J. Membrane Biol. 135–150 (1978)

Effect of Amiloride on Catecholamine-Induced Changes in Ion Transport in Short-Circuited Frog Skin

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Received 30 November 1977

Summary. The effect of amiloride (10^{-6} M), added either before or after the catecholamine, on the adrenaline- or isoprenaline-induced changes in short-circuit current and Na and Cl fluxes of isolated skin of *Rana temporaria* was investigated. At the catecholamine concentration used the increment in short-circuit current was the same in the absence or presence of amiloride (ca. 7.1 neq·cm^{-2}·min^{-1}) and the amiloride inhibition was the same in the absence or presence of catecholamine (ca. 9.4 neq·cm^{-2}·min^{-1}). Amiloride inhibited the Na and Cl influxes of the control period (by -8.63 ± 1.28 and -2.08 ± 0.75 neq·cm^{-2}·min^{-1}, respectively) but did not prevent the increase of these fluxes on the addition of adrenaline. There was no evidence of amiloride inhibition of the Na and Cl effluxes. There was an association between the increase of Na efflux and *net* Cl efflux following adrenaline, which if secreted together by a neutral NaCl pump would not contribute to the increased short-circuit current. The increased short-circuit current was correlated with the increased Na influx throughout the experiment if allowance is made for the periods where there is a lag between the current and isotopic measurement (i.e., the period immediately after the addition of a drug). It is tentatively suggested that the catecholamine-induced increase in Na influx is not altered by the amiloride concentration used in this study. In addition the magnitude of the changes induced by catecholamine in the influx and efflux of both Na and Cl seem to be unaffected.

In a previous paper (Tomlinson & Wood, 1976) it was shown that 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. In addition there was evidence for a small and variable increase of shunt permeability, reflected in changes in Cl influx and perhaps accounting in a minor part for changes in Na efflux. All these changes were brought about by β-adrenergic stimulation.

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0022-2631/78/S040-0135 $02.80

In this paper we present preliminary results of the effect of amiloride on the changes in Na and Cl fluxes induced by adrenaline and isoprenaline.

Materials and Methods

Samples of skin from *Rana temporaria* were mounted between glass chambers (area 6.7 cm^2), and the short-circuit current (I_{sc}) was monitored automatically. The membrane conductance (G_m) was measured as the fall in I_{sc} due to a 10 mV depolarization.

Unidirectional Na fluxes were measured using the radionuclide $^{22}NaCl$ and Cl fluxes with $Na^{36}Cl$ and $Na^{82}Br$, correcting the ^{82}Br fluxes with the appropriate relative rate factor (f). The values of f for untreated and catecholamine treated frog skin have been published previously (Tomlinson & Wood, 1972).

The activity of γ-emissions from ^{82}Br were measured with a Nuclear Enterprises automatic gamma spectrometer and that of β-emissions from ^{36}Cl and ^{22}Na with a Packard 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's solution was as follows (in mM): NaCl, 111.2; KCl, 1.9; $NaHCO_3$, 2.4; $CaCl_2$, 1.0. The solutions within the chambers were mixed and oxygenated by a stream of air saturated with water.

Catecholamines used were: adrenaline (1:1000 British Pharmacopoeia, Antigen, Ltd.) and isoprenaline (as sulphate; Wellcome). The amiloride was obtained from Merck, Sharp and Dohme, Ltd. The final concentrations used in these experiments were: adrenaline 1.5×10^{-5} M, isoprenaline 9.6×10^{-7} M and amiloride 0.92×10^{-6} M.

These concentrations were chosen to give maximum response (Fassina, Carpenedo & Fiandini, 1968; Ambalavanar, Foster & Schnieden, 1973) and sufficiently high to cause a net Cl efflux (Bastide & Jard, 1968) in the case of the catecholamines and to produce a marked inhibition of Na influx in the case of amiloride.

Two experimental protocols were used; the first 4–20 min periods acted as a control period and this was followed by either (i) two 20-min periods of amiloride followed by three 20-min periods of catecholamine or (ii) two 20-min periods of catecholamine followed by three 20-min periods of amiloride. The catecholamines were added to the inside and the amiloride to the outside bathing solutions. Once added the drugs were present for the duration of the experiment.

For the histological examination of the skin mucous glands, six paired skins were mounted, one of the pair acting as a control while to the other isoprenaline was added. After approximately 75 min the skins were removed, fixed, paraffin sections cut, and the sections stained with haematoxylin and eosin. Microphotographs were taken of each complete transverse section and two diameters of each mucous gland measured. From these diameters the surface area of each gland was calculated.

Results

Short-Circuit Current Response

The typical patterns of I_{sc} response in the two experimental protocols are depicted in Fig. 1 for adrenaline and Figs. 6 and 8 for isoprenaline.

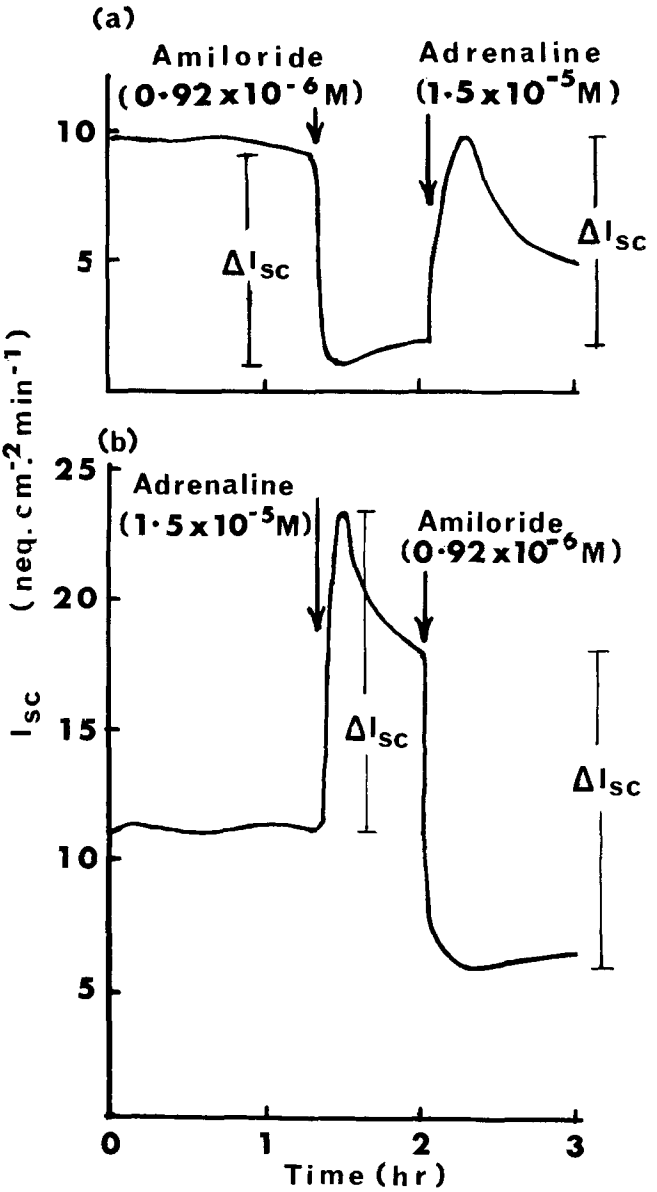

Fig. 1. Typical responses of the short-circuit current (I_{sc}) to (*a*) amiloride then adrenaline (protocol 1) and (*b*) adrenaline then amiloride (protocol 2). ΔI_{sc} is the change in I_{sc} from its value just before the addition of the drug and the drug's maximum effect

Comparison of the catecholamine response between the two protocols showed that the addition of amiloride before the catecholamine tended to retard the achievement of the maximum I_{sc} response. This was more noticeable in the case of isoprenaline where the sharp rise in I_{sc} to

Table 1. The mean (±SE) change in short-circuit current caused by amiloride, adrenaline, or isoprenaline in the two experimental protocols[a]

Protocol		1 (amiloride then catechol.)	2 (catechol. then amiloride)
Amiloride inhibition	ΔI_{sc}	8.51± 0.62 (22)	10.21±0.76 (9)
	%	81 ± 2 (22)	78 ±4 (9)
Adrenaline increase	ΔI_{sc}	6.73± 0.76 (22)	7.72±1.01 (9)
	%	349 ±50 (22)	83 ±6 (17)
Amiloride inhibition	ΔI_{sc}	11.15± 1.05 (6)	11.6, 10.0 (2)
	%	83 ± 4 (6)	83, 83 (2)
Isoprenaline increase	ΔI_{sc}	6.94± 0.71 (6)	9.7, 7.4 (2)
	%	285 ±55 (6)	145, 110 (2)

[a] Values given as % change and ΔI_{sc} in neq·cm^{-2}·min^{-1}. Figure in parentheses is number of observations.

a maximum and the beginning of the decline which occurred within 20 min in protocol 2 (Fig. 8) was replaced by a more gradual rise which reached a maximum between 30–60 min when using protocol 1 (Fig. 6).

The degree of inhibition of I_{sc} by amiloride and increase of I_{sc} by the catecholamines are shown in Table 1.

There was no significant difference between the ΔI_{sc} (amiloride) obtained from the two protocols nor the ΔI_{sc} (adrenaline).

There was a highly significant correlation between ΔI_{sc} (amiloride) and the I_{sc} immediately before the addition of amiloride ($r=0.91$, $P<0.001$ for protocol 1, and $r=0.90$, $P<0.001$ for protocol 2 using adrenaline).

Following the first protocol, there was no significant correlation between ΔI_{sc} (amiloride) and ΔI_{sc} (adrenaline) ($r=-0.20$, $P>0.1$), between ΔI_{sc} (amiloride) and ΔI_{sc} (isoprenaline ($r=0.45$, $P>0.1$), nor between the amiloride I_{sc} immediately before the addition of catecholamine and the ΔI_{sc} (adrenaline) or the ΔI_{sc} (isoprenaline) ($r=0.08$, $P>0.1$; $r=0.55$, $P>0.1$, respectively).

With the second protocol, there was a probably significant correlation between ΔI_{sc} (amiloride) and ΔI_{sc} (adrenaline) ($r=0.71$, $P=0.03$) and between ΔI_{sc} (adrenaline) and the control I_{sc} immediately before the addition of adrenaline ($r=0.70$, $P=0.04$).

Sodium Fluxes

The results of the measurement of Na influx and efflux separately on different skins for nine 20-min periods are shown in Fig. 2. The

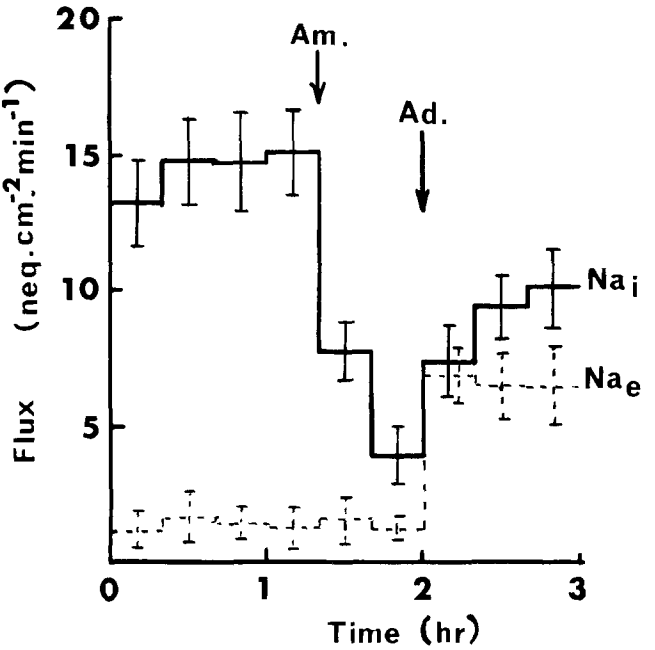

Fig. 2. Mean (±SE) sodium influx (Na_i) and efflux (Na_e) for each period of 20 min before and after the addition of amiloride (*Am.*) and adrenaline (*Ad.*). Two separate groups of skins were used for the influx and efflux measurements

Table 2. Mean (±SE) Na and Cl fluxes for the control, amiloride, and adrenaline periods[a]

	Control	Amiloride	Adrenaline
Na influx (8)	14.47±0.79	5.84±0.88	8.91±0.77
Na efflux (6)	1.42±0.34	1.39±0.46	6.59±0.68
ΔNa	13.05[c]	4.45[c]	2.32[b]
Cl influx (9)	5.15±0.49	3.07±0.41	4.40±0.33
Cl efflux (6)	2.57±0.35	1.93±0.38	9.44±0.66
ΔCl	2.58[b]	1.14	−5.04[c]

[a] Values expressed as neq·cm^{-2}·min^{-1}. The number in parentheses represents the number of experiments. In each experiment there were four 20-min periods for the control, two 20-min periods for amiloride and three 20-min periods for adrenaline.
[b] $0.01 < P < 0.05$.
[c] $P < 0.001$.

mean results for the control, amiloride and adrenaline periods are set out in Table 2. Following the addition of amiloride there was a marked decrease of Na influx with no change in the Na efflux. The addition of adrenaline resulted in an increase of both Na influx and Na efflux; the increase in influx being maintained over the 1-hr period and the

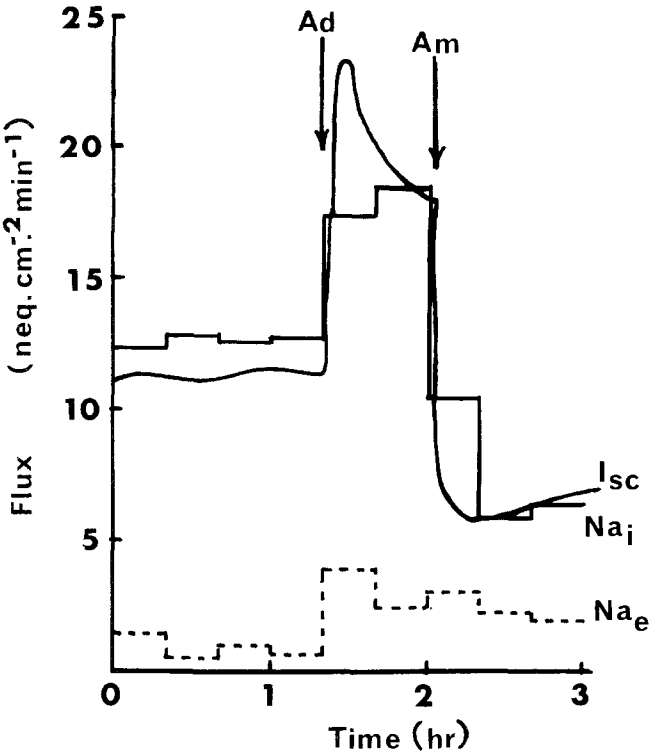

Fig. 3. Effect of adrenaline (*Ad.*) and amiloride (*Am.*) on the bidirectional fluxes of Na measured separately in a paired skin sample. The short-circuit current (I_{sc}) for the skin used for the Na influx (Na_i) measurement is also shown

efflux reaching a plateau during the first 20-min period after the addition of the adrenaline.

Consideration of each of the nine 20-min periods showed that there was a significant net Na influx in the first five only (i.e., the four control periods and the first amiloride period). The correlation between I_{sc} and Na influx was highly significant ($P<0.001$) for the four control periods ($r=0.87$, 0.90, 0.94, and 0.93), not significant ($P>0.05$) for the two amiloride and first adrenaline period ($r=0.54$, 0.24 and 0.15, respectively), and significant ($P<0.01$) for the last two adrenaline periods ($r=$ 0.81 and 0.86).

Isoprenaline (4 experiments) produced similar changes in Na influx and I_{sc} as adrenaline.

A typical result of using the second protocol of adrenaline first and then amiloride on sodium fluxes is shown in Fig. 3. Adrenaline increased both Na influx and efflux the influx being decreased by the addition of amiloride whereas the efflux was virtually unchanged.

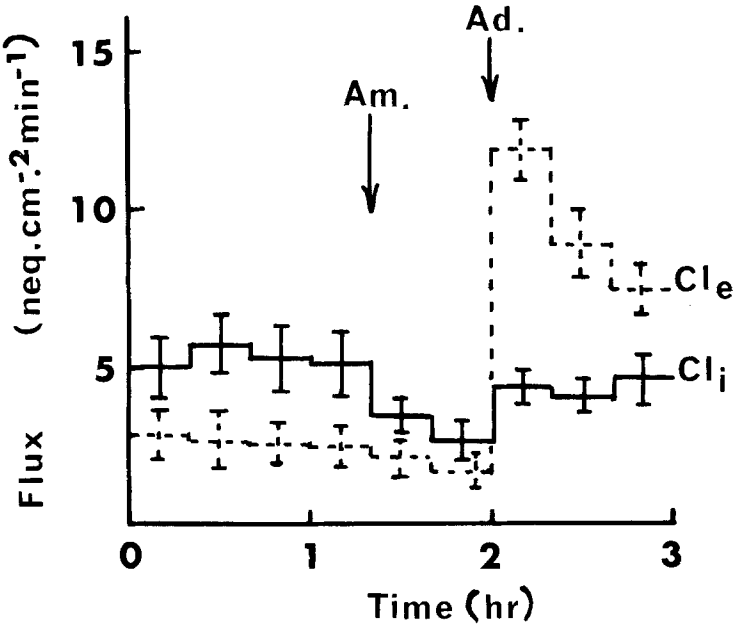

Fig. 4. Mean (±SE) chloride influx (Cl_i) and efflux (Cl_e) for each period of 20 min before and after the addition of amiloride (*Am.*) and adrenaline (*Ad.*). Two separate groups of skins were used for the influx and efflux measurements

Chloride Fluxes

The results of the measurement of Cl influx and efflux separately on different skins and in a few cases simultaneously on the same skin are shown in Fig. 4. The mean results for the control, amiloride and adrenaline periods are set out in Table 2. It will be seen that the skins on which the influx measurements were made were more permeable to chloride (range 1.21–10.46 neq·cm^{-2} min^{-1}) than those used for efflux measurements (range 1.18–6.45 neq·cm^{-2} min^{-1}). Comparison of the two groups of results thus shows an apparent probably significant net Cl influx for the control periods. However, experiments in which the bidirectional fluxes were measured on the same preparation showed no net Cl influx (Fig. 5).

Consideration of each of the nine 20-min periods showed a probably significant net Cl influx for periods 2, 3 and 4 (control) and a significant net Cl efflux for periods 7, 8 and 9 (adrenaline).

Amiloride produced a significant decrease in Cl influx, whereas adrenaline produced a probably significant increase in Cl influx and a highly significant increase in Cl efflux (Table 3).

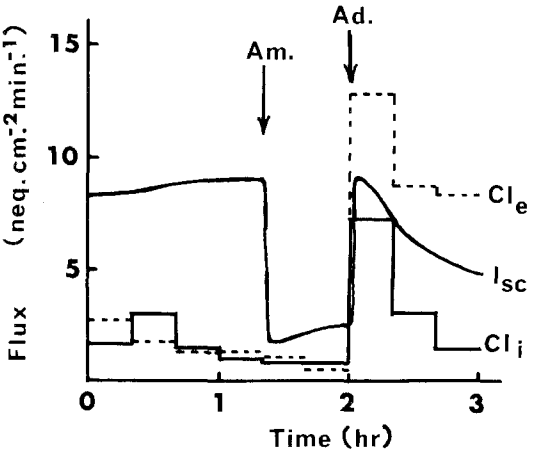

Fig. 5. Effect of amiloride (*Am.*) and adrenaline (*Ad.*) on the bidirectional fluxes of Cl measured simultaneously in short-circuited skin. Cl_i is ^{82}Br-traced Cl influx; Cl_e is ^{36}Cl-traced Cl efflux

Table 3. Mean differences Δ ($\pm$SEM) between the control-amiloride and amiloride-adrenaline periods for Na and Cl fluxes[a]

	Control-amiloride	Amiloride-adrenaline
ΔNa influx	-8.63 ± 1.28[d]	$+3.07 \pm 1.18$[c]
ΔNa efflux	-0.03 ± 0.59	$+5.20 \pm 0.89$[d]
ΔCl influx	-2.08 ± 0.75[c]	$+1.33 \pm 0.52$[b]
ΔCl efflux	-0.64 ± 0.56	$+7.51 \pm 0.87$[d]

[a] Values in neq $\cdot$ cm^{-2} $\cdot$ min^{-1}. This datum is derived from the results presented in Table 2.
[b] $0.01 < P < 0.05$.
[c] $0.001 < P < 0.01$.
[d] $P < 0.001$.

Isoprenaline (2 bidirectional measurements) produced similar changes (Fig. 6).

A typical result of using the second protocol of adrenaline or isoprenaline first and then amiloride on Cl bidirectional fluxes are shown in Figs. 7 and 8. In four such experiments the catecholamine produced a marked increase in Cl efflux and a variable response in Cl influx (either no change or an increase). In all four experiments the Cl efflux remained elevated over the control periods for the remainder of the experiment and did not appear to be affected by the addition of amiloride. Similarly the Cl influx when it was increased (Fig. 7) remained elevated

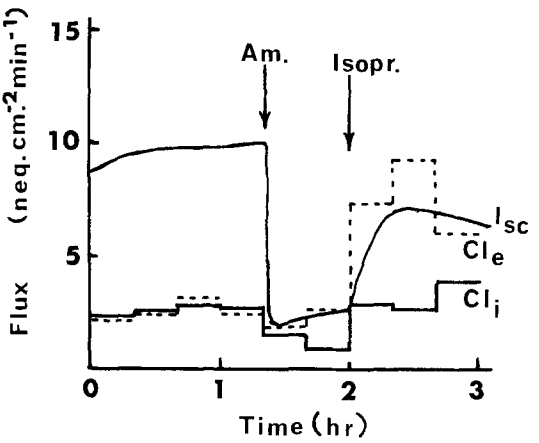

Fig. 6. Effect of amiloride (*Am.*) and isoprenaline (*Isopr.*) on bidirectional fluxes of Cl measured simultaneously in short-circuited skin. Cl_i is ^{36}Cl-traced Cl influx; Cl_e is ^{82}Br-traced efflux

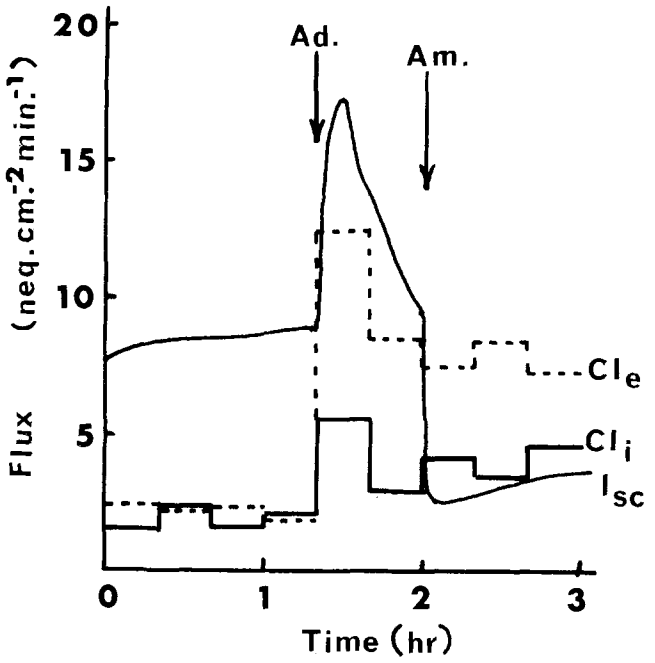

Fig. 7. Effect of adrenaline (*Ad.*) and amiloride (*Am.*) on the bidirectional fluxes of Cl measured simultaneously in short-circuited skin. Cl_i is ^{36}Cl-traced Cl influx; Cl_e is ^{82}Br-traced efflux

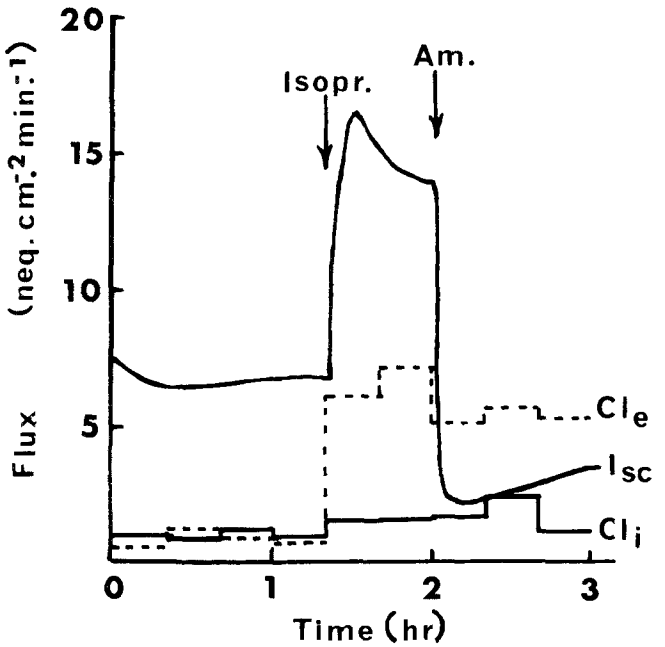

Fig. 8. Effect of isoprenaline (*Isopr.*) and amiloride (*Am.*) on the bidirectional fluxes of Cl measured simultaneously in short-circuited skin. Cl_i is ^{82}Br-traced Cl influx; Cl_e is ^{36}Cl-traced efflux

over the control periods for the remainder of the experiment. Whether the Cl influx was increased or not, there was no indication of it being inhibited by amiloride.

Comparison of I_{sc} with Na Influx

As mentioned in the section on sodium fluxes, a good correlation was obtained between the average short-circuit current and the Na influx for the four control periods and the last two adrenaline periods only. To estimate the extent to which Na influx mirrors changes in short-circuit current, the mean value of I_{sc} for each 20-min period was subtracted from the value of Na influx for that period and the value of this quantity (j_s) compared for the control, amiloride, and catecholamine periods (Table 4).

Addition of amiloride produced a probably significant increase in the value of j_s averaged over the control and amiloride periods ($P=0.02$).

Table 4. Comparison of the difference between Na influx and average short-circuit current ($j_s = Na_i - \bar{I}_{sc}$) in the nine 20-min periods (4 control, 2 amiloride, and 3 catecholamine)[a]

	Control				Amiloride		Catecholamine		
	1	2	3	4	5	6	7	8	9
Adrenaline (8)	1.29[b]	2.51	2.28	2.64	5.50[b]	1.75[b]	0.64	1.24	2.57
Group mean ±SE	2.18±0.43				3.62±0.82		1.48±0.71		
Isoprenaline (4)	1.87	2.81	3.06	2.45	5.87	2.66	−0.52	−0.78	2.54[b]
Group mean ±SE	2.55±0.37				4.26±0.86		0.42±0.84		
Combined (12)	1.48[b]	2.61	2.54	2.58	5.62[b]	2.05	–		
Group mean ±SE	2.30±0.31				3.84±0.61				

[a] Values in neq·cm^{-2}·min^{-1}. Number of experiments represented shown in parentheses.
[b] These values more than 2 SE from the group mean.

The first amiloride period was more than 2 SE away from the group mean for the combined experiments, indicating perhaps a lag of Na influx measurement behind current measurement.

Addition of adrenaline did not produce any significant change in j_s compared with the value for either the control or amiloride periods ($P=0.4$ and 0.06, respectively). The value of j_s for isopremaline decreased probably significantly from the control value ($P=0.02$) and decreased significantly from the amiloride value ($P=0.006$).

In an analysis of variance the presence of amiloride and catecholamine was found not to be a significant source of variance in the case of adrenaline treated skins and only probably significant ($P=0.02$) in the case of isoprenaline. The major source of variance was that between experiments. Inspection of Table 4 further shows that the range of j_s is rather small (1.24–3.06) when the first amiloride, first adrenaline, and first and second isoprenaline periods are omitted. As mentioned previously this may be justified because of the lag of Na influx measurement behind I_{sc} when the I_{sc} changes suddenly upon the addition of a drug. In the case of isoprenaline the retarded response after amiloride may produce a lag effect into the second isoprenaline period. Any lag effect in Na influx measurement behind I_{sc} will result in poorer precision of measurement of j_s for that period.

Comparison of Na and Cl Efflux

Inspection of the data presented in Table 3 shows that adrenaline produced a mean (±SEM) increase in Na efflux of 5.20 ± 0.89 neq·cm^{-2} min^{-1} over the amiloride value, which was of the same order of magnitude as the mean (±SE of the difference) *net* Cl efflux of 6.18 ± 0.95 neq·cm^{-2} min^{-1}.

In two experiments in which bidirectional Cl fluxes and Na efflux were measured simultaneously, the mean changes in Na efflux for the adrenaline periods over the amiloride period were 4.41, 6.57 neq·cm^{-2} min^{-1} and the mean *net* Cl efflux 6.06, 5.95 neq·cm^{-2} min^{-1}, respectively.

Conductance Changes

The skin conductance was measured during each 20-min period of both experimental protocols and the change (ΔG_m) in the mean value for the control, amiloride, and adrenaline periods compared. The values are expressed in kΩ^{-1}·cm^{-2}. The mean (±SE) ΔG_m was -0.19 ± 0.03 ($n=17$) from the control to amiloride period and $+0.38 \pm 0.05$ ($n=17$) from amiloride to adrenaline period (first experimental protocol). For the second experimental protocol the ΔG_m was $+0.32 \pm 0.06$ ($n=7$) from the control to adrenaline period and -0.20 ± 0.04 ($n=7$) from the adrenaline to amiloride period. There is no significant difference in the ΔG_m produced by amiloride added after the control period or after the adrenaline period. Similarly there is no significant difference in ΔG_m produced by adrenaline added after the control period or after the amiloride period.

In both experimental protocols amiloride significantly decreased and adrenaline significantly elevated the skin conductance.

Histological Changes in Mucous Glands

Six paired skin samples (one of each pair acting as a control, the other exposed to isoprenaline for approximately 75 min) were prepared for light microscopy examination. The number of mucous glands in a transverse section of the skin was counted and their diameters measured. From the measured diameters the area of each gland was calculated. It was calculated that 44% of the glands would be within 10% of their maximum diameter in any particular transverse section. The results are

Table 5. The mean (±SE) area of the mucous glands seen in transverse sections of control and isoprenaline treated skins[a]

	Area (nm^2)		Significance P
	Control	Isoprenaline	
Number of glands	476	535	
Outside surface area	1480±52	2001±63	<0.001
Inside surface area	445±29	1077±47	<0.001
Outside−inside surface area (cell thickness)	1034±34	924±22	0.07

[a] The significance (P) was computed after a square root transformation of the areas to normalize the distribution of the areas.

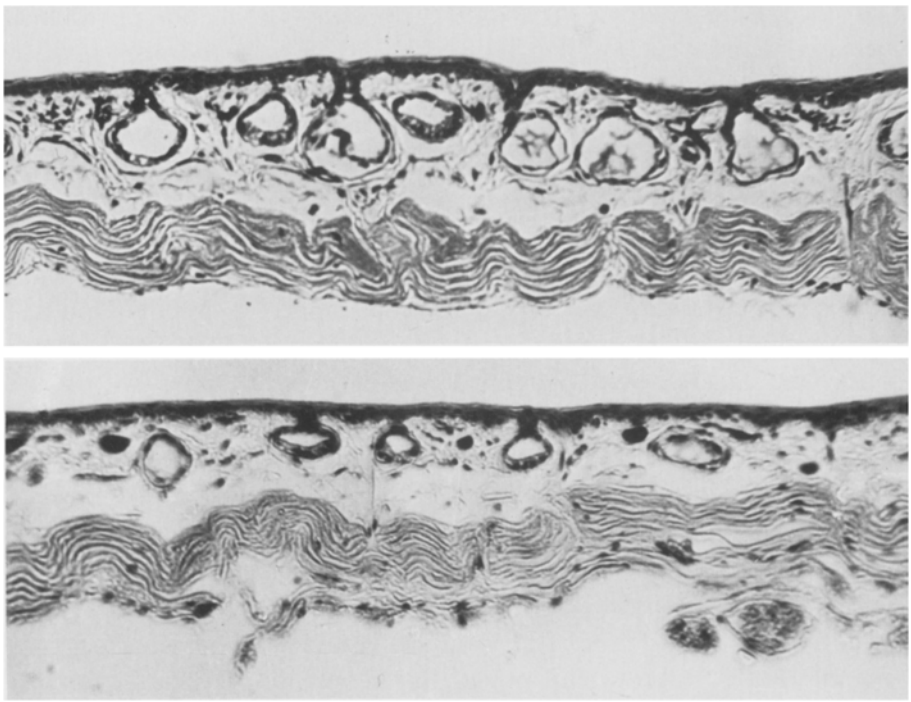

Fig. 9. Microphotographs of transverse sections of isoprenaline treated (*upper*) and control (*lower*) samples of same skin. The isoprenaline treated mucous glands are much enlarged and their contents discharged. Magnification (180×)

presented in Table 5 and representative portions of the section shown in Fig. 9.

In the skin samples exposed to isoprenaline the mucous glands were significantly larger with some diminution of the cellular thickness of

the gland lining (Table 5). Many of isoprenaline treated glands appeared to have discharged their contents.

Discussion

In this study it has been shown that the catecholamines adrenaline and isoprenaline produce the same magnitude of change in short-circuit current (ΔI_{sc}) whether or not amiloride is present. Similarly, amiloride produced the same magnitude of ΔI_{sc} whether added before or during the catecholamine response (Table 1). The ΔI_{sc} (amiloride) was significantly correlated with the I_{sc} just before the amiloride was added, i.e., with the control I_{sc} in protocol 1 and the catecholamine stimulated I_{sc} in protocol 2. In contrast the ΔI_{sc} (catecholamine) was not significantly correlated with the amiloride-produced I_{sc} (protocol 1) and only probably so with the control I_{sc} (protocol 2). In a previous paper (Tomlinson & Wood, 1976) we found no significant correlation between the magnitude of the initial rise and the value of either the pre-adrenaline or pre-isoprenaline I_{sc}.

An implication of the above observations is that adrenaline and isoprenaline when added induce an increment of approximately 7 neq$\cdot$ cm$^{-2}\cdot$min^{-1} to the I_{sc}, whether amiloride is present or not. However, when amiloride was not present and is subsequently added it still inhibits the I_{sc} (i.e., catecholamine increment plus basal I_{sc}) by 83%.

The addition of amiloride produced the expected marked inhibition of Na influx but had no effect on the basal Na efflux (Table 3) and no obvious effect on the elevated Na efflux produced by catecholamines (Fig. 3). This lack of inhibition of Na efflux by amiloride was found previously (Nielsen & Tomlinson, 1970) but is in contrast to that reported by some other workers (e.g., Salako & Smith, 1970). As mentioned previously the Cl unidirectional flux values given in Table 2 were obtained from two separate groups of skins, the higher Cl permeability of the skins used for the influx determinations probably being responsible for a fortuitous net Cl influx. The addition of amiloride to these skins resulted in a significant inhibition of Cl influx (Table 3) which resulted in no net Cl influx for the amiloride period. Schneider (1975) has reported a net Cl influx in isolated skin of *Rana esculenta* at the Cl concentration of a normal NaCl Ringer's solution, which was inhibited to 50% by 10^{-4} M amiloride. Furthermore, the inhibition was more readily demonstrated in skins with initial large Cl influxes. In the present study and

a previous one (Tomlinson & Wood, 1976) where Cl bidirectional fluxes were measured on the same skin sample, no net Cl influx was demonstrated. In some of the bidirectional Cl flux measurements performed for this paper, amiloride did appear to inhibit the Cl influx (e.g., Fig. 6); however, it did not appear to inhibit the elevated Cl influx induced by adrenaline (Fig. 7).

The addition of adrenaline (10^{-5} M) to short-circuited preparations of intact frog skin is known to produce a net efflux of Cl, a variable change in net Na influx and an increase in skin conductance, reflected in increased Na and Cl unidirectional fluxes (Koefoed-Johnsen, Ussing & Zerahn, 1952). The actions of isoprenaline at 10^{-5} M are similar to those of adrenaline (McAfee, 1970). The net Cl efflux is produced by β-stimulation (Watlington, 1968), an observation confirmed by Tomlinson & Wood (1976) who also found a strong association between Na efflux increase and the net Cl efflux. This association was also found in the experiments with amiloride reported in this paper. Such an electroneutral NaCl pump would not, of course, contribute to the increased I_{sc} nor would it be inhibited by amiloride. This would mean that the increased I_{sc} produced by the catecholamines was due to an increased Na influx.

Since the ΔI_{sc} increment produced by the catecholamines was the same in the absence or presence of amiloride, this would mean that they open up new Na channels not blocked by the amiloride present (experimental protocol 1). However, the ΔI_{sc} inhibition produced by amiloride is the same whether or not catecholamine is present, so that amiloride is only blocking the Na channels carrying the basal Na influx.

Several other workers (including Koefoed-Johnson *et al.*, 1952; Watlington, 1968; Lindley, 1969) favor an electrogenic Cl pump mechanism. If the increased I_{sc} is due to the production of a net Cl efflux, then the ΔI_{sc} increment caused by catecholamine would be unaffected by amiloride, the inhibition of I_{sc} being due to the inhibition of the basal Na influx.

The data in this paper would support either mechanism. However, in a previous paper (Tomlinson & Wood, 1976) the increase in I_{sc} produced by isoprenaline was almost twice the net Cl efflux. In addition, the findings of McAfee (1970) and Lang, Sjöberg & Skoglund (1975) that the glandular secretions of *Ranidae* contain equal amounts of Na and Cl favors a neutral NaCl extrusion from the glands. Such an extrusion would seem to occur without contractions of the smooth muscle surrounding the gland since Lindley (1969) found no change in the shape

of the gland after catecholamine treatment, and we found that approximately 75 min after the addition of isoprenaline when the I_{sc} was still markedly elevated the mucous glands were much larger than those in the control sample of skin (Fig. 9).

One of the problems of interpretation of the data is the lag of the isotopic measurement behind the I_{sc}. If this is taken into account in considering the values of j_s (Table 4) and in the correlations found between Na influx and I_{sc} measurements, then it would seem that the increase of I_{sc} is a reflection of increased Na influx, which can still occur in the presence of amiloride at the concentration used in this study. The β-adrenergic receptor responsible for the stimulation of Na influx across the skin epithelial layer and the outward secretion of NaCl from the mucous glands would have to be insensitive to other hormones which alter I_{sc} without stimulation of a net Cl movement (ADH and aldosterone) and insensitive to the presence of amiloride.

Financial support from the Wellcome Trust is gratefully acknowledged.

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