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CATECHOLAMINE-INDUCED CHANGES IN ION TRANSPORT IN SHORT-CIRCUITED FROG SKIN AND THE EFFECT OF β-BLOCKADE

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SUMMARY

1. A method for measuring bidirectional Cl fluxes has been used to estimate net Cl movements in short-circuited frog skin and to compare these with the short-circuit current (I_{sc}) and Na fluxes.

2. In some experiments bidirectional fluxes of both Na and Cl were measured simultaneously. It was found that the algebraic sum of the net fluxes of these two ions did not differ significantly from the values of I_{sc}, either in untreated or catecholamine-treated skins, except for the half-hour period immediately after catecholamine addition.

3. The net effluxes of Cl produced by noradrenaline ($1{\cdot}6 \times 10^{-5}$ M), isoprenaline (8×10^{-7} M) and adrenaline (6 and 15×10^{-6} M) were of similar magnitude for each catecholamine. The magnitude of the Cl response measured as a flux ratio was related to a certain extent to the pre-catecholamine Cl conductance.

4. The net Na influx was increased by isoprenaline and reduced by noradrenaline.

5. Addition of the β-adrenergic blocking agent oxprenolol ($4{\cdot}5 \times 10^{-5}$ M) to skins stimulated by catecholamine resulted in the disappearance of the net Cl movement and fall in skin conductance and I_{sc}. This fall was similar in magnitude to, and correlated with the mean rise in I_{sc} produced by isoprenaline, but of significantly greater magnitude in the case of noradrenaline.

6. The changes in Na influx were strongly associated with the changes in I_{sc} following catecholamine addition. Similarly, the changes in Na efflux and Cl efflux were correlated, suggesting the Na fluxes to be dissociated, influx and efflux changes perhaps taking place at different loci.

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7. Acetazolamide ($1{\cdot}2 \times 10^{-4}$ M), added either before or during the noradrenaline stimulation, had no effect on the Cl efflux response.

8. The tissue exchange of Cl from the outside bathing medium after 4 hr was greater in catecholamine-stimulated skins than in those in which the response had been blocked by oxprenolol.

9. These findings were taken to support a model entailing a neutral NaCl pump resident in the mucous glands and an epithelial Na pump enhanced by β- and inhibited by α-adrenergic stimulation.

INTRODUCTION

In short-circuited preparations of intact frog skin, adrenaline (10^{-5} M) produces (i) a net efflux of Cl (ii) a variable change in the net Na influx and (iii) an increase in skin conductance, reflected in increased Na and Cl unidirectional fluxes (Koefoed-Johnsen, Ussing & Zerahn, 1952). The actions of noradrenaline at concentrations greater than 10^{-5} M (Bastide & Jard, 1968) and isoprenaline at 10^{-5} M (McAfee, 1970) are similar to those of adrenaline. The responses have been separated into α- and β-adrenergic effects by Watlington (1968) who by measuring bidirectional Na fluxes showed that the net 'non-Na' movement was associated with β-adrenergic stimulation.

There is, however, some divergence of opinion in relation to the question of the origin of the net Cl flux. Several authors agree that it is associated with the extrusion of mucus from the skin glands. McAfee (1970) found Na to be extruded in equal amounts with Cl (an electro-neutral NaCl pump), whereas other workers (including Koefoed-Johnsen *et al.* 1952; Watlington, 1968; Lindley, 1969) favour an electrogenic Cl pump mechanism. House (1971) presented evidence against an electrogenic Cl pump and postulated a model involving the opening up of glandular shunt pathways and the alteration of ionic selectivity properties at the permeability barriers.

Since the measurement of net Cl flux and also simultaneous comparison with net Na flux has proved somewhat difficult even in paired skin preparations, the present series of experiments was designed to overcome this difficulty and also to investigate the effect on Na and Cl fluxes of the addition of a β-adrenergic blocking agent before or after the addition of various catecholamines.

METHODS

Samples of skin from *Rana temporaria* were mounted between glass chambers (area 6·7 cm^2) and the short-circuit current (I_{sc}) monitored automatically. The open-circuit potential (V) and membrane conductance (G_m) either as the ratio I_{sc}/V or as the fall in I_{sc} due to a 10 mV depolarization, were also measured. Wherever possible paired skin samples were obtained by dividing the skin along the dorsal and ventral

mid lines, thus including in either sample an area of dorsal skin, where many granular (serous) glands occur. In the ventral regions, the glands are almost exclusively of the mucous type.

Bidirectional Na fluxes were measured using the radionuclides ^{22}NaCl and ^{24}NaCl and Cl fluxes with Na^{36}Cl and Na^{82}Br, correcting the ^{82}Br fluxes with the appropriate relative rate factor (f) for the two radionuclides traversing the skin from the same side. The values of f for untreated- and catecholamine-treated frog skin have been published previously (Tomlinson & Wood, 1972). The over-all error on each individual flux measurement was estimated to be approximately 20%.

In some experiments ^{22}Na and ^{36}Cl were added together to the solution bathing one side of the skin with ^{82}Br and ^{24}Na added to the other. In the samples withdrawn at successive time intervals, the halides were precipitated with 0·1 M-$AgNO_3$ and the activity of each radionuclide measured as described previously (Tomlinson & Wood, 1974).

The activity of γ-emissions from ^{82}Br and ^{24}Na were measured with a Nuclear Enterprises 'Edinburgh' series automatic gamma spectrometer and that of β-emissions from ^{36}Cl and ^{22}Na with a Beckman LS 200 automatic liquid scintillation system, the volume and composition of all samples being uniform. Samples in which ^{22}Na and ^{36}Cl were to be measured were placed also in a coincidence detecting arrangement in which the positron annihilation γ-rays from the ^{22}Na were counted.

The composition of the Ringer solution was as follows: NaCl, 111·2 mM; KCl, 1·9 mM; $NaHCO_3$, 2·4 mM; $CaCl_2$, 1·0 mM. The solutions within the chambers were mixed and oxygenated by a stream of air saturated with water.

Catecholamines used were: noradrenaline (Levophed injection; Winthrop), adrenaline (1:1000 B.P. injection or bitartrate salt in DL form) and isoprenaline (as sulphate; Wellcome). The final concentrations of catecholamines were: adrenaline 6×10^{-6} M and $1{\cdot}5 \times 10^{-5}$ M, noradrenaline $1{\cdot}6 \times 10^{-5}$ M and isoprenaline 8×10^{-7} M. These concentrations were chosen to give maximum response (Fassima, Carpenedo & Fiandini, 1968; Ambalavanar, Foster & Schnieden, 1973) and sufficiently high to cause a net Cl efflux (Bastide & Jard, 1968). The β-adrenergic blocking agent used was oxprenolol (Trasicor, Ciba) rather than propranolol because of its rather more marked effects on I_{sc} in catecholamine-stimulated skin (unpublished observation). At the end of each experiment the skins were excised on the chambers, weighed, assayed for γ-activity (^{82}Br), dried and reweighed. The dried skins were then dissolved in N-NaOH, an aliquot dissolved in liquid scintillant and the β-activity measured. The fall in count rate due to 'quenching' was of the order of 3% compared to ^{36}Cl Ringer solution samples. Duplicate aliquots were neutralized and the Cl content estimated using a test kit (Sigma).

RESULTS

Short-circuit current response

The patterns of I_{sc} response were rather similar for the three catecholamines (Figs. 1–3) although the response in each individual preparation was rather variable. In about half the skins there was a second maximum about 1 hr after the first but in only one response out of over seventy observed was it greater than the first. The magnitude of the initial rise was not correlated either with the values of pre-catecholamine current (I^o_{sc}) or conductance (G^o_m), nor with their inverse, with the exception of noradrenaline, where the correlation with $1/G^o_m$ was probably significant.

There was a significant difference between the % increase in I_{sc} to the maximum in noradrenaline and isoprenaline treated skins (73 ± 7 and 99 ± 9% respectively) and the values of I_{sc} 2 hr after catecholamine addition, expressed in the same way ($\Delta I_{sc} \times 100/I_{sc}^{o}$), were significantly different (noradrenaline 28 ± 7%; isoprenaline 61 ± 10%).

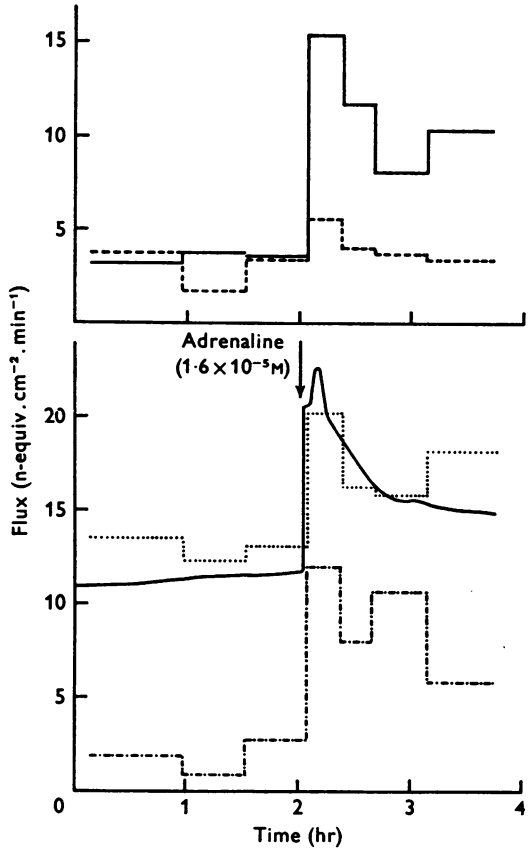

Fig. 1. Effect of adrenaline on the bidirectional fluxes of Na and Cl measured simultaneously in short-circuited frog skin. Lower: ———, short-circuit current; ·····, ^{24}Na-traced Na influx; ·-·-·-, ^{22}Na-traced Na efflux. Upper: ———, ^{36}Cl-traced Cl efflux; ------, ^{82}Br-traced Cl influx.

Chloride fluxes

Before the addition of catecholamine no net flux of Cl was apparent (for the hour period before catecholamine addition the net flux was 0·08 ± 0·08 n-equiv. cm^{-2} min^{-1} inwards, n = 41). The values for unidirectional flux ranged from 0·34 to 8·75 n-equiv. cm^{-2} min^{-1} (mean 3·15 ± 0·17).

After catecholamine addition, the efflux of Cl (j_{Cl}^{out})reached a maximum in either the first or second half-hour period and increased up to fifteen times its resting value. On the other hand, Cl influx (j_{Cl}^{in}: small letters denote unidirectional fluxes, capitals net fluxes) responded in a more variable way, but there was a tendency for j_{Cl}^{in} to rise to a maximum before falling again in the case of noradrenaline stimulation, and to remain more or less unchanged following the addition of isoprenaline.

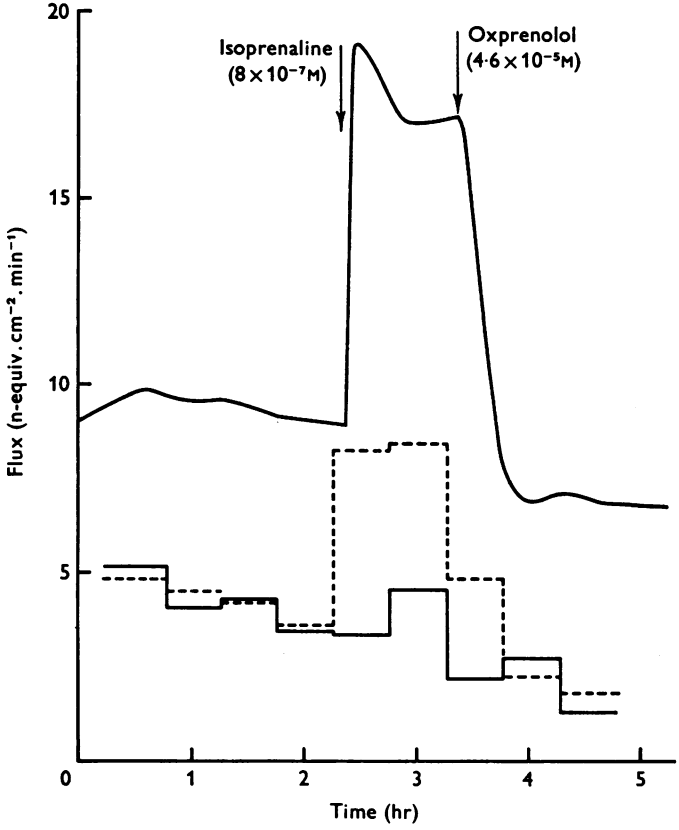

Fig. 2. Effect of isoprenaline and subsequent β-blockade by oxprenolol on short-circuit current and bidirectional Cl fluxes in frog skin. Curve: short-circuit current. Bars: ———, ^{36}Cl-traced Cl influx; ------, ^{82}Br-traced Cl efflux.

The average net flux of Cl (J_{Cl}) was estimated for the first and second hour periods following catecholamine addition (Table 1). During the first hour J_{Cl} was similar for all three catecholamines at these concentrations. In a few experiments with a lower concentration of 8×10^{-7} M noradrenaline it was found that J_{Cl} was greatly diminished ($1{\cdot}07 \pm 0{\cdot}20$ n-equiv.

cm^{-2} min^{-1}, $n = 4$). The % rise in I_{sc} was $60 \pm 20\%$ which was not significantly less than that at the higher concentration.

The fractional increase of j_{Cl}^{out} to the maximum value (j_{Cl}^{max})after catecholamine addition (usually the first or second half-hour period) compared to the average pre-catecholamine value (j_{Cl}^{o}) decreased with increasing j_{Cl}. Since pre-catecholamine values of Cl efflux and influx were approximately equal ($= j_{Cl}^{o}$), the reciprocal, $1/j_{Cl}^{o}$, is proportional to the resting passive resistance to Cl ($R_{Cl}^{o} = RT/F^2 j_{Cl}^{o}$). The regression slopes for the three catecholamines did not differ significantly and the pooled data gave the equation

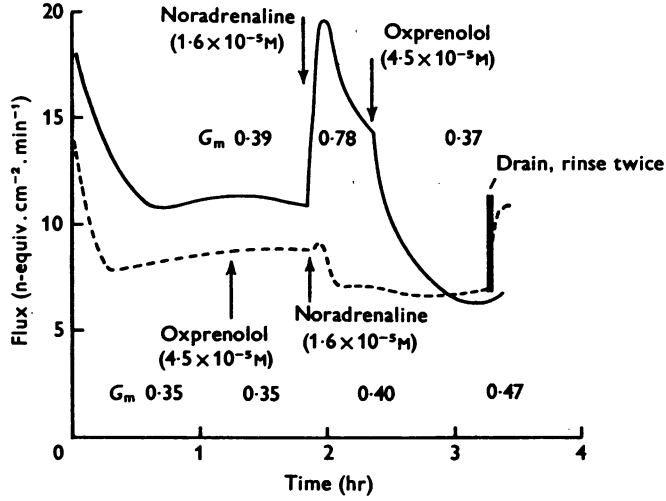

Fig. 3. Effect of β-blockade on short-circuit current response to noradrenaline in duplicate samples of skin from one animal: in the first (continuous curve) oxprenolol added subsequent to noradrenaline, in the second (interrupted curve) before noradrenaline, as indicated. At double line: chambers drained, washed twice and re-filled with Ringer solution. G_m ($k\Omega^{-1}$. cm^{-2}) is the conductances of the skins at the times shown.

$$(j_{Cl}^{max} - j_{Cl}^{o})/j_{Cl}^{o} = (8{\cdot}1 \pm 1{\cdot}1)/j_{Cl}^{o} - (0{\cdot}0 \pm 0{\cdot}6),$$

$P = 0{\cdot}001$, $n = 50$. Rearrangement of this equation would suggest that the rise in j_{Cl}^{out} tends to be 8·1 n-equiv. cm^{-2} min^{-1} irrespective of initial conditions. On the other hand, there was a significant correlation between the maximum value of 'Cl transport potential' V_{Cl} ($= -RT \ln(j_{Cl}^{in}/j_{Cl}^{out})/F$) and $1/j_{Cl}^{o}$ (isoprenaline $P < 0{\cdot}001$; noradrenaline $P = 0{\cdot}03$) which suggests that the Cl response is modified by the size of the resting shunt pathway. The mean net efflux J_{Cl} was not significantly correlated either with $1/j_{Cl}^{o}$, (isoprenaline $r = 0{\cdot}52$, $P = 0{\cdot}06$; noradrenaline $r = 0{\cdot}35$, $P = 0{\cdot}25$), or with ΔI_{sc}, the rise in the mean I_{sc} during the first hour after catechol-

TABLE 1. Values of net Cl efflux (J_{Cl}), *increase* in values of 'non-Cl' short-circuit current (ΔJ_X) and net Na influx (ΔJ_{Na})in the first (1) and second (2) hour period following the addition of catecholamines (expressed as the increase from the values in the hour period before catecholamine addition). $\Delta \bar{I}_{sc}$ is the change in short-circuit current averaged over the hour before and the hour after catecholamine addition (by graphical integration). J_{Cl} and J_{Na} were estimated from bidirectional fluxes during periods of approximately 30 min integrated over the hour before and the first and second hours after catecholamine addition. Numbers of experiments given in parentheses. Values in n-equiv. cm.$^{-2}$ min^{-1} and are mean (± S.E.)

Catecholamine	$\Delta \bar{I}_{sc}$	$J_{Cl}^{(1)}$	$J_{Cl}^{(2)}$	$\Delta J_X^{(1)}$	$\Delta J_X^{(2)}$	$\Delta J_{Na}^{(1)}$	$\Delta J_{Na}^{(2)}$
Noradrenaline $1{\cdot}6 \times 10^{-5}$ M	$4{\cdot}5 \pm 0{\cdot}7$ (14)	$5{\cdot}8 \pm 0{\cdot}6$ (14)	$3{\cdot}0 \pm 0{\cdot}4$ (5)	$-2{\cdot}1 \pm 0{\cdot}8$ (14)*	$0{\cdot}3 \pm 2{\cdot}3$ (5)	$+0{\cdot}1 \pm 0{\cdot}6$ (6)	$-1{\cdot}7 \pm 0{\cdot}8$ (4)
Isoprenaline 8×10^{-7} M	$8{\cdot}3 \pm 1{\cdot}1$ (14)	$4{\cdot}2 \pm 0{\cdot}4$ (14)	$3{\cdot}4 \pm 0{\cdot}7$ (8)	$3{\cdot}9 \pm 1{\cdot}2$ (14)**	$4{\cdot}5 \pm 1{\cdot}4$ (8)**	$6{\cdot}8 \pm 1{\cdot}1$ (6)***	$6{\cdot}2 \pm 1{\cdot}6$ (6)***
Adrenaline 6×10^{-6} M	$5{\cdot}8 \pm 1{\cdot}3$ (9)	$5{\cdot}1 \pm 1{\cdot}4$ (9)	$2{\cdot}3 \pm 0{\cdot}9$ (5)	$0{\cdot}3 \pm 1{\cdot}6$ (9)	$3{\cdot}0 \pm 1{\cdot}3$ (5)	—	—
Adrenaline $1{\cdot}5 \times 10^{-5}$ M	$7{\cdot}2 \pm 0{\cdot}7$ (5)	$7{\cdot}1 \pm 0{\cdot}3$ (5)	4·6 (3)	$0{\cdot}2 \pm 1{\cdot}0$ (5)	$-1{\cdot}7$ (3)	$-8{\cdot}8$, $-3{\cdot}8$ (2)	$-10{\cdot}0$, $0{\cdot}5$ (2)

In last four columns: significance levels of difference from zero: * $0{\cdot}01 < P < 0{\cdot}05$
** $0{\cdot}001 < P < 0{\cdot}01$
*** $P < 0{\cdot}001$.

amine addition (isoprenaline $r = 0{\cdot}25$, $P = 0{\cdot}4$; noradrenaline $r = 0{\cdot}21$, $P = 0{\cdot}5$). It would appear from the differences in significance levels that there are more components in the response to noradrenaline than to isoprenaline.

The net flux of Na^+

In six experiments (two each of adrenaline, noradrenaline and isoprenaline) bidirectional fluxes of Na and Cl were measured simultaneously and the total charge transfer $J_{Na}+J_{Cl}$ compared with the mean I_{sc} for each period. The mean discrepancy between these two quantities ($I_{sc}-(J_{Na}+J_{Cl})$) in the three half-hour periods before, and periods II–IV after catecholamine addition was $-0{\cdot}47 \pm 0{\cdot}45$, and $0{\cdot}61 \pm 0{\cdot}81$ n-equiv. cm^{-2} min^{-1} respectively, neither of which were significantly different from zero. In the first period after catecholamine addition (period I) the mean discrepancy was significant ($4{\cdot}4 \pm 2{\cdot}1$ n-equiv. cm^{-2} min^{-1}), although still much smaller than the I_{sc} value at this point (about 20 n-equiv. cm^{-2} min^{-1}). In none of the succeeding three periods was the mean for any period more than 2 S.E. different from the over-all mean. It would seem that the net movements of Na and Cl account for the major, if not the entire part of the short-circuit current response. This being so, in the experiments in which bidirectional Cl fluxes were measured, an estimate of J_{Na} can be made from the quantity $I_{sc}-J_{Cl}$ in each period. There was a significant fall in the mean value of this quantity J_X (defined as $I_{sc}-J_{Cl}$) during the first hour after noradrenaline, after isoprenaline a significant rise and after adrenaline little change (Table 1). In individual experiments, no association between changes in J_X and J_{Cl} was apparent. In some experiments J_X could be estimated for a second hour also; in the case of isoprenaline the significant increase was maintained. This difference was confirmed in several experiments in which the bidirectional Na fluxes were measured directly, although the fall in J_{Na} following noradrenaline was not apparent until the second hour, and the rise was rather greater following isoprenaline.

Effects of a β-adrenergic blocking agent

Addition of oxprenolol ($4{\cdot}6 \times 10^{-5}$ M) 1 hr aftert he addition of catecholamine produced a prompt fall in I_{sc} usually to values approximately equal to or less than those before catecholamine addition (Fig. 2). This reduction in I_{sc} was reflected in the disappearance of net Cl efflux (Table 2) and a reduction of J_X to pre-catecholamine values in the case of isoprenaline, and below them in the case of noradrenaline and adrenaline. The magnitude of the fall in I_{sc} due to oxprenolol (ΔI_{sc}^{β}) was compared with the rise in mean I_{sc} (by graphical integration) during the first hour after catecholamine addition ($\Delta \bar{I}_{sc}$). These values were significantly correlated

in the case of isoprenaline ($r = 0{\cdot}95$; $P = 0{\cdot}004$), but not in the case of noradrenaline ($r = 0{\cdot}07$; $P = 0{\cdot}9$). The mean $\Delta\bar{I}_{sc}$ was significantly less than ΔI_{sc}^{β} in the case of noradrenaline but not in the case of isoprenaline, again indicating that another component, acting to decrease the short-circuit current, was present in the noradrenaline response.

TABLE 2. The effects of the addition of $4{\cdot}6 \times 10^{-5}$ M oxprenolol 1 hr after catecholamine addition. The mean ($\pm$ S.E.) rise in I_{sc} during the first hour ($\Delta\bar{I}_{sc}$) is compared to the fall in I_{sc} to steady values following β-blockade, ΔI_{sc}^{β}. $J_{Cl}(\beta)$ is the mean value of net Cl efflux, and $\Delta J_{x}(\beta)$ the change in 'non-Cl' short-circuit current in the hour following β-blockade, compared with the value during the hour before catecholamine addition. All values in n-equiv. cm^{-2} min^{-1}. Concentrations as in Table 1, adrenaline at lower of the two concentrations

Catecholamine	$\Delta\bar{I}_{sc}$	ΔI_{sc}^{β}	$J_{Cl}(\beta)$	$\Delta J_{x}(\beta)$
Noradrenaline	$3{\cdot}6 \pm 0{\cdot}7$ (6)	$6{\cdot}9 \pm 0{\cdot}8$ (6)	$0{\cdot}4 \pm 0{\cdot}3$ (6)	$-4{\cdot}8 \pm 0{\cdot}9$ (5)***
Isoprenaline	$8{\cdot}7 \pm 1{\cdot}5$ (6)	$10{\cdot}0 \pm 1{\cdot}6$ (6)	$0{\cdot}6 \pm 0{\cdot}3$ (6)	$-0{\cdot}5 \pm 0{\cdot}5$ (6)
Adrenaline	3·4, 8·7	6·3, 11·6	−2·5, 0·3	−1·6, −4·9

In last column significance level of change from zero: *** $P < 0{\cdot}001$.

TABLE 3. Comparison of increase in Na efflux (averaged over the first and second hour period after catecholamine addition) compared with the mean values of net Cl efflux during the same periods in a separate series of experiments. Values in n-equiv. cm^{-2}. min^{-1}. Numbers of experiments shown in parentheses

		Δj_{Na}^{out}	J_{Cl}
Noradrenaline	1st hour	$4{\cdot}3 \pm 0{\cdot}8$ (6)	$5{\cdot}8 \pm 0{\cdot}6$ (14)
	2nd hour	$2{\cdot}4 \pm 0{\cdot}3$ (4)	$3{\cdot}0 \pm 0{\cdot}4$ (5)
Isoprenaline	1st hour	$5{\cdot}8 \pm 0{\cdot}9$ (6)	$4{\cdot}2 \pm 0{\cdot}4$ (14)
	2nd hour	$4{\cdot}8 \pm 0{\cdot}9$ (6)	$3{\cdot}4 \pm 0{\cdot}7$ (8)
Adrenaline	1st hour	7·3 (2)	$7{\cdot}1 \pm 0{\cdot}3$ (5)
	2nd hour	4·7 (2)	4·6 (3)

For $n > 3$ the values are significantly different from zero. None of the differences between corresponding values in the two columns are significant; for pooled data $P = 0{\cdot}95$ for 1st hour, $P = 0{\cdot}45$ for 2nd hour.

Oxprenolol added 1 hr before the addition of catecholamine generally abolished the response to isoprenaline. There was an inhibition of I_{sc} following the addition of noradrenaline to previously β-blocked skins (Fig. 3), but this effect was more marked in some cases than in others, and in some, the β-blockade was overcome after about 10 min, leading to a stimulation.

Comparison of Na and Cl efflux

A close association between the magnitude of the rise in j_{Cl}^{out} and j_{Na}^{out} was apparent in the case of all three catecholamines in those experiments in which these two fluxes were measured simultaneously, over four half-hour periods. In these experiments the rise in Na and Cl fluxes did not vary appreciably with time. The pooled data ($n = 33$ pairs) showed a highly significant correlation ($r = 0{\cdot}56$, $P < 0{\cdot}001$) between the magnitude of the rises in Na and Cl efflux. Taking all experiments in which j_{Na}^{out} was measured, the mean increase in the first and second hours after catecholamine addition did not differ significantly from the mean values of *net* Cl efflux (J_{Cl}) in separate series of experiments (Table 3).

TABLE 4. Comparison of the difference between Na influx and average short-circuit current ($j_s = j_{Na}^{in} - \bar{I}_{sc}$) in the three half-hour periods before and four after catecholamine addition. Values in n-equiv. cm^{-2} min^{-1}. Numbers of experiments represented shown in parentheses

Catecholamine	Before			After			
	I	II	III	I	II	III	IV
Noradrenaline (9)	2·10	1·09	2·23	0·18*	3·46*	2·70	1·31
Group mean ± S.E.	1·78 ± 0·41			1·95 ± 0·50			
Isoprenaline (10)	0·84	1·60	1·49	−0·57*	2·66	3·57*	2·02
Group mean ± S.E.	1·30 ± 0·31			1·92 ± 0·49			
Adrenaline (2)	0·80	2·75	0·85	−7·20	1·00	0·40	−0·40

* These values more than 2 S.E. from the group mean.

Comparison of I_{sc} with Na influx

The parallel changes in I_{sc} and j_{Na}^{in} were a feature of the response to catecholamines. To estimate the extent to which j_{Na}^{in} mirrors changes in short-circuit current, the mean value of I_{sc} for each half-hour period was subtracted from the value of j_{Na}^{in} for that period and the value of this quantity (j_s) in the periods before and after catecholamine addition compared (Table 4). Addition of noradrenaline or isoprenaline did not significantly change the value of j_s averaged over the periods, although individual period means were more than 2 S.E. away from the group means, indicating perhaps a lag of Na influx measurement behind current measurement. In an analysis of variance (omitting period I after catecholamine addition because of the poorer precision of measurement of j_s in that period) the presence of catecholamine was found not to be a significant source of variance in the case of noradrenaline, and only probably

significantly so in the case of isoprenaline. The major source of variance was that between experiments. The values of j_s were much less than the change in the value of j^{in}_{Na} due to catecholamine addition, which typically rose from about 8 to 16 n-equiv. cm^{-2} min^{-1}.

Conductance changes

The values of total membrane conductance, G_m, remain elevated over a period of at least 2 hr after catecholamine addition (Table 5). In those experiments in which oxprenolol was added after 1 hr, G_m was reduced to control levels, or perhaps to below them in the case of noradrenaline. The values of g^o_{Cl} (mean Cl conductance before catecholamine addition) are included as a comparison. Since Na fluxes (and Cl fluxes after catecholamine addition) have active components, it is impossible to estimate these conductances without a value for the e.m.f. of the active component or a reliable method for estimating the proportion of these fluxes which occur via passive 'leak' or 'shunt' pathways. In those experiments in which catecholamine was added to skin to which oxprenolol had been added 1 hr previously, there was no significant change in G^o_m either with the addition of oxprenolol or catecholamine ($n = 6$).

TABLE 5. Values of skin conductance (mean ± S.E.) before catecholamine addition (G^o_m) compared with those during the first or second hour periods after addition ($G^{(1)}_m$ and $G^{(2)}_m$ respectively). In some experiments ($+\beta$), oxprenolol ($4{\cdot}6 \times 10^{-5}$ M) was added at the end of the first hour period. Numbers of experiments shown in parentheses. Values in $k\Omega^{-1}.cm^{-2}$. Values of initial Cl conductance, g^o_{Cl} included for comparison (estimated from $g^o_{Cl} = F^2 j^o_{Cl}/RT$)

		G^o_m	$G^{(1)}_m$	$G^{(2)}_m$	g^o_{Cl}
Noradrenaline	(5)	0·37 ± 0·04	0·72 ± 0·04	0·66 ± 0·04	0·13 ± 0·04
Noradrenaline + β	(5)	0·35 ± 0·02	0·61 ± 0·06	0·28 ± 0·02	0·21 ± 0·04
Isoprenaline	(6)	0·47 ± 0·05	0·79 ± 0·04	0·76 ± 0·06	0·19 ± 0·07
Isoprenaline + β	(6)	0·40 ± 0·06	0·72 ± 0·08	0·31 ± 0·04	0·19 ± 0·04
Adrenaline	(3)	0·31 ± 0·03	0·59 ± 0·15	0·55 ± 0·17	0·12 ± 0·02
Adrenaline + β	(2)	0·48	0·71	0·30	0·14
Adrenaline′	(3)	0·60 ± 0·04	1·37 ± 0·16	1·23 ± 0·12	0·28 ± 0·04

Concentrations: noradrenaline, $1{\cdot}6 \times 10^{-5}$ M; isoprenaline, 8×10^{-7} M; adrenaline, 6×10^{-6} M; adrenaline, $1{\cdot}5 \times 10^{-5}$ M.

Action of acetazolamide

In three experiments, acetazolamide (to give a final concentration of $1{\cdot}1 \times 10^{-4}$ M) was added ½ hr before, and in two experiments ½ hr after the addition of catecholamine. In none of these was there any effect on the short-circuit current, nor on the Cl efflux response to noradrenaline addition (Fig. 4).

Tissue labelling

The level of exchange of ^{36}Cl (or ^{82}Br) at the end of each experiment was estimated from the ratio of tissue : bathing solution specific activities for each radionuclide. Those skins in which the β-stimulation (and hence net Cl movement) had been inhibited by prior treatment with oxyprenolol were found to have a significantly lower value of exchange from the outside solution than that for β-stimulated skins ($0{\cdot}6 \pm 0{\cdot}1$ and $1{\cdot}3 \pm 0{\cdot}1$% respectively). There was no significant difference in the exchange from the inside solution (32 ± 5 and 28 ± 2% respectively), nor in water content (measured as weight of tissue water per unit dry weight), nor in Cl content.

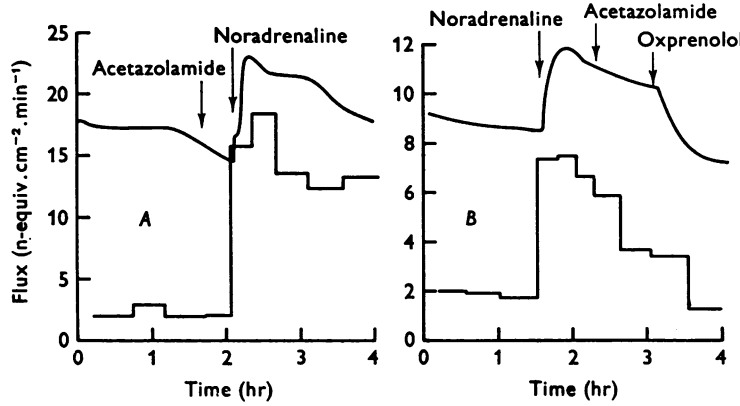

Fig. 4. Absence of effect of acetazolamide ($1{\cdot}1 \times 10^{-4}$ M) on the short-circuit current (curve) or Cl efflux (bars) in frog skin. *A*, acetazolamide added before noradrenaline ($1{\cdot}6 \times 10^{-5}$ M). *B*, acetazolamide added subsequent to noradrenaline. Oxprenolol ($4{\cdot}5 \times 10^{-5}$ M) added subsequent to both produced a reduction of short-circuit current and Cl efflux to their initial levels, as usually observed.

DISCUSSION

The Cl flux data presented in this study support the hypothesis (Watlington, 1968) that β-stimulation produces a net Cl efflux and an increase in passive conductivity, and α-stimulation produces no net Cl efflux and a decrease in short-circuit current. The α-adrenergic inhibitory effect was much less marked in *Rana temporaria* than in *R. pipiens*, the species used in Watlington's study. However, there is good evidence from this study that the Na influx and efflux are dissociated, with a strong association between Na efflux increase and net Cl efflux, which is in agreement with the findings of McAfee (1970) and Lang, Sjöberg & Skoglund (1975) that the glandular secretions of *Ranidae* contain equal amounts of Na and Cl. The findings in the present experiments were that isoprenaline increased

net Na flux, whereas Watlington (1968) reported that this did not change in *R. pipiens* preparations. This may be of little consequence, however, since net Na transport may be compounded of two independent processes and not really a characteristic measure of the response.

There are a number of factors which make the response of amphibian skin to catecholamines somewhat complex. In the first place, these compounds not only have an action on the skin glands but also on the non-glandular or transporting epithelium. This is evidenced by the work of Rajerison, Montegut, Jard & Morel (1972) who showed that noradrenaline increased net Na flux in a short-circuited preparation of *R. esculenta* skin epithelium, split away from the underlying corium containing the glands. However, the net Cl efflux observed in intact skin did not occur in these preparations. Secondly, the glands themselves are of two types, mucous and serous, the latter being more common in the dorsal regions of *R. temporaria* skin. Benson & Hadley (1969) observed (in *R. pipiens*) the appearance of the milky serous secretion directly, and concluded that only α-adrenergic stimulation produced a serous secretion response. Lindley (1969) and Seldin & Hoshiko (1966) observed the production of droplets of mucous secretion in response to adrenaline and noradrenaline. The analyses of Watlington & Huf (1971), House (1969*a*, *b*) and McAfee (1970) were carried out on frog and toad skin secretions collected from both types of gland.

Thirdly, the time course of the ion flux changes (at least 3 hr in duration) is two orders of magnitude greater than that for the appearance of secretion droplets, no more of which appear after about 2 min exposure to adrenaline (Seldin & Hoshiko, 1966). The electrical response to the skin to an electrical stimulus of the skin nerve is of about the same order of magnitude in duration (2–3 min) as the appearance of droplets (Schoffeniels & Salée, 1965; Lindley, 1969). However, Watlington & Huf (1971) showed that after an initial peak, the secretion rate was still several times greater 3 hr after catecholamine addition than before.

There are two types of mechanism whereby the flow of electrolyte from mucous glands could be stimulated: (i) a mechanical contraction of smooth muscle surrounding the gland, or opening of the gland neck, allowing the gland contents to escape, and (ii) a neurochemical stimulation of the movement of ions into the gland lumen. The first of these is unlikely since similar amounts of stainable material were present in mucous glands of both untreated- and catecholamine-treated skins (Benson & Hadley, 1969) and since the mucous glands did not change shape after catecholamine treatment (Lindley, 1969). If on the other hand a stimulation of ion movement is responsible, the receptor involved would have to be insensitive to the other hormones which alter short-circuit current without

stimulation of a net Cl movement (ADH and aldosterone, for example). It is possible that certain cells in the glands are specialized to secrete Cl, perhaps in a manner similar to the 'chloride cells' in fish gills (Philpott, 1966). The increased Na efflux as an accompanying cation would be a consequence of increased passive shunt pathways through the epithelium. However, acetazolamide, which does inhibit the net Cl influx which occurs under certain circumstances (Erlij, 1971; Kristensen, 1972) had no effect on Cl efflux in stimulated skins. Secondly, the amount of Cl exchange to the tissue from the outside solution indicates that catecholamine stimulation favours Cl uptake from the outside solution, since the exchange from the inner solution and specific volume remain unchanged. If the increased exchange from the outside arises from an increase in electropositivity in the lumen of the glands, this would be the reverse of what might be expected with a lumen-directed Cl pump. In fact, Lindley (1969) found that the potential in stimulated glands changed from being negative to positive with respect to the inner surface of the skin, although this might be a feature only of events in the first few minutes after stimulation. These findings make an electrogenic Cl pump unlikely, but do not rule it out.

Other possibilities are either that Na is pumped into the lumen, (it being pumped in two directions at once), or that NaCl is secreted by a neutral pump as indicated earlier. A neutral pump has been suggested to operate in the intestinal mucosa (Taylor, Wright, Schultz & Curran, 1968) and would account for the close association of the individual ion effluxes, and also that Na is required in the inside solution for a net Cl efflux to occur (Pinschmidt, Campbell & Huf, 1973).

The serous glands, on the other hand, are relatively few in number in *R. temporaria* skin and would not be expected to contribute much to the total ion flux. It is likely that the gland contents of these glands are extruded by mechanical contraction in a relatively short period of time.

From the foregoing considerations it would appear that the ionic changes described here are essentially: (i) a β-adrenergic stimulation of j_{Na}^{in} across the skin epithelial layer, (ii) a β-adrenergic stimulation of an outward secretion of NaCl from the mucous glands, (iii) a small α-adrenergic inhibition of j_{Na}^{in}, moderating the β-adrenergic effect, and (iv) a small transient β-adrenergic mediated increase of shunt permeability, reflected in changes in j_{Cl}^{in}, and perhaps accounting in part for changes in j_{Na}^{out}. It is unlikely that some of these changes are simply the consequences of changes in ionic selectivity properties, since in several experiments j_{Na}^{out} increased tenfold, and j_{Cl}^{in} not at all.

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