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Catecholamine stimulation of ion transport in the toad urinary bladder

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We have observed that serosal catecholamines increase the amplitude of the short-circuit current (I_{sc}) in the toad urinary bladder by as much as 450%. Chemical sympathectomy with 10^{-6} M 6-hydroxydopamine and the sympathomimetic effects of 10^{-5} M tyramine indicate a reservoir of amines in the serosal stroma of the tissue. The urinary epithelium from the toad responds to six adrenoceptor agonists: (−)-epinephrine, (−)-norepinephrine, (−)-phenylephrine, clonidine, methoxamine and oxymetazoline. The α_2-adrenoceptor agonist clonidine is most potent for stimulating I_{sc}. Some agonists were found to diminish I_{sc}. Apparently this is related to a simultaneous increase in the transepithelial flux of both chloride and sodium. The I_{sc} response to the catecholamines is also inhibited by several adrenoceptor antagonists. The α_2-adrenoceptor antagonist yohimbine is more effective than the α_1-antagonist prazosin for blocking the stimulation of epithelial transport. As a result of these studies, we have tentatively classified the serosal adrenoceptor of the toad urinary bladder as α_2.

Introduction

The basic mechanisms underlying sodium transport through the toad urinary bladder epithelium are described by the Koefoed-Johnsen–Ussing model [1]. Cells of the epithelium are polarized: the apical membrane is permselective for Na^+ while the basolateral is permeable to K^+. Na^+ diffuses down an electrochemical gradient from the luminal solution into the cytoplasmic space. Once near the basolateral membrane, the Na^+ is actively pumped out of the cell in exchange for K^+ by a Na^+/K^+-ATPase. K^+ accumulations in the cell diffuse down an electrochemical gradient across the basolateral membrane. One electrical consequence of Na^+ transport is an apical to basolateral cation current. Normally, this electrolyte flux creates a potential difference across the 'open-circuit' urinary bladder (lumen negative to serosa). The magnitudes of the short-circuit current and transepithelial voltage are increased by aldosterone and antidiuretic hormone [2]. Since adrenohypophysectomized toads do not experience complete natriuresis or diuresis [3], an additional mechanism appears to regulate salt balance. An adrenoceptor-mediated mechanism may complement vasopressin and aldosterone in the amphibian urinary system [4].

The effects of catecholamines on sodium flux and short-circuit current in frog skin are well established [5–9]. Leaf et al. [10] reported that epinephrine had no significant effect on the short-circuit current of urinary bladders from the toad *Bufo marinus*. Later, Handler et al. [11] found that norepinephrine simultaneously inhibited the

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vasopressin-mediated hydro-osmotic response of the tissue while stimulating short-circuit current by about 20%. Wood and Tomlinson [12] reported that micromolar concentrations of either catecholamine increased the short-circuit current (I_{sc}) of the bladder by as much as 500%. Using isotopic measurements, increases in the unidirectional flux of both Na^+ and Cl^- appeared to be mediated by an α-adrenergic mechanism. The α-adrenoceptor agonist, isoproterenol, had no effect on the I_{sc}. Using a radioimmune assay, Turtle and Kipnis [13] concluded that catecholamines inhibit the bladder's hydro-osmotic response to vasopressin by blocking cAMP production. Wong et al. [14], on the other hand, found that catecholamines increased cAMP. In light of recent developments concerning the nature of adrenoceptor-second messenger coupling [15–18], the effect of catecholamines on epithelial transport in the toad urinary bladder remains to be clarified.

In this article, we present the results of our investigations on the in vitro response of the toad bladder to several α-adrenergic agonists and antagonists. We have found that the I_{sc} is altered by various catecholamines and several of their analogs. Using tyramine and 6-hydroxydopamine, we have produced physiological evidence indicating the presence of sympathetic neurotransmitters in the tissue. The magnitude of the catecholamine effect is influenced by the spontaneous rate of sodium transport in the tissue and the presence of chloride ions in the mucosal bathing solution. If Cl^- in the apical Ringer's is replaced with an impermeant anion, the tissue's response to catecholamines is enhanced. This observation is consistent with the notion that the adrenoceptors may stimulate the unidirectional flux of both Na^+ and Cl^- [12]. Although the response of the bladder to sympathomimetic agents is complicated by Cl^- permeability, we tentatively consider the adrenoceptors to be α_2.

Materials and Methods

Bufo marinus of Mexican origin were purchased from either Riverside Biological (Somerset, WI) or Nasco (Ft. Garrison, WI) and maintained on Sanicel moistened with 10% amphibian Ringer's. Except during the summer, toads were not fed or kept for more than 1 month. During July and August, they were given minced liver or crickets every 3 weeks.

Some urinary bladders were removed from doubly pithed toads and mounted vertically in an Ussing apparatus that isolated the hemibladders into paired control and experimental portions with surface areas of 3.4 cm^2. The surfaces of the tissue were bathed continuously with 4.0 ml of stirred Ringer's solution. Apical and serosal solutions were changed simultaneously with two 5 ml syringes. In each experiment, n indicates the number of hemibladders with common control and experimental portions. In other cases, the tissues were mounted horizontally in a modified chamber exposing a 3.14 cm^2 area of tissue as previously described by Higgins et al. [19]. The spontaneous transepithelial voltage (V_t) was measured with calomel half-cell electrodes in saturated KCl connected to the Ussing chamber by 5% NaCl/agar bridges. An electrical current, applied either manually or automatically through Ag/AgCl electrodes, was measured with a digital multimeter to evaluate the magnitude of I_{sc} for 3.4 cm^2. A VCC 600 voltage clamp (Physiologic Instruments; Houston, TX) was used as a square-wave pulse generator for estimating tissue resistance. Transepithelial resistance (R_t) was calculated as k $\Omega \cdot cm^2$ and transepithelial conductance (G_t) as mS/cm^2. The initial or basal I_{sc} was averaged over 15-min intervals for 1 h after the tissue received three washings with isotonic Ringer's. For dose-response studies, percent maximum I_{sc} was calculated by:

$$\%I_{sc}^{max} = \frac{I_{sc} - I_{sc}^{basal}}{I_{sc}^{max} - I_{sc}^{basal}} \times 10^2$$

In the formula, I_{sc} values are responses to drug at particular concentration, I_{sc}^{max} is the maximum I_{sc} recorded and I_{sc}^{basal} is the initial I_{sc}. All values are reported as $\bar{X} \pm$ S.E. of $\mu A/3.4\ cm^2$. Probit analysis and linear regression [20] were used to determine the 'best-fit' dose-response curve and median effective concentration (EC_{50}) for each agonist and antagonist. Statistical significance (P) for accepting a null hypothesis (H_0) was determined by the two-tailed Student's t-test. Significantly different values are indicated by an asterisk.

The bladder preparations were bathed in Ringer's with the following ion composition (mM): 109 Na^+, 2.5 K^+, 0.95 Ca^{2+}, 110.9 Cl^-, 2.5 HCO_3^- and 5 glucose (osmolarity, 215 mosM). For a Cl^--free Ringer's solution, gluconate compounds were substituted isotonically for Cl^- salts. 5 mM calcium gluconate was used to maintain free calcium levels. The solutions were bubbled with 100% O_2 and the final pH adjusted with 0.01 M HCl to range between 7.4 and 7.6. A small junctional potential (1.3 ± 0.3 mV) developed between the NaCl/agar bridges and Cl-free Ringer's solutions. Since there was no measurable resistance difference between NaCl and gluconate Ringer's solutions, this potential did not introduce significant artifacts into V_t or I_{sc} estimates.

(−)-Norepinephrine, (−)-epinephrine, (±)-isoproterenol, tyramine, 6-hydroxydopamine, propranolol, yohimbine, (−)-phenylephrine and ascorbic acid were purchased from Sigma (St. Louis, MO). Clonidine-HCl, methoxamine-HCl and oxymetazoline were donated by Boehringer Ingelheim (New York, NY), Burroughs Wellcome (Research Triangle, NC) and Shering Corporation (Bloomfield, NJ), respectively. Amiloride was a gift of Merck, Sharp and Dohme (West Point, PA). Phentolamine was given by Ciba Pharmaceutical (Summit, NJ) while Pfizer, Inc. (Brooklyn, NY) donated the prazosin. All adrenoceptor agonists and antagonists were prepared fresh daily in Ringer's containing 100 mg/l ascorbic acid. 6-Hydroxydopamine was dissolved in nitrogen-saturated Ringer's and applied immediately to the tissue. Prazosin and yohimbine were initially solubilized in 1 ml of ethanol before dissolving in Ringer's. With the exception of amiloride, which was added to the tissue only through mucosal solutions, all drugs were applied unilaterally to the serosa of the bladders.

All experiments were conducted at room temperature (21–24°C).

Results

Effect of epinephrine and norepinephrine on the electrophysiology of toad urinary bladder

Catecholamine stimulation of I_{sc} and Na^+-transport

When 10^{-5} M epinephrine is applied to the serosa of the toad urinary bladder (Fig. 1A), the I_{sc} increases from 10.8 ± 2.4 to 32.8 ± 5.9 μA ($P < 0.001$, $N = 5$) while R_t declines from 3.43 ± 0.64 to 3.05 ± 0.54 kΩ · cm² ($\Delta R_t = 0.38 \pm 0.12$ kΩ · cm²; $P < 0.01$). Since I_{sc} measurements taken in the first 15-min interval contain artifacts from changing the solutions, these were disregarded. The tissue responds to epinephrine with a gradual increase in I_{sc} that reaches a stable, maximum value of 30 μA within 1 h. Since the I_{sc} value represents the net ionic flux across the bladder, a 10^{-4} M concentration of amiloride was added to the mucosal solutions to selectively inhibit transepithelial sodium flux [21]. As the catecholamine enhancement of I_{sc} is blocked by amiloride (Fig. 1A), R_t increases to 9.6 ± 0.7 kΩ · cm² ($P < 0.001$; $N = 5$). If amiloride is applied first, then epinephrine, as illustrated in Fig. 1B, there is no significant increase in I_{sc} following exposure to the catecholamine. Failure of the catecholamine to alter I_{sc} in the presence of amiloride indicates that stimulation of the I_{sc} by epinephrine represents an increase in sodium transport through the urinary bladder.

To determine whether the action of catecholamines alters transcellular or paracellular electrical properties, the relationships between V_t, R_t (G_t) and I_{sc} were examined. When toad bladders were first mounted in the horizontal Ussing chamber and bathed with NaCl/amphibian Ringer's, we observed an inverse relationship between V_t and R_t (Fig. 2A). If plotted as a function of R_t (i.e. transepithelial conductance; $G_t = 1/R_t$), the I_{sc} increases with transepithelial conductance (Fig. 2B). In general, the $V_t - I_{sc}$ relationship appears to be linear between ±100 mV [22]. Applications of amiloride decrease I_{sc} and V_t while increasing R_t. Higgins et al. [19] have suggested that such relationships among the electrical properties of amphibian bladder tissues imply that the rate of Na^+-transport depends primarily on the conductance of the luminal cell membrane in the epithelium.

Variation in the epinephrine-response was noted between tissues. One source of variation is related to the initial spontaneous electrical properties of the bladders as described above. Bladder tissues with conductance less than 0.6 $k\Omega^{-1} \cdot cm^{-2}$ ($R_t > 1.7$ kΩ · cm²) respond to epinephrine with a greater degree of change than those with higher conduc-

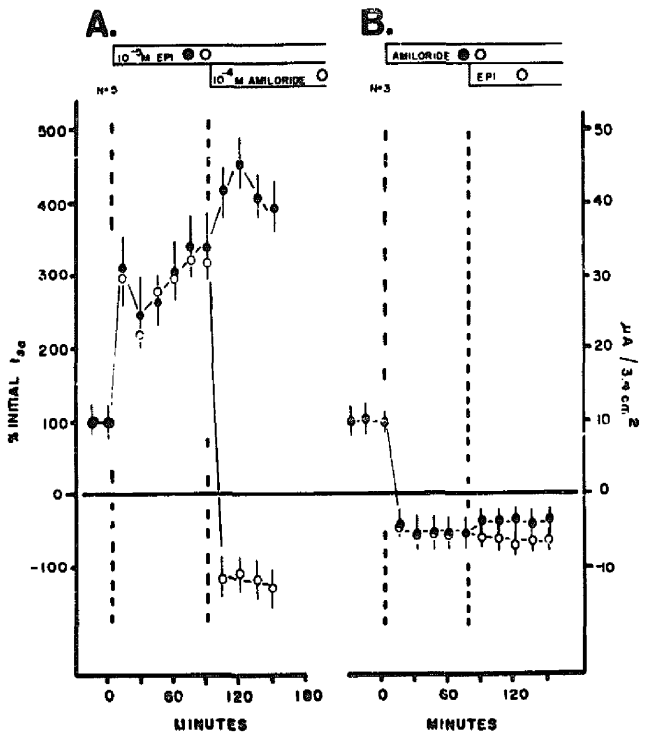

Fig. 1. Effects of 10^{-5} M epinephrine (EPI) and 10^{-4} M amiloride on the short-circuit current of the toad urinary bladder. Open or closed circles in upper bars indicate corresponding treatments of paired sections of bladder. (A) The effects of epinephrine followed by amiloride application. Values are $\bar{X} \pm$ S.E. for 5 bladders. (B) The effects of amiloride followed by epinephrine application. Values are $\bar{X} \pm$ S.E. for 3 bladders.

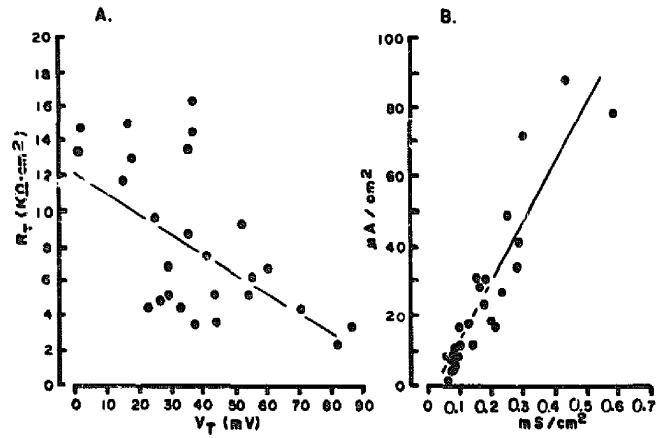

Fig. 2. Transepithelial electrical properties of the toad urinary bladder. (A) Relationship between spontaneous potential difference (V_t) and resistance (R_t) for 26 bladders. Linear regression ($y = -0.12x + 12.6$) $P < 0.01$, $r^2 = 0.317$. (B) Relationship between spontaneous transepithelial conductance (G_t) (i.e. $1/R_t$) and I_{sc}. Linear regression ($y = 170.3x - 5.2$) $P < 0.01$, $r^2 = 0.841$.

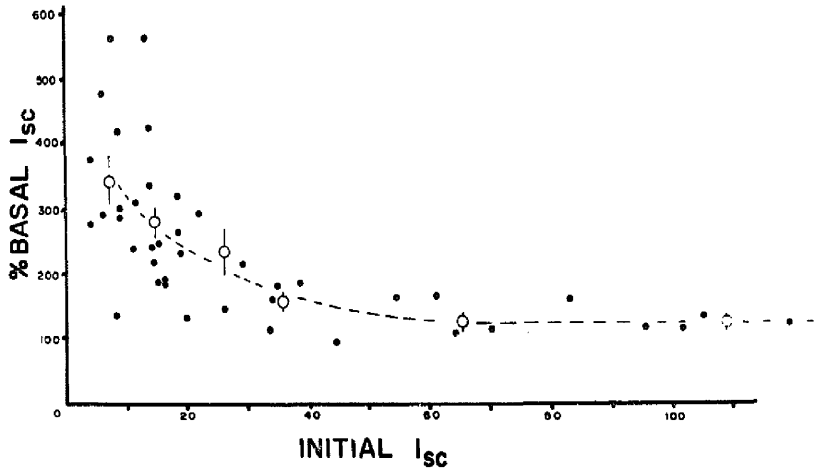

Fig. 3. Relationship between initial spontaneous I_{sc} and percent basal I_{sc} after stimulation with 10^{-5} M epinephrine. Closed circles are measurements from 41 bladders. Open circles are $\bar{X} \pm$S.E. for 10 μA intervals of I_{sc} between 0 and 40 μA. Single values are presented as the mean for I_{sc} greater than 40 and 80 μA, respectively.

tance [12]. In support of this, we found the I_{sc}-augmenting ability of epinephrine to be inversely related to the initial spontaneous I_{sc} in the bladder. Bladders with low conductance generally have a small I_{sc}. As illustrated in Fig. 3, bladders with I_{sc} below 30 μA and R_t greater than 4.5 k$\Omega \cdot$cm^2 respond to epinephrine with a larger increase in I_{sc} than those with initially high V_t or I_{sc}. Factors controlling the size of the initial I_{sc} and V_t are not known.

Effects of Cl^--free Ringer's

To explore the relationship between apical solution Cl^- concentration and catecholamine stimulation of I_{sc}, sections of tissue were bathed with Ringer's containing no Cl^- (Fig. 4). When Cl^- was present in the apical Ringer's, 10^{-4} M norepinephrine increases I_{sc} from 13.5 to 46.2 $\pm$ 6.2 μA ($P < 0.01$) while decreasing R_t from 5.1 $\pm$ 0.7 to 3.0 $\pm$ 0.6 k$\Omega \cdot$cm^2. With Cl^--free Ringer's bathing the luminal membrane and normal Ringer's bathing the serosa, the substitution of gluconate for Cl^- initially decreased I_{sc} from 23.5 $\pm$ 9.6 to 15.3 $\pm$ 4.3 μA ($P < 0.20$) while increasing R_t from 3.2 $\pm$ 1.0 to 4.0 $\pm$ 1.4 k$\Omega \cdot$cm^2 ($\Delta R_t = 0.8 \pm 0.3$ k$\Omega \cdot$cm^2; $P < 0.05$). However, the I_{sc} increased to 74.6 $\pm$ 8.0 μA ($P < 0.001$) and R_t decreased to 2.6 $\pm$ 0.8 k$\Omega \cdot$cm^2 ($P < 0.001$) when exposed to 10^{-4} M norepinephrine. In both Ringer's, I_{sc} increases were completely blocked by 10^{-5} M amiloride and R_t increased to 6.2 $\pm$ 1.1 k$\Omega \cdot$cm^2. The diminished I_{sc} response upon exposure to norepinephrine in Cl^- media supports the notion that catecholamines increase Cl^- as well as Na^+ flux through the epithelium [12].

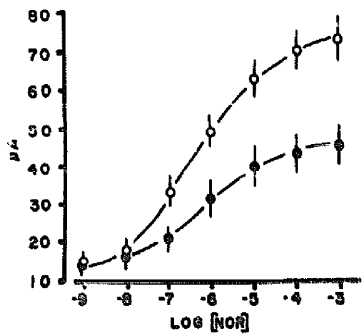

Fig. 4. I_{sc}-response of toad urinary bladder to stromal norepinephrine (NOR) in apical Ringer's with (●, $N = 11$) or without (○, $N = 12$) chloride. Serosa bathed with complete amphibian Ringer's.

Pharmacology of the catecholamine response

Effects of tyramine and 6-hydroxydopamine

The presence of an endogenous source of catecholamine within the bladder tissue was revealed through application of tyramine and 6-hydroxydopamine to the serosa of the bladder (Table I). Prior to tyramine-treatment, a solution (10^{-6} M) containing the α-adrenoceptor blocker phentolamine was added to some bladder sections. Subsequently all portions of the bladder received tyramine. Significant increases were observed when tyramine was applied in the absence of the α-adrenoceptor antagonist but phentolamine blocked tyramine-stimulation of I_{sc}. When tissues are treated with 10^{-5} M 6-hydroxydopamine, peripheral sympathetic neurons are selectively destroyed within 30 min [23,24]. Pretreatment of the toad bladder for 45 min with 10^{-6} M 6-hydroxydopamine blocked the increase in I_{sc} stimulated by tyramine (Table I). Control tissues not receiving 6-hydroxydopamine exhibited a significant increase in I_{sc} when treated with tyramine.

On examining the possibility that 6-hydroxydopamine directly blocks postsynaptic adrenoceptors, we found that norepinephrine could stimulate I_{sc} in the bladders following 6-hydroxydopamine treatment (Table II). Although the I_{sc} response is reduced, it is not eliminated by the same concentration of 6-hydroxydopamine used in the experiments described in Table I. Norepinephrine (10^{-5} M) increased significantly the bladder's I_{sc} by 10 μA in the presence of 10^{-6} M 6-hydroxydopamine, indicating that the receptor is not blocked completely by the sympathectomizing agent. Since the effects of both tyramine and 6-hydroxydopamine are expressed on presynaptic membranes, a reservoir of catecholamine occurs proximal to a synapse between sympathetic neurons and sodium transporting epithelial cells.

TABLE I

EFFECT OF TYRAMINE ON I_{sc} OF THE TOAD URINARY BLADDER

Values are means ± S.E. (μA/3.4 cm^2) in each case. * Indicates P is significant for rejection of the null hypothesis.

	Initial I_{sc}	10^{-5} M tyramine, 30 min	n	P
Pretreatment (control)	67.3 ±11.4	89.1±14.8	12	0.01 *
Phentolamine, 10^{-6} M	63.25±15.5	62.6±21.4	12	0.90
Pretreatment (control)	57.4 ± 6.2	74.2±12.1	6	0.05 *
Hydroxydopamine, 10^{-6} M	61.1 ± 5.6	53.3± 5.0	6	0.10

TABLE II

EFFECT OF 6-HYDROXYDOPAMINE ON ABILITY OF NOREPINEPHRINE TO ALTER I_{sc}

See Table I for explanation of values.

	Initial I_{sc}	10^{-5} M norepinephrine, 60 min	n	P
Pretreatment (control)	29.0±5.8	47.8±5.8	8	0.001 *
6-Hydroxydopamine, 10^{-6} M	32.4±3.5	40.3±2.7	8	0.01 *

Catecholamine dose-response

In Fig. 5A, the catecholamine dose-response of the epithelium is related to the initial spontaneous I_{sc} in Cl^--rich Ringer's. At 10^{-6} M concentrations, maximal increases in I_{sc} are seen with phenylephrine and clonidine. These compounds can double the magnitude of the initial I_{sc}. Epinephrine, norepinephrine and methoxamine are less effective for increasing I_{sc} magnitude. With all agonists except norepinephrine, inhibition of I_{sc} was observed at supramaximal concentration suggesting receptor desensitization [25]. For each agonist, the percent-response data in Fig. 5A was transformed to probit units [20] then analyzed by least-squares regression to obtain a 'best-fit' equation and median effective concentration (EC_{50}). The best-fit dose-response curves for the data in Fig. 5A are illustrated in Fig. 5B. This forms the basis for identifying adrenoceptor subtypes. Methoxamine is deleted from the scheme since it produces minimal changes in the magnitude of I_{sc}.

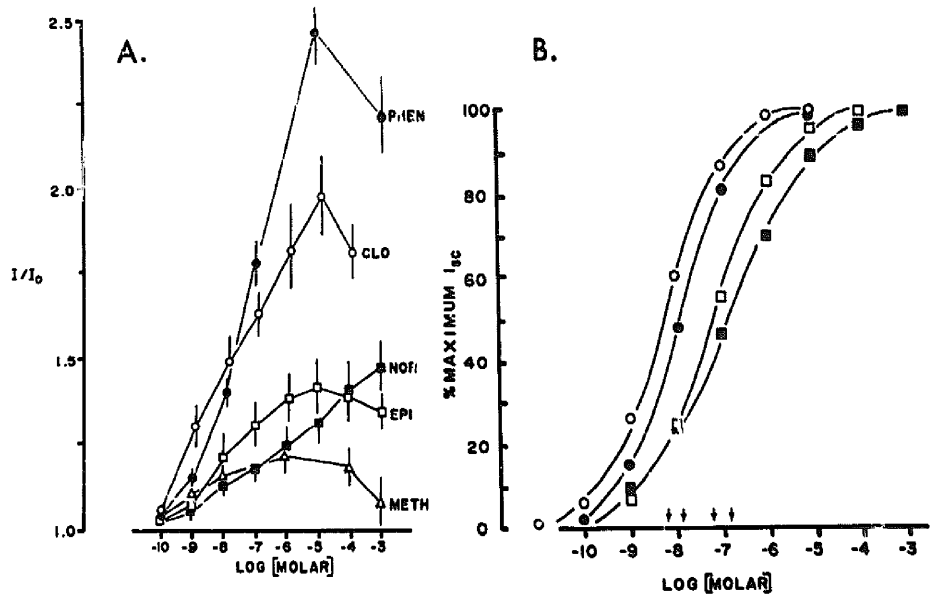

Fig. 5. (A) Response of the toad urinary bladder to α-adrenoceptor agonists. I_{sc}/I_0 is the ratio of drug-stimulated to spontaneous I_{sc}. Symbols: (○) clonidine (CLO), $N = 9$; (△) methoxamine (METH), $N = 7$; (●) phenylephrine (PHEN), $N = 9$; (□) epinephrine (EPI), $N = 8$ and (■), norepinephrine (NOR), $N = 11$. (B) Best-fit dose-response curves for catecholamines in (A) based on least-squares regression by probit transformation [20]. (○) clonidine (CLO), $r^2 = 0.997$; (●) epinephrine (EPI), $r^2 = 0.912$; (□) phenylephrine (PHEN), $r^2 = 0.740$; and (■) norepinephrine (NOR), $r^2 = 0.815$. Arrows indicate EC_{50} determined by probit analysis [20].

Based on median effective concentration (EC_{50}), the receptor affinity is clonidine > epinephrine > phenylephrine > norepinephrine, indicating that the adrenoceptor is most effectively stimulated by α_2-adrenoceptor agonists. The EC_{50} for clonidine is approximately $5 \cdot 10^{-9}$ M with maximum effects at 10^{-5} M.

Effects of α-adrenoceptor antagonists

The α-adrenoceptor blocker phentolamine can inhibit epinephrine-stimulated tissues. Tissues ($N = 4$) were initially stimulated with 10^{-5} M epinephrine then treated 60 min later with $5 \cdot 10^{-5}$ M phentolamine for 1 h. The I_{sc} declined from 41.6 ± 4.2 to 28.4 ± 2.7 μA ($P < 0.01$) in phentolamine-treated tissues. On the other hand, the I_{sc} remained at 40.8 ± 3.5 μA in untreated, control tissues. Receptor antagonism was also tested with 10^{-4} M yohimbine (Table III). In these experiments, yohimbine was applied to the tissue 45 min prior to stimulation with epinephrine. Yohimbine successfully blocked catecholamine stimulation of I_{sc}. Since clonidine, an α_2-selective adrenoceptor agonist, was most effective for increasing I_{sc} and possessed the lowest EC_{50}, it was used as a stimulus in a study of receptor antagonism by the α_1-adrenoceptor agonist prazosin. The tissues were initially bathed in complete Ringer's solutions until stable transepithelial voltages and I_{sc} were measured. Prazosin was then added to the serosal solutions to give a final concentration of 10^{-5} M.

TABLE III

EFFECT OF THE α_2-ADRENOCEPTOR ANTAGONIST YOHIMBINE ON EPINEPHRINE-STIMULATED I_{sc}

See Table I for explanation of values.

	Initial I_{sc}	10^{-5} M epinephrine, 30 min	N	P
Pretreatment				
(no yohimbine)	42.8 ± 6.5	68.2 ± 11.6	8	0.01 *
Yohimbine, 10^{-4} M	51.3 ± 5.7	50.0 ± 4.8	8	0.90

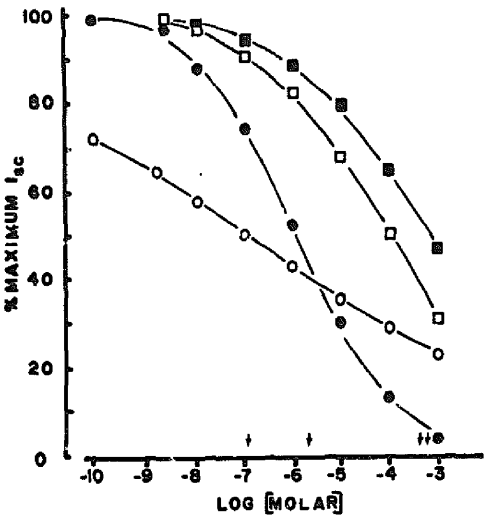

Fig. 6. Best-fit by linear regression curves of I_{sc} inhibition by adrenoceptor agonists in complete amphibian Ringer's. (●) isoproterenol, $N = 6$, $r^2 = 0.835$; (□) oxymetazoline, $N = 7$, $r^2 = 0.868$; (○) phenylephrine, $N = 9$, $r^2 = 0.826$; and (■) norepinephrine, $N = 3$, $r^2 = 0.705$. Arrows indicate IC_{50} determined by probit analysis [20].

Prazosin had no significant influence on basal I_{sc} or the ability of clonidine to stimulate Na^+-transport and I_{sc}.

Catecholamine antagonism of I_{sc}

In a few cases, bladders responded by exhibiting a decline in I_{sc} with increasing catecholamine dosage. This phenomenon was observed in Cl^--rich but not Cl^--free Ringer's. The behavior did not correlate with any obvious condition like sex, size or season. Examples of the catecholamine antagonizing effects on I_{sc} are shown in Fig. 6. Percent-responses were transformed into probit units then analyzed by least-squares regression to obtain best-fit equations and median inhibitor concentrations [20]. Phenylephrine and norepinephrine could inhibit I_{sc} in some tissues while stimulating it in others (e.g. Figs. 5A and B). Isoproterenol and oxymetazoline, on the other hand, were always inhibitory. The lowest IC_{50} values were observed with phenylephrine ($IC_{50} = 1.2 \cdot 10^{-7}$ M) and isoproterenol ($IC_{50} = 2.1 \cdot 10^{-6}$ M). The tissues were much less sensitive to inhibition by oxymetazoline and norepinephrine with IC_{50} values greater than 10^{-4} M.

Discussion

Several substances are known to stimulate Na^+-transport in the toad urinary bladder [2,4,11]. Of these, less is known about the physiological effect of catecholamines on the amphibian urinary system. Our observations indicate that in vivo catecholamines play an important role in regulating ion transport in the bladder. Sympathomimetic agents elicit dose-dependent responses between 10^{-8} and 10^{-3} M. Furthermore, the action of both tyramine and 6-hydroxydopamine implies an endogenous reservoir of catecholamines in the tissue. Epinephrine and norepinephrine stimulate the I_{sc} associated with active Na^+-transport while increasing passive Cl^- flux [12]. The magnitude of the catecholamine-mediated response is influenced by several factors. These factors interact to determine the net electrophysiological properties of the toad bladder.

The focus of our investigation has been to classify the adrenoceptor coupled to Na^+-transport in the toad urinary bladder. In a pharmacological sense, determination of agonist-affinity sequences is complicated since the specific effects of catecholamines on either Na^+ or Cl^- flux are not distinctly separate. When Cl^- is present in the mucosal medium, certain adrenergic agents may either stimulate or inhibit the bladder's I_{sc}. If Cl^- is removed from the apical Ringer's, the agents always increase I_{sc}. The α_2-adrenoceptor agonist, clonidine, possesses the minimum EC_{50} for stimulating I_{sc}. Since it never inhibited the I_{sc}, this compound appears to represent the most potent class of adrenoceptor agonists for stimulating Na^+-transport in Cl^--rich Ringer's. The adrenergic response is blocked by α- but not β-adrenoceptor antagonists [11,12]. Yohimbine-blockade of catecholamine stimulation is more effective than prazosin. In this light, the receptor mediating Na^+-transport in the bladder is tentatively classified as α_2 [26,27].

Catecholamine mode of action in the epithelium is only partially resolved by this study. In

our experiments, epinephrine increased only the amiloride-sensitive component of the I_{sc} in the toad urinary bladder. This response differs from that of other epithelia like toad or frog skin where catecholamine stimulation of I_{sc} is resistant to amiloride inhibition. In these preparations, norepinephrine either produces new sodium channels which are insensitive to amiloride inhibition or increases electrogenic Cl^- efflux [28]. In the toad urinary bladder, there is no evidence of amiloride-insensitive sodium transport other than Na^+/H^+ and Na^+/Ca^{2+} exchange. Although electrogenic Cl^- chloride transport has been reported in the bladder [29], its relation to hydrogen/bicarbonate ion transport remains obscure. Soboslai et al. [30] reported that serosally directed active Cl^--transport in the bladder is stimulated by blocking Na^+-transport and inhibited by acetazolamide. Since removal of Cl^- from mucosal solutions did not alter the reversed short-circuit current, serosally directed Cl^--transport must be electroneutral. In opposition to these observations, the increase in amiloride-sensitive I_{sc} upon exposure to catecholamines in our experiments is due only to a change in the serosally directed rate of Na^+-transport. Similar observations have been made in the rabbit colon [31].

Since catecholamines directly increase active Na^+ flux, Cl^- apparently follows an electrochemical gradient along either cellular or paracellular routes. In general, the major conductance pathway in apical membrane of the toad bladder epithelium is utilized by Na^+ [32,33]. The presence of Cl^- in the apical Ringer's solution regulates the magnitude of basal Na^+-transport. Removal of Cl^- from mucosal solutions normally reduces V_t and I_{sc} while increasing R_t by altering single channel conductance or electrochemical gradients [34,35]. In addition, there is reasonable evidence that other ionic conductances may parallel that for sodium in the epithelium [36]. From their isotope flux studies with the toad bladder, Wood and Tomlinson [12] concluded that norepinephrine stimulated unidirectional active sodium transport as well as the passive Cl^- permeability. Walser [37] found an electroneutral Na^+ and Cl^- influx in the bladder. Navarte and Finn [38] and Nelson et al. [39], using different techniques, have found a Cl^- conductance in the apical membrane of toad cells. On the other hand, in studies comparing control and vasopressin-stimulated epithelia, MacKnight [40] found that Cl^- movement onto epithelial cells occurred only across the basolateral membrane. In the present case, increasing doses of some adrenergic agonists were found to decrease I_{sc} by as much as 50%. Such behavior is related to the effects of norepinephrine on transepithelial Cl^- flux. Since the magnitude of the I_{sc} response to a catecholamine is greater in the absence of apical Cl^- (Fig. 4), our observations support the possibility of Cl^- permeability in the toad bladder. Cl^- flux increases concomitantly with stimulation of active Na^+ transport to diminish the I_{sc}. Cl^- influx must be coupled in an obligatory fashion to active Na^+ influx since amiloride blocked the flux of both ions.

In the absence of acceptable microelectrode measurements, the V_t–R_t–I_{sc} relationship observed in the toad urinary epithelium implies that the overall rate of ion transport is regulated by apical membrane conductance [19,41]. Our observations support the notion of a transcellular route for both Na^+ and Cl^- permeability [38,39] rather than a paracellular [42]. R_t may be determined by the conductance properties of either the trans- or paracellular pathway (i.e. R_a or R_p). Consequently, epinephrine could modify R_t by altering either R_a or R_p. Assuming that R_a remains constant, decreasing R_p would result in decreased V_t while requiring a large I_{sc}. Non-selective Na^+ and Cl^- conductance through the paracellular pathway (R_p) would electrically short-circuit increases in active Na^+ influx through the cellular pathway. Amiloride would have little or no effect on R_t. With a constant R_p, on the other hand, decreasing R_a would increase both I_{sc} and V_t while decreasing R_t. Amiloride will increase R_t significantly following catecholamine stimulation. Our observations support the latter of the two relations, where as I_{sc} increases, R_a decreases over R_p. The adrenergic response is greatest in high-resistance (R_t) tissues with low V_t and I_{sc}. Since I_{sc} and V_t increase with decreasing R_t, the resistance of the transcellular pathway is most likely altered by epinephrine-treatment since R_t is high after amiloride.

Na^+-transport in the toad urinary bladder may be regulated by at least three humoral factors:

aldosterone, vasopressin and the catecholamines. The cellular mechanisms coupled to aldosterone and vasopressin stimulation of I_{sc} and V_t have been described [2]. The nature of cellular changes associated with catecholamine stimulation of transport in the bladder is not known. The initial enhancement of tranepithelial Na^+ flux appears to be accomplished by increasing apical membrane Na^+ permeability. The results of the present study imply that as sympathetic agents stimulate active Na^+-transport, passive Cl^- flux increases through apical membrane channels. Catecholamines can thus increase the flux of both Na^+ and Cl^-. This represents a major difference between vasopressin and catecholamine stimulation of ion transport. Vasopressin increases both Na^+-transport and hydro-osmotic conductivity in the bladder. In addition, the hormone increases basolateral rather than apical or paracellular Cl^- permeability [40]. On the other hand, the catecholamines increase Na^+ and Cl^- flux while inhibiting water flux [11,12]. The mechanism controlling transepithelial chloride flux remains to be identified.

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References

1 Koefoed-Johnsen, V. and Ussing, H.H. (1958) Acta Physiol. Scand. 42, 298–308.
2 Lewis, S.A. (1983) J. Exp. Biol. 106, 9–24.
3 Meddler, S.A., Kleeman, C.R. and Edwards, E. (1967) Gen. Comp. Endocrinol. 9, 38–48.
4 Pang, P.K.T. (1983) J. Exp. Biol. 106, 283–299.
5 Gonzales, C.S., Concha, J.B. and Fernandez, N.N. (1981) Cell. Mol. Biol. 27, 1–6.
6 House, C.R. (1970) J Physiol. (Lond.) 209, 513–537.
7 Koefoed-Johnsen, V., Ussing, H.H. and Zerahn, K. (1953) Acta Physiol. Scand. 27, 38–48.
8 Schoffeniels, E. and Salee, M.C. (1965) Comp. Biochem. Physiol. 14, 587–602.
9 Tomlinson, R.W.S. and Wood, A.W. (1976) J. Physiol. (Lond.) 257, 515–530.
10 Leaf, A., Anderson, J. and Page,L.B. (1958) J. Gen. Physiol. 41, 657–668.
11 Handler, J.S., Bensinger, R and Orloff, J. (1968) Am. J. Physiol. 215, 1014–1031.
12 Wood, A.W. and Tomlinson, R.W.S. (1974) Biochim. Biophys. Acta 367, 375–381.
13 Turtle, J.R. and Kipnis, D.M. (1967) Biochem. Biophys. Res. Commun. 28, 797–802.
14 Wong, P.Y.D., Bedwani, J.R. and Cuthbert, A.W. (1972) nature New Biol. 238, 27–31.
15 Lees, G.M. (1981) Br. Med. J. 283, 173–178.
16 Lefkowitz, R.J. and Hoffman, B.B. (1980) Trends Pharm. Sci. 1, 314–318.
17 Pettinger, W.A., Umemura, S., Smyth, D.D. and Jefferies, W.B. (1987) Am. J. Physiol. 252, F199–F208.
18 Exten, J.H. (1985) Am. J.Physiol. 248, E633–E547.
19 Higgins, J.T., Cesaro, L., Gebler, B. and Fromter, E. (1975) Pfluger's Arch. 358, 41–56.
20 Finney, D.J. (1952) Probit Analysis, Cambridge University Press, New York.
21 Benos, D.J. (1982) Am. J. Physiol. 242, C131–C145.
22 Civan, M.M. (1970) Am. J. Physiol. 219, 234–245.
23 Jonsson, C. and Sachs, C. (1970) Eur. J. Pharmacol. 9, 141–155.
24 Kostrzewa, R.M. and Jacobowitz, D.M. (1974) Pharmacol. Rev. 26, 199–288.
25 Sibley, D.R., Nambi, P. and Lefkowitz, R.J. (1985) in Molecular Mechanisms of Transmembrane Signalling (Cohen, A. and Housley, C., eds.), pp. 359–374, Elsevier Scientific Publications (Biomedical Division), Amsterdam.
26 Berthelsen, S. and Pettinger, W.A. (1977) Life Sci. 21, 595–606.
27 McGrawth, J.C. (1982) Biochem. Pharmacol. 31, 467–484.
28 Tomlinson, R.W.S. and Wood, A.W. (1978) J. Membrane Biol. 40, 135–150.
29 Finn A.L., Handler, J.S. and Orloff, J. (1967) Am. J. Physiol. 184, 179–184.
30 Solomslai, G.B., McTigue, M. and Weiner, M.W. (1977) Am. J. Physiol. 233, F421–F427.
31 Alvan, D. and Gutman, Y. (1980) Biochem. Pharmacol. 29, 1271–1273.
32 Van Driessche, W. and Zeiske, W. (1985) Physiol. Rev. 65, 833–903.
33 Wills, N.K. and Zweifach, A. (1987) Biochim. Biophys. Acta 906, 1–31.
34 Singer, I. and Civan, M.M. (1971) Am. J.Physiol. 221, 1019–1026.
35 Lewis, S.A., Butt, A.G., Bowler, M.J., Leader, J.R. and Macknight, A.D.C. (1985) J. Membrane Biol. 83, 119–137.
36 Palmer, L.G. (1982) J. Membrane Biol. 92, 217–226.
37 Walser, M. (1972) Biophys. J. 12, 351–368.

38 Navarte, J. and Finn, A.L. (1980) J. Gen Physiol. 76, 69–81.
39 Nelson, D.J., Tang, J.M. and Palmer, L.G. (1984) J. Membrane Biol. 80, 81–89.
40 Macknight, A.D.C. (1977) J. Membrane Biol. 36, 55–63.
41 Macknight, A.D.C., Dibona, D.R. and Leaf, A. (1980) Physiol. Rev. 60, 615–715.
42 Civan, M.M. and Dibona, D.R. (1978) J. Membrane Biol. 38, 359–386.