weeks for decay of $^{24}Na^+$.

Electrical measurements

For skin potential difference and I_s measurements, calomel half-cells were connected by Ringer–agar bridges to the skin chambers. With no skin between the chambers, potential difference values were 1 mV or less. The skin was continuously short-circuited except that every 6 min the open-circuit skin potential difference was determined for 30 sec.

To compare I_s to ion flux, I_s was expressed as the amount of positive charge in cation equivalents, that moved inward across 1 cm^2 of skin per h. It is designated as the short-circuit current equivalent. The percent change in I_s was calculated by using the value at some time t_0 as a reference point, *i.e.*, $\Delta\ \%\ I_s = (I_s$ at time t / I_s at time $t_0) \times 100\ \% - 100\ \%$. In paired skin studies, $\Delta\ \%\ I_s$ was calculated for each skin at time t and the difference of the experimental (E) and control (C) skins in each pair denoted as $\Delta\ \%\ I_s$(E − C).

RESULTS

Effects of phenylephrine and adrenergic-blocking agents

In preliminary studies, phenylephrine (in the range of 10–100 μM) produced a decrease in I_s which was similar in magnitude and time-course to previous findings with α-adrenergic stimulation obtained with epinephrine and a β-blocking agent[4]. The I_s change began 3–5 min after drug administration and reached a maximum at approx. 30 min. Occasionally, a slight increase in I_s occurred for the first 3–6 min of the response.

In paired skin studies, the presence of the β-adrenergic blocking agent, propranalol, may have accentuated the decrease in I_s produced by phenylephrine (Table IA). At 1.0 h the $\Delta\ \%\ I_s$ was significantly lower than in the skin which received phenylephrine alone ($P = 0.05$). This finding suggests some β-adrenergic effect of phenylephrine (see DISCUSSION). Therefore, propranalol was used with phenylephrine in all subsequent experiments. Phenylephrine did not produce a decrease in I_s in the presence of phenoxybenzamine, an α-blocker. The $\Delta\ \%\ I_s$ in skins receiving the catecholamine *plus* the α-adrenergic blocker was not significantly different from the control which received blocking agents only (Table IB).

In preliminary experiments of dose–response relationships, it was found that decreases in I_s with relatively large concentrations of phenylephrine were not re-

TABLE I

EFFECT OF ADRENERGIC BLOCKING AGENTS ON THE CHANGE IN I_s (Δ% I_s(E − C)) PRODUCED BY PHENYLEPHRINE IN PAIRED SKINS

Phenylephrine placed into inside chamber 1.0 h after mounting skin. See METHODS for dosage and time of administration of adrenergic blocking agents. Values given are the mean ± S.E. of difference in control (C) and experimental (E) paired skin halves for the number (n) of experiments shown. Results were analyzed statistically as paired data. See METHODS for mode of calculation of Δ% I_s(E − C).

	Adrenergic blocking agent	*Phenylephrine concn.* (μM)	n	Δ% I_s(E − C) *after phenylephrine*	
				0.5 h	*1.0 h*
A. Control (C)	None	50	9	−6.5 ± 4.4*	−8.5 ± 4.0**
Experimental (E)	Propranalol	50			
B. Control (C)	Propranalol + phenoxybenzamine	None	7	+3.6 ± 0.5*	−3.1 ± 7.1*
Experimental (E)	Propranalol + phenoxybenzamine	100			

* Not significant.
** $P = 0.05$.

producible in the same skin, even if the solution in the inside chamber was replaced up to 3 times with Ringer's solution containing no catecholamine and if the I_s had returned to approximately control value. A return of roughly comparable responsiveness required as much as 2–3 h. For this reason, each concentration tested in the dose–response study in Fig. 1 represents a different series of skin pairs. The magnitude of

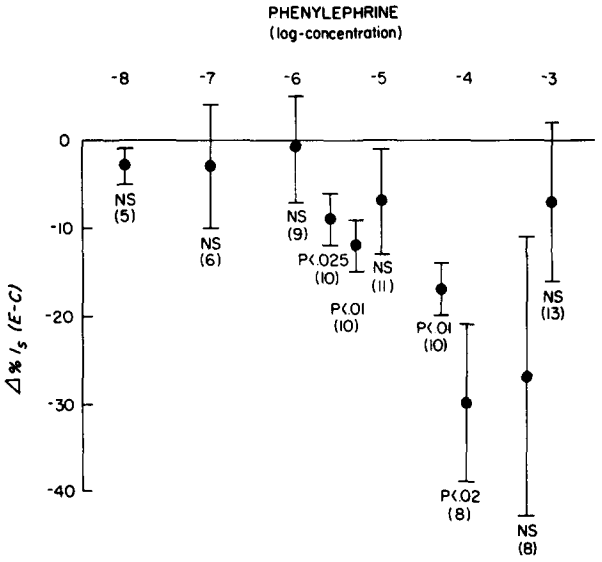

Fig. 1. Dose–response relationship of effect of phenylephrine on I_s in paired skins Δ % I_s (E − C)). Phenylephrine was placed in inside chamber of experimental skins (E) 1.0 h after mounting. Both experimental (E) and control (C) skins received propranalol. See METHODS for dosage and time of administration of blocking agent and for mode of calculation of Δ % I_s (E − C). Number of experiments for each concentration shown in parentheses. Results analyzed statistically as paired data. P values greater than 0.05 considered not significant (NS).

decrease in I_s was quite variable at any given concentration. However, the minimum and maximum effective concentrations seem to be in the range of 1–100 μM. Results at concentrations above 0.1 mM are of particular interest. In several experiments, phenylephrine produced a definite and sustained increase in I_s which never occurred at 0.1 mM or below. This occasional increase in I_s probably explains, at least in part, the decrease in mean response. The highest concentration of phenylephrine used (1 mM) was shown not to change the pH of the bathing solution.

Effects of phenylephrine, vasopressin and 3',5'-AMP

In the presence of a relatively low concentration of vasopressin (1 munit/ml) a submaximal concentration of phenylephrine (2.5 μM) produced a significant decrease in I_s (Table IIA). In the presence of the higher vasopressin concentration (100 munits/ml, Table IIB) the submaximal concentration of phenylephrine did not significantly decrease I_s. However, the maximally effective concentration of the catecholamine (0.1 mM) clearly decreased I_s.

TABLE II

CHANGE IN I_s (Δ % I_s) AFTER PHENYLEPHRINE IN THE PRESENCE OF VASOPRESSIN AND 3',5'-AMP

Propranalol and vasopressin (or 3',5'-AMP) placed in inside chamber at 1.0 and phenylephrine at 1.5 h after mounting skin. Values in each group represent the mean ± S.E. for the number (n) of experiments indicated. See METHODS for mode of calculation of Δ % I_s.

Stimulatory substance	*Phenylephrine concn. (μM)*	*n*	*Δ % I_s*		*P**
			0.5 h after stimulation	*0.5 h after phenylephrine*	
A. Vasopressin (1 munit/ml)	None	11	+29 ± 4.1	− 1 ± 4.4	
	2.5	13	+25 ± 7.3	−18 ± 3.2	<0.005
B. Vasopressin (100 munits/ml)	None	12	+38 ± 15	− 3 ± 5.9	
	2.5	13	+36 ± 7.0	− 3 ± 5.0	Not significant
	100	11	+44 ± 11	−41 ± 4.9	<0.001
C. 3',5'-AMP (5 mM)	None	7	+16 ± 7.2	− 1 ± 2.8	
	2.5	8	+12 ± 14	− 1 ± 4.0	Not significant
	100	7	+22 ± 7.4	−16 ± 3.8	<0.02

* Refers to t test comparing change after phenylephrine to change in control group which received the same concentrations of vasopressin or 3',5'-AMP but not phenylephrine. Values greater than 0.05 considered not significant.

The mean Δ % I_s after 3',5'-AMP administration for all experiments was 17 ± 5.8, $n = 22$ (Table IIC). This was a smaller increase of I_s but statistically not significantly different from the mean response to 1 munit/ml vasopressin (27 ± 4.3). However, the submaximal concentration of phenylephrine (2.5 μM) produced no significant decrease in Δ % I_s in the presence of 3',5'-AMP in contrast to its effect in the presence of the low vasopressin concentration.

In the studies in Fig. 2, the frog skin was exposed to phenylephrine and vasopressin or 3',5'-AMP simultaneously. There was no evidence of an effect of vasopressin (1 munit/ml) on I_s in the presence of phenylephrine. However, the Δ % I_s after phenylephrine and 3',5'-AMP was significantly higher than after phenylephrine alone. In

other words 3',5'-AMP relatively increased Δ % I_s in the presence of phenylephrine, yet a concentration of vasopressin which was at least equipotent (Table IIA and IIC) produced no change.

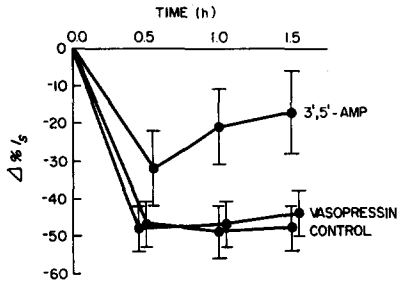

Fig. 2. Effect of vasopressin and 3',5'-AMP on I_s (Δ % I_s) in the presence of phenylephrine. In all experiments, 0.1 mM phenylephrine was placed into inside chamber at time zero, which was 1 h after mounting skins. Control series (N = 10) received phenylephrine alone. In the other two series (N = 8 for each) vasopressin (1 munit/ml) or 3',5'-AMP (5 mM) was also placed in the inside chamber at time zero. Values are the mean ± S.E. for each series. The Δ % I_s in the 3',5'-AMP series was significantly higher than control at 1.0 and 1.5 h after substances administered ($P <$ 0.025 and $<$ 0.02, respectively).

Net Na^+ flux and the short-circuit current equivalent were approximately equal in the presence of relatively high concentrations of phenylephrine *plus* vasopressin (Table IIIA). This equality is also probable in the case of concomitant administration of phenylephrine and 3',5'-AMP (Table IIIB), for the mean Na^+ influx was only 6 % higher than the short-circuit current equivalent. This difference is in the expected range of Na^+ outflux[4].

TABLE III

Na^+ FLUX AND I_s IN THE PRESENCE OF PHENYLEPHRINE *plus* VASOPRESSIN OR 3',5'-AMP

All values are mean ±S.E. for the number of experiments (n) expressed as μequiv/cm^2 per h. Bidirectional $^{22}Na^+$ and $^{24}Na^+$ fluxes in A performed over 1.0-h period after phenylephrine on skins shown in Table IIB. Unidirectional $^{22}Na^+$ fluxes in B performed over 1.0-h period after phenylephrine on skins shown in Table IIC.

	N	*Na⁺ influx*	*Na⁺ outflux*	*Net flux*	*Short-circuit current equivalent*
A. Phenylephrine (0.1 mM) + vasopressin (100 munits/ml)	8	1.67 ± 0.29	0.040 ± 0.007	1.63 ± 0.30	1.59 ± 0.29
B. Phenylephrine (0.1 mM) + 3',5'-AMP (5 mM)	7	1.42 ± 0.18	—	—	1.34 ± 0.20

DISCUSSION

Phenylephrine was chosen for these studies since it produces predominately α-adrenergic stimulation[10]. However, the studies in Table IA suggest that phenyl-

ephrine does does exert some β-adrenergic effect on ion transport. The decrease in I_s was probably accentuated by addition of a β-adrenergic-blocking agent. β-Adrenergic stimulation increases I_s and would be expected to partially inhibit a predominant decrease in I_s produced by α-stimulation[4]. This opposing β-effect would then be unmasked by β-blockade.

The prevention of a decrease in I_s after phenylephrine by phenoxybenzamine, an α-blocking agent (Table IB), indicates that the decrease is the result of α-adrenergic stimulation.

The degree of variation in magnitude of decrease in I_s at the various concentrations (Fig. 1) does not allow an interpretation of the nature of the dose–response curve. The increase, rather than decrease, in I_s occasionally observed at concentrations above 0.1 mM was of particular interest. As described above, this is the result expected from β-adrenergic stimulation. Propranalol is a competitive blocking agent[10] and at these high concentrations may have been partially displaced from β-receptor sites by phenylephrine. There was no evidence that this change in response was the result of pH alteration in the internal medium produced by the phenylephrine·HCl.

The effect of phenylephrine on I_s in the presence of vasopressin (Table IIA and IIB) is compatible with some type of competition in the same regulatory system for Na^+ transport. A submaximally effective concentration of phenylephrine decreased I_s in the presence of a low concentration of vasopressin but not when the concentration of vasopressin was higher. This inhibition of the phenylephrine effect by the higher vasopressin concentration was overcome by the maximal effective concentration of phenylephrine. These findings are in contrast to the effects of Ca^{2+} in the outside bathing medium[11,12]. Increased Ca^{2+} concentration produces changes in Na^+ transport which are similar to α-stimulation, *i.e.*, an equivalent decrease in I_s and net Na^+ transport and concomitant decrease in the rate coefficient for Na^+ entry into the skin transporting compartment. However, vasopressin at low or high concentration does not inhibit or alter the sensitivity of the skin to Ca^{2+} concentration change, indicating different mechanisms of action of these two substances.

It was suggested that a decrease in tissue 3',5'-AMP concentration may be the mode of mediation of the decrease in Na^+ transport produced by α-stimulation[3,4]. This is opposite to the proposed mechanism of vasopressin stimulation of Na^+ transport in toad bladder[13] and frog skin[14]. If α-stimulation by phenylephrine acted at the step of vasopressin responsive synthesis of 3',5'-AMP or prior to this step, it should lead to inhibition of the vasopressin effect. However, phenylephrine should not prevent an effect of an equipotent concentration of 3',5'-AMP on Na^+ transport. In other words, α-adrenergic inhibition of synthesis of the presumed mediator of vasopressin's action, 3',5'-AMP should not prevent the action of an extracellular source of the nucleotide. The results in Fig. 2 show this to be the case. Vasopressin (1 munit/ml) had no effect on I_s in the presence of phenylephrine. However, an equipotent or less potent concentration of 3',5'-AMP produced a relative increase in I_s in the presence of the same phenylephrine concentration. These findings support the hypothesis that the decrease in Na^+ transport produced by α-adrenergic stimulation is mediated by a decrease in epidermal 3',5'-AMP synthesis. HANDLER *et al.*[7] have made similar findings in studies of the effects of α-adrenergic stimulation on vasopressin-induced increase in water permeability in toad bladder. They also studied the interaction of theophylline and α-stimulation. Theophylline was not used in the studies presented here as it

increases Cl^- transport[15], presumably by stimulating mucous glands[6]. This would complicate interpretation of I_s data.

Interpretation of the experiments on the interaction of phenylephrine, vasopressin and 3',5'-AMP assumes that the I_s change is a measure of net Na^+ transport change under these conditions. α-Adrenergic stimulation, vasopressin, and 3',5'-AMP have each been shown to produce an equal alteration in net Na^+ transport and the short-circuit current equivalent[4,8,14]. The results in Table III indicate that this is also the case for α-stimulation concomitant with vasopressin or 3',5'-AMP administration.

The original observations that indicated opposing effects of α- and β-adrenergic stimulation on Na^+ permeability[3,4] lead to the proposal that these transport changes might be mediated by opposing effects on the adenyl cyclase system. They suggested that α-stimulation altered epidermal transport and β-stimulation altered mucous gland transport. BASTIDE AND JARD[16] have shown that concentrations of norepinephrine (0.01–1 μM) lower than those used in the studies mentioned above mimicked the effect of vasopressin by increasing epidermal Na^+ transport only. They presented evidence that this increase in net Na^+ transport was a β-adrenergic effect and was mediated by 3',5'-AMP. At the low concentration, there was no evidence of Cl^- transport change. This suggests absence of mucous gland stimulation by the low concentration of the catecholamine since increase of Cl^- transport is considered a result of glandular stimulation[4,6,17]. Thus, it is likely that the mucous gland β-receptors (affecting Na^+ and Cl^- transport) are less sensitive to catecholamines than the epidermal receptor under the conditions used. α-Adrenergic effects were not noted and also seem to require higher catecholamine concentrations. Still, there is no apparent explanation of the absence of evidence for epidermal β-adrenergic stimulation at high catecholamine concentration in the studies performed in this laboratory[4]. Fig. 3 diagramatically describes these postulated modes and sites of action of catecholamines on Na^+ transport across frog skin.

It may be that opposing effects of α- and β-adrenergic stimulation on the adenyl cyclase system and resultant opposing effects on membrane permeability is a general

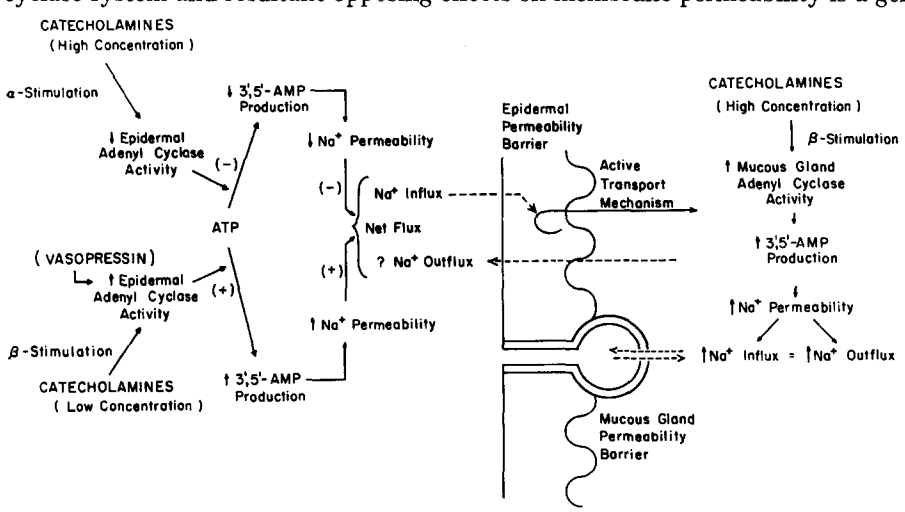

Fig. 3. Postulated mode of action of catecholamines on Na^+ transport in frog skin.

phenomenon produced by catecholamines in many other tissues. For instance, the two classes of stimuli are opposed in action on smooth muscle contraction[10] and pancreatic insulin release[18]. The latter phenomenon does appear to be the result of opposing effects on adenyl cyclase activity[19].

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