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THE EFFECT OF CATECHOLAMINES ON ION TRANSPORT IN THE TOAD BLADDER

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SUMMARY

Noradrenalin ($8 \cdot 10^{-6}$ M) and adrenalin ($6 \cdot 10^{-6}$ and $6 \cdot 10^{-7}$ M) were found to cause marked stimulation of short-circuit current (S.C.C.) in isolated toad bladder, but isoprenalin ($8 \cdot 10^{-7}$ M) was found to be without effect. The percentage rise in S.C.C. due to noradrenalin was found to be inversely proportional to the initial S.C.C. or total conductance of the bladder. Again in the case of noradrenalin the rise in S.C.C. was almost completely abolished by α-adrenergic blockade but not by β-blockade. This rise in S.C.C. was found not to be significantly different from the rise in net Na^+ flux. Bidirectional Cl^- fluxes were estimated using ^{82}Br as a companion radionuclide to ^{36}Cl. No significant net Cl^- flux was apparent, either before or after addition of any of the three catecholamines tested. However, in some cases the unidirectional Cl^- fluxes rose markedly following addition of noradrenalin or of adrenalin and this change was not reflected in a change in total conductance. This anomaly was noted to occur in bladders whose initial conductance was of the order of 0.5 $k\Omega^{-1} \cdot cm^{-2}$ or greater. The evidence presented suggests that two actions of catecholamines on ion transport in toad bladder are (a) to increase Na^+ transport via stimulation of α-adrenergic sites and (b) at the concentrations tested to cause an increase in passive Cl permeability in bladders whose initial conductance is high.

INTRODUCTION

The effects of catecholamines on Na^+ and Cl^- fluxes and on the short-circuit current (S.C.C.) of isolated frog skin have been well documented [1–4]. Few measurements have been made on isolated toad bladder, since Leaf et al. [5] stated that adrenalin ($2.5 \cdot 10^{-5}$ M) had no effect on S.C.C. Indeed the lack of response of toad bladder to catecholamines has been used to support the hypothesis that in frog skin the action of catecholamines is to stimulate the skin glands, producing a net outward movement of Cl^-. However, Handler et al. [6] reported that at certain times of the year there was a 30% increase in S.C.C. compared to a paired control bladder in

Abbreviations: S.C.C., short-circuit current; P.D., open-circuit potential.

response to noradrenalin. Ion fluxes were not measured in this study, but Bastide and Jard [2] reported that there was no significant change in Na^+ influx in frog bladder following addition of 10^{-4} M noradrenalin.

The experiments described in this paper were designed to test whether, in toad bladder, any increased S.C.C. due to addition of catecholamine could be accounted for by a similar increase in Na^+ influx, and whether the Cl^- fluxes were affected in any way, as they are in frog skin [1–4].

METHODS

Animals

Toads (*Bufo marinus*) originating from Colombia [7] were obtained from the Pet Farm Inc., Florida, U.S.A., kept on moist peat at 22 °C and fed on meal worms once a month. Animals were sacrificed by double-pithing and the bladder lobes excised. In a few earlier experiments toads were obtained from a supplier in Queensland, Australia.

Short-circuit current technique

Each lobe was mounted as a flat sheet between glass chambers (area 6.7 cm^2) each containing identical Ringer's solution in an arrangement similar to that described by Ussing and Zerahn [8]. The membrane potential was clamped automatically to zero [9] and the resulting S.C.C. monitored continuously, except for a few tens of seconds thrice in each experiment, when the open-circuit potential (P.D.) across the bladder was recorded. From this the membrane conductance (G_m) was estimated as the ratio of S.C.C. to P.D. In some experiments the conductance was also estimated as the drop in S.C.C. due to a 10-mV depolarisation. Conductance measured by the first method was lower than that by the second, by a mean (± S.E.) of 8 ± 1 % (28 pairs of observations).

Ionic-flux measurements

Bidirectional Na^+ fluxes were measured as described by Levi and Ussing [10] using the radionuclides ^{22}Na and ^{24}Na. Unidirectional Cl^- fluxes were measured with the pure β-emitter ^{36}Cl, and in several experiments ^{82}Br (half life: 37.5 h) was used as a companion radionuclide to ^{36}Cl. In experiments similar to those previously described in frog skin [11], ^{36}Cl and ^{82}Br were added together to the solution bathing one face of the short-circuited bladder and each 20 min samples withdrawn and their radioactivity measured on γ- and β-spectrometers. The flux of ^{36}Cl (j^*_{Cl}), ^{82}Br (j^*_{Br}) and their ratio ($f = j^*_{Cl}/j^*_{Br}$) was determined for several periods in each experiment. The mean ratio, $\bar{f}$, was used to estimate the equivalent unidirectional Cl^- flux from ^{82}Br flux data in subsequent experiments with the two radionuclides added to opposite faces of the bladder.

Materials

The composition of the Ringer's solution was as follows: NaCl, 111.2 mM; KCl, 1.9 mM; $NaHCO_3$, 2.4 mM; $CaCl_2$, 1.0 mM. Catecholamines used were: noradrenalin as Levophed injection (Winthrop); adrenalin injection 1:1000 (B.P.); isoprenalin sulphate (Burroughs-Wellcome). Adrenergic blocking agents used were:

propranolol as Inderal injection (I.C.I.); phentolamine mesylate (CIBA). Isoprenalin or phentolamine solutions were prepared fresh each experiment by dissolving the salt in Ringer's solution. Drug diluents were found to be without effect on the S.C.C. Radionuclides were obtained as NaCl solution with the exception of ^{82}Br, obtained as 70 mM NH_4Br. On receipt, this solution was passed through a column of cation-exchange beads in the Na^+ form. In the final bathing solution the concentration of Br^- was approximately 0.2 mM and that of NH_4^+ much less than this.

RESULTS

^{36}Cl:^{82}Br flux ratio

In three experiments in which tracers were added to the mucosal bathing solution of short-circuited bladder the mean ($\pm$ S.E.) ratio of ^{36}Cl:^{82}Br flux ($\bar{f} = j^*_{Cl}/j^*_{Br}$) was 0.97 ± 0.07 in a total of 12 periods of approx. 20 min. In four experiments (giving a total of 16 periods) with the tracers added to the serosal bathing solution this ratio ($\pm$ S.E.) was 0.98 ± 0.04. In two experiments noradrenalin $1.6 \cdot 10^{-5}$ M was added to the serosal solution and the ^{36}Cl:^{82}Br flux ratio determined for five periods of approx. 20 min. In the first, the tracers were added to the mucosal solution (results are shown in Fig. 4b), and the mean ratio was 0.99 ± 0.03, and in the second (tracers added to serosal solution) the mean ratio 0.98 ± 0.04. The values of the ratio are higher than those for frog skin [11], but there is no change in the ratio with the addition of catecholamine, as found in frog skin where the value was lower in the presence of catecholamines.

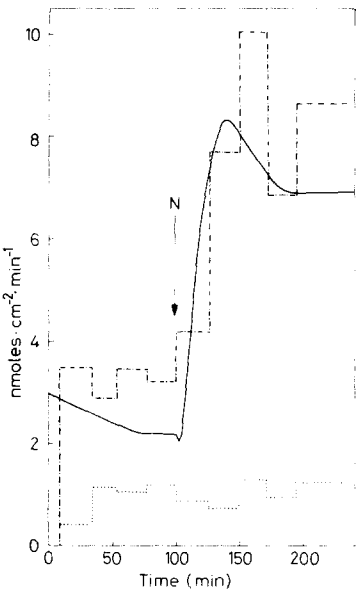
In subsequent experiments in which the radionuclides ^{36}Cl and ^{82}Br were added to opposite sides of the bladder, the factor 0.98 was used to estimate Cl^- flux from ^{82}Br data irrespective of the side to which the ^{82}Br was added, or whether noradrenalin was present or not.

The effect of catecholamines on S.C.C.

Noradrenalin was added to the serosal bathing solution to give a final concentration of $8 \cdot 10^{-6}$ M approx. 2 h after commencing short-circuiting. In all the 28 experiments S.C.C. rose to a maximum after about 30 min (Fig. 1). The mean ($\pm$ S.E.) percentage maximum increase over the value of the S.C.C. immediately before the addition of noradrenalin ($100\ (I_m - I_0)/I_0$) was $163 \pm 25\%$. The largest percentage increase observed was 480 %. There was a tendency for the greatest increases in S.C.C. to take place in bladders of the lowest initial conductance. In Fig. 2 the percentage increase in S.C.C. for each experiment is plotted against R^0_m, the initial resistance of the membrane (measured as the ratio P.D./S.C.C.). Above a resistance of $2 k\Omega \cdot cm^2$ the two parameters are significantly correlated ($P < 0.001$; $r = 0.79$; $n = 21$). This is reflected in a highly significant correlation between I_m and I_0 ($r = 0.93$; $P < 0.001$). The regression equation is:

$$I_m = 1.49\ (\pm 0.14)\ I_0 + 2.9\ (\pm 1.0) \qquad (1)$$

Clearly, dividing this equation by I_0, the lower the value of I_0, the greater the percentage stimulation. The correlations between I_m and V_0 (the P.D. before noradrenalin addition) and also $\log((I_m - I_0)/I_0)$ and $1/V_0$, are significant ($r = 0.81$, $r = 0.71$, respectively; $n = 21$; $P < 0.001$ in both cases). Hence large stimulations of S.C.C.



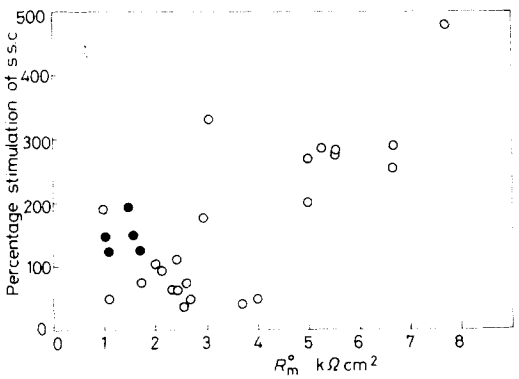


Fig. 1. Effect of noradrenalin on Na^+ fluxes in short-circuited toad bladder: typical experiment. At arrow: noradrenalin added to serosal bathing solution ($8 \cdot 10^{-6}$ M final concentration). ———, S.C.C.; - - - - -, ^{24}Na-traced Na^+ influx (mucosal to serosal flux); ····, ^{22}Na-traced Na^+ efflux, both plotted in same units as S.C.C.

Fig. 2. The variation of percentage maximal stimulation of S.C.C. in response to noradrenalin (100 $(I_m - I_0)/I_0$), with initial membrane resistance $R_m^0 (= 1/G_m^0 =$ P.D./S.C.C.). Initial P.D. of bladders > 10 mV except those represented by filled circles ●. Each point represents results from one experiment.

were associated with those bladders whose initial S.C.C. is low, or P.D. high.

Isoprenalin at concentrations of $8 \cdot 10^{-7}$ M (n = seven experiments) $8 \cdot 10^{-6}$ M ($n = 1$) and $1.6 \cdot 10^{-5}$ M ($n = 2$) produced little change in S.C.C., or at the lowest concentration a transient decrease (Fig. 4a). A lower concentration of noradrenalin ($3.2 \cdot 10^{-7}$ M) produced increases of 23 % and 81 % when added subsequent to the addition of $1.6 \cdot 10^{-5}$ M isoprenalin (which had no effect).

Adrenalin produced a rise in S.C.C. similar to that occurring with noradrenalin. At a concentration of $6 \cdot 10^{-7}$ M the mean ($\pm$ S.E.) increase was 32 ± 9 % ($n = 4$) and at $6 \cdot 10^{-6}$ M 86 ± 52 % ($n = 3$; individual values were 190, 21 and 47 %).

The effect of blocking agents was tested using the two bladder lobes from one animal. The increase in S.C.C. produced by noradrenalin alone in one half was compared with its effect after the addition of the blocking agent on the other half.

The increase in S.C.C. produced by noradrenalin was inhibited by the α-adrenergic blocking agent phentolamine when added either before noradrenalin or during a noradrenalin response ($n = 4$ pairs). Phentolamine alone had a negligible effect on the S.C.C. of unstimulated bladders. A typical experiment is depicted in Fig. 3a. The β-blocking agent, propranolol ($n = 3$ pairs) failed to markedly inhibit the increase in S.C.C. produced by noradrenalin (Fig. 3b), although in one experiment the S.C.C. was diminished by propranolol itself. In all three cases the percentage

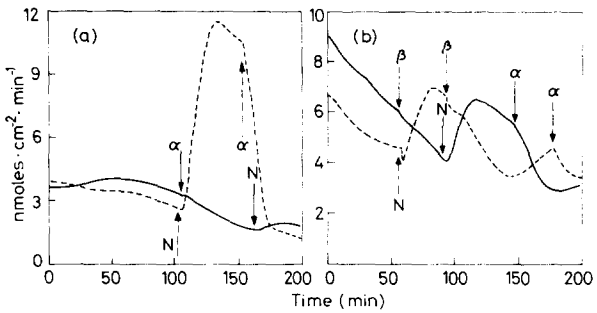


Fig. 3. The effect of (a) α- and (b) β-adrenergic blocking agents on the response of S.C.C. to noradrenalin. Two typical experiments. At arrows: noradrenalin (N), phentolamine (α) or propranolol (β) added to serosal solution to give final concentrations of $8 \cdot 10^{-6}$ M, $7 \cdot 10^{-5}$ M and $7 \cdot 10^{-5}$ M, respectively. ——— and - - -, S.C.C. in two half bladder preparations from the same animal.

increase due to noradrenalin after β-blockade was in accordance with values expected from R_m^0 in Fig. 2.

Ionic flux: net

The net Na^+ flux (J_{Na}), defined as the difference between Na^+ influx* (j_{Na}^{influx}) and efflux (j_{Na}^{efflux}) was not significantly different from the S.C.C. either before or after the addition of noradrenalin (Fig. 1, Table I). It would appear, therefore, that the S.C.C. response to noradrenalin can be completely accounted for by net Na^+ influx. However, there was the possibility of there being, in the response, a change in Cl^- permeability, or an appearance of a net movement of Cl^- (small compared to

TABLE I

NET Na^+ FLUX: EFFECT OF NORADRENALIN

Values are mean (±S.E.) of four experiments, in nmoles · cm^{-2} · min^{-1}. Net Na^+ flux (J_{Na}) estimated as ^{24}Na-traced influx (j_{Na}^{influx}) minus ^{22}Na-traced efflux (j_{Na}^{efflux}). Δ is the discrepancy between $\bar{I}$, the average S.C.C., and J_{Na} in each 20-min period ($\Delta = \bar{I} - J_{Na}$). Values in the four periods before noradrenalin addition are pooled, since the ratio of variance between periods to that between experiments is not significant. Values for the five periods (I–V) following noradrenalin addition are given separately, with the level of significance (P) of their difference from zero. Final concentration of noradrenalin: $8 \cdot 10^{-6}$ M.

Periods before noradrenalin		Periods after noradrenalin				
		I	II	III	IV	V
J_{Na}	4.70 ±0.48	5.03 ±1.10	7.65 ±0.33	8.53 ±0.61	7.00 ±1.08	7.45 ±0.60
Δ	0.14 ±0.23	0.80 ±0.46	0.60 ±0.23	−0.65 ±0.30	0.33 ±0.78	−0.20 ±0.38
P	0.45	0.15	0.05	0.08	0.70	0.60

* Influx denotes flux from mucosal to serosal bathing solutions and efflux denotes flux in the opposite direction.

TABLE II

NET Cl^- FLUX: EFFECT OF CATECHOLAMINES

Values are mean (±S.E.) net influx of Cl^- (J_{Cl}) in nmoles · cm^{-2} · min^{-1}, the numbers of experiments are given in parentheses, each experiment consisting of four 20-min periods before and usually five periods after catecholamine addition. Bidirectional Cl^- fluxes estimated from ^{36}Cl and ^{82}Br data, as indicated in the text. ΔJ_{Cl} is the mean of changes of the mean net flux before and after addition of catecholamine for each experiment, and P the level of significance of this change. Final concentrations of catecholamines: noradrenalin, $8 \cdot 10^{-6}$ M; isoprenalin, $8 \cdot 10^{-7}$ M; adrenalin, $6 \cdot 10^{-7}$ M.

Catecholamine	J_{Cl}		ΔJ_{Cl}	P
	Before	After		
Noradrenalin (5)	0.05±0.24	0.26±0.26	−0.26±0.44	0.57
Isoprenalin (5)	0.39±0.16	0.80±0.22	0.41±0.32	0.22
Adrenalin (3)	0.19±0.19	0.17±0.24	0.01±0.26	0.97

that of Na^+). There was in fact an overall mean (± S.E.) net Cl^- influx of 0.21±0.15 nmoles · cm^{-2} · min^{-1} (which is non-significant) and there were no significant changes in net Cl^- flux after the addition of catecholamines (Table II).

Ionic flux: unidirectional

In unstimulated bladder unidirectional Cl^- fluxes were in the range 1.3–20 nmoles · cm^{-2} · min^{-1}. In five out of ten experiments there was an appreciable rise in Cl^- influx (or efflux) after addition of noradrenalin (Fig. 4b). In all these cases the Cl^- flux was steady in the periods preceding the addition of noradrenalin, then rose

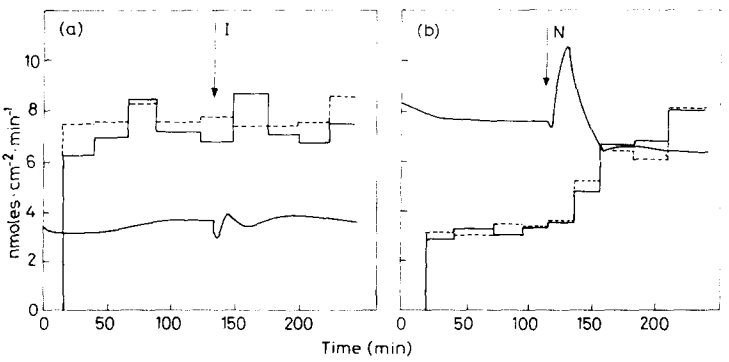


Fig. 4. Effect of catecholamines on Cl^- fluxes in short-circuited toad bladder. Unbroken curve: S.C.C. At arrow: (a) isoprenalin (I), (b) noradrenalin (N) added to serosal solution ($8 \cdot 10^{-7}$ and $8 \cdot 10^{-6}$ M, respectively). In (a), radionuclides added to opposite faces of the bladder; ⌐¬, ^{36}Cl-estimated Cl^- efflux; :···:, ^{82}Br-estimated Cl^- influx (correction factor 0.98), as described in text. In (b) both radionuclides added to mucosal bathing solution, i.e. Cl^- influx estimated by: ⌐¬, ^{36}Cl; :···:, ^{82}Br.

continuously in the periods following addition, without the appearance of a net Cl^- flux. This pattern was also observed in three out of five experiments with adrenalin added but in none of the five isoprenalin experiments.

In contrast there was no significant tendency for Na^+ efflux to increase following noradrenalin stimulation. In the experiment depicted in Fig. 1, and in 11 other experiments, the mean Na^+ efflux in the five periods following noradrenalin addition was not significantly different from that in the four preceding periods. The Na^+ efflux in all except one of these experiments was in the range 0.53–1.96 nmoles $\cdot$ cm^{-2} $\cdot$ min^{-1}. However, in the remaining experiment the Na^+ efflux, initially 5.77 nmoles $\cdot$ $cm^{-2} \cdot min^{-1}$ (mean of four periods) after noradrenalin rose in successive periods to 7.22 nmoles $\cdot$ $cm^{-2} \cdot min^{-1}$ in the final period, but the difference in the mean values failed to reach significance.

Conductance

Total conductance (G_m) as the ratio of the S.C.C. to P.D. was plotted against Cl^- conductance (Fig. 5). Since Cl^- fluxes were the same in both directions, the Cl^- conductance was measured (assuming independent diffusion) as [12]:

$$g_{Cl} = \frac{F^2}{RT} j_{Cl} = 0.064\, j_{Cl} \tag{2}$$

where j_{Cl} is the mean unidirectional Cl^- flux measured by ^{36}Cl before the addition of catecholamine. Below G_m or $g_{Cl} = 0.6 k\Omega^{-1} \cdot cm^{-2}$, g_{Cl} is less than G_m, and the two quantities are correlated ($r = 0.84$, $P < 0.001$, slope = 0.90), but higher values (arising from in many cases a low P.D.) are divergent.

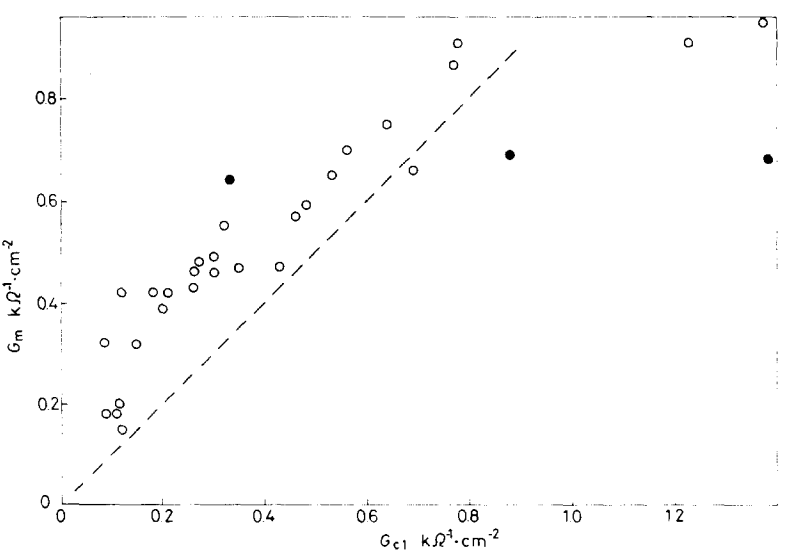


Fig. 5. The relationship between total conductance G_m^0 (= S.C.C./P.D.) and mean Cl^- conductance g_{Cl}^0 in unstimulated toad bladder. Each point represents results from one experiment. g_{Cl}^0 estimated from Eqn 2, using the mean ^{36}Cl-estimated Cl^- flux in four 20-min periods. Initial P.D. of bladders > 10 mV, except those represented by ●. - - -, line of slope = unity.

TABLE III

CHANGES IN TOTAL CONDUCTANCE AFTER NORADRENALIN

Conductance, G_m, measured as ratio S.C.C./P.D. Values are increase in G_m after addition of noradrenalin. Bladders whose initial conductance is high ($G_m^0 > 0.6\ k\Omega^{-1} \cdot cm^{-2}$) are treated separately; this value was chosen as a discriminator on basis of Figs 2 and 5. Concentration of noradrenalin $8 \cdot 10^{-6}$ M. P is level of significance of the change being different from zero. N.S., not significant.

	N	Mean (±S.E.) conductance increase (ΔG_m) after noradrenalin ($\Omega^{-1} \cdot cm^{-2}$)			
		At S.C.C. max.	P	2 h after addition	P
$G_m^0 < 0.6\ m\Omega^{-1}cm^{-2}$	15	0.064±0.010	0.001	0.024±0.011	0.04
$G_m^0 > 0.6\ m\Omega^{-1}cm^{-2}$	5	−0.002±0.019	N.S.	−0.112±0.034	0.03

Total conductance was increased significantly at the height of the S.C.C. response to noradrenalin (Table III) if only those values of G_m less than $0.6\ \Omega^{-1} \cdot cm^{-2}$ are considered. In many cases (Table IV) the appreciable increase in Cl^- conductance already referred to was not reflected by a similar increase in total conductance G_m, and at the end of these experiments g_{Cl} was in excess of the estimated value of G_m. It should be emphasised that in all cases the P.D. exceeded 10 mV, a reasonable criterion for the acceptability of toad bladder preparations.

TABLE IV

CHANGES IN Cl^- CONDUCTANCE AFTER NORADRENALIN

Results from eight experiments, all values in $\Omega^{-1} \cdot cm^{-2}$. First two columns: Cl^- conductance before (g_{Cl}^0) and after (g_{Cl}^f) addition of noradrenalin ($8 \cdot 10^{-6}$ M). Third column: change in Cl^- conductance, Δg_{Cl} ($= g_{Cl}^f - g_{Cl}^0$), compared (last two columns) with value of total conductance, G_m^f, and the change in total conductance, ΔG_m, 2 h after addition of noradrenalin. g_{Cl}^0 estimated from Eqn 2, taking the mean value of $^{36}Cl^-$ flux in the four 20-min periods before, and noradrenalin addition, and g_{Cl}^f from the value of ^{36}Cl flux in the final (or last two) periods after noradrenalin addition.

g_{Cl}^0	g_{Cl}^f	Δg_{Cl}	G_m^f	ΔG_m
0.30	0.66	0.36	0.44	−0.06
0.21	0.26	0.05	0.44	0.02
0.20	0.51	0.31	0.45	0.06
0.11	0.10	−0.01	0.15	0.00
0.11	0.14	0.03	0.26	0.08
0.26	0.24	−0.02	0.30	−0.03
*1.37	1.88	0.51	0.80	−0.19
*1.23	2.57	1.34	0.88	−0.11

* Data from bladders whose initial total conductance (G_m^0) was greater than $0.6\ \Omega^{-1} \cdot cm^{-2}$.

DISCUSSION

In this study, it has been shown that high concentrations of noradrenalin produce a marked increase in Na^+ transport, mirrored by increased S.C.C. Since the S.C.C. increase (i) did not occur with the β-stimulator isoprenalin, (ii) was almost

completely abolished when noradrenalin was added to bladders previously treated with the α-adrenergic blocker, phentolamine, and (iii) was not abolished in those treated with the β-blocker, propranolol, it would seem that the increase Na^+ transport is associated with α-adrenergic receptor sites.

In contrast, α-stimulation (β-blocker+noradrenalin) of the intact skin produced a fall in net Na^+ transport in several species (*Rana pipiens*[13], *Rana esculenta*, *Rana temporaria* and *Bufo marinus* (Wood, A. W., unpublished). However, this effect may be a feature of the glandular, rather than transporting epithelium. In the "split" frog skin preparations of Rajerison et al. [14] with the epithelial layer removed intact from the glands and surrounding dermis, the effect of noradrenalin to increase the S.C.C. is abolished by the β-blocker, propranolol, which is in contrast to the effects (ii) and (iii) noted above.

It has been noted by Handler et al. [6] that in toad bladder α-receptors were predominant in the action of noradrenalin to inhibit the water-permeability response to vasopressin or theophylline. The changes in S.C.C. due to noradrenalin reported by these authors did not occur in certain months of the year, whereas the present series of experiments performed throughout the year, showed no significant seasonal variation, although several abnormally large increases in magnitude of S.C.C. were noted to occur during the months of March and April. The changes were also much smaller than those reported in this study. In contrast, their reported increase in S.C.C. due to isoprenalin was not observed in these experiments; however, they used much higher concentrations. There is a possibility that the difference in transport characteristics between *B. marinus* of Dominican and Colombian origin could account for the lack of response reported by Leaf et al. [5]. As in this study, toads used by Handler and coworkers originated from Colombia, whereas those of Leaf et al. were from the Dominican Republic [7]. However, the increased S.C.C. due to adrenalin reported here occurred in earlier experiments in which Australian toads were used. These toads probably originated from the same area as the Dominican toads [7] (the Amazon basin)*. Bladders from these Australian toads did not show the reversed potentials in K^+-free Ringer's solution characteristic of Colombian toads.

Another possible explanation for the lack of a noradrenalin response reported by others arises from the present finding that the percentage increase is lower for high initial values of S.C.C. (or conductance). The values of S.C.C. reported by Leaf et al. were of the order of 20 nmoles $\cdot$ cm^{-2} $\cdot$ min^{-1}, which are larger than those observed in this study. If, as in Fig. 2, bladders with a high conductance were only stimulated by about 30–40 %, this would not appear significant, unless a large number of experiments were performed. Many of the bladders in the present series of experiments had initial values of S.C.C. less than 5 nmoles $\cdot$ cm^{-2} $\cdot$ min^{-1} (Fig. 1 for example). If the criterion used for an acceptable bladder preparation is that of a high S.C.C. (rather than as in these experiments a high P.D. or low G_m) then bladders which could have a marked S.C.C. response to catecholamines would be rejected.

The increase in S.C.C. caused by noradrenalin is accounted for by the increased Na^+ influx, there being no significant change in Na^+ efflux. The inhibition by noradre-

* We would like to thank Mr Colin McCarthy of the British Museum (Natural History) for tracing the probable origin of *B. marinus* in Queensland, Australia, to the area surrounding Georgetown, Guyana.

nalin of the basal and vasopressin-induced increase in water permeability has been explained in terms of decreased cyclic AMP production [6]. Wong et al. [15] found no evidence that noradrenalin affects cyclic AMP levels in toad bladder in the absence of vasopressin, nor was there a significant difference in cyclic AMP concentrations in vasopressin-treated bladders, with and without noradrenalin, although there was a significant inhibition of water flow. The increase in Na^+ influx by noradrenalin could be mediated by an increase in the cyclic AMP pool devoted to Na^+ transport or it may have a mechanism of action other than via adenyl cyclase.

It is not clear why in certain bladders (those with initial conductance greater than around 0.5 $k\Omega^{-1} \cdot cm^{-2}$) noradrenalin and adrenalin should produce marked increases in Cl^- fluxes, while at the same time leaving the total conductance unchanged. There were also cases in unstimulated bladders where $g_{Cl} > G_m$. The values of g_{Cl} in Table IV and Fig. 5 were calculated assuming that all of the Cl^- flux (as traced by radionuclides) contributed to the electrical conductance, which need not be so if, for example, carrier-mediated diffusion occurs. Changes in passive conductance would also be reflected in changes in Na^+ efflux, but in the twelve experiments in which Na^+ efflux was measured and found not to change significantly, the bladders had initial conductances less than 0.6 $k\Omega^{-1} \cdot cm^{-2}$.

The small (non-significant) net influx of Cl^- observed in these experiments may arise as a consequence of insufficient short-circuiting due to the conductance of the electrolyte and passive cellular layers between the potential sensing electrodes. The P.D. drop for a S.C.C. of 10 nmoles $\cdot cm^{-2} \cdot min^{-1}$ would be about 1 mV, which would account for this. The absence of net Cl^- movement after noradrenalin stimulation strengthens the argument that the movement observed in frog skin is associated with glandular secretion.

ACKNOWLEDGEMENT

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