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THE EFFECT OF NORADRENALINE ON THE TOAD SKIN POTENTIAL

BY C. R. HOUSE

From the Department of Physiology, Royal (Dick) School of Veterinary Studies, University of Edinburgh, Edinburgh, EH 9 1 QH

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SUMMARY

1. The electrical response of isolated toad skins to the presence of 4×10^{-5} M noradrenaline in the inner medium has been studied.

2. When skins were bathed in Ringer solution, noradrenaline initiated a partial depolarization of the skin potential (inside surface becoming less positive) followed by a hyperpolarization; however, noradrenaline depolarized skins in sulphate Ringer.

3. The origin of the hyperpolarizing phase of the response to noradrenaline was studied by comparing the size of perturbations in the skin potential, produced by identical changes in external sodium, external chloride or internal potassium concentrations, before and during the response to noradrenaline.

4. Measurements of skin conductance were made in different sulphate media in order to estimate the magnitudes of the conductance of the shunt pathway through the skin and the conductance of the pathway for actively transported sodium ions.

5. Interpretation of both the variations in the perturbations of skin potential and the skin conductance measurements led to the conclusion that the hyperpolarizing phase of the response to noradrenaline was generated by an increase in the sodium to chloride permeability ratio for the outer barrier. It was considered that other evidence was compatible with this view.

6. Similar experimental methods were employed to study the action of antidiuretic hormone (ADH) and an elevated external concentration of calcium on the outer barrier. It was found that ADH increased the sodium to chloride permeability ratio whereas calcium decreased it. The separate actions of ADH and calcium on the sodium permeability of the outer barrier did not interfere apparently with the subsequent ability of noradrenaline to increase the sodium to chloride permeability ratio for this barrier in the skin.

INTRODUCTION

Both Schoffeniels & Salee (1965) and Salee & Vidrequin-Deliege (1967) have reported that the stimulation of cutaneous nerves produced a cyclic variation in the frog skin potential. Moreover, these workers found that the application of noradrenaline to the inner surface mimicked the action of nervous stimulation on the skin potential. The electrical response to either nervous stimulation or noradrenaline was composed of an initial depolarization (inner surface becoming less positive) followed by a hyperpolarization. Recently Lindley (1969) and House (1969) have confirmed the electrical characteristics of the responses to these sources of stimulation.

Schoffeniels & Salee (1965) considered that the depolarizing phase of the response was generated by an increase in the sodium permeability of the inward-facing membranes of the epithelial cells and/or from an increase in the potassium permeability of the outer membranes of the epithelial cells. These proposals are opposed by the experimental evidence of House (1969). Salee & Vidrequin-Deliege (1967) concluded that the depolarization resulted from the activation of an active influx of chloride ions. This hypothesis is not supported by the experimental data of Lindley (1969) and House (1969) and, in particular, the latter study revealed the emergence of a transient shunt in the skin which was approximately contemporaneous with glandular secretion from the skin. House (1969) proposed that the initial depolarizing phase of the response was generated partially by an increase in the permeability of the outer membranes to chloride ions and predominantly by a transient shunt pathway through the skin glands.

The origin of the hyperpolarizing phase of the response is more dubious than that of the initial depolarization. Schoffeniels & Salee (1965) suggested that either a decrease in the passive permeability of the frog skin to chloride ions or an active efflux of this ion might be responsible for the hyperpolarization. The former part of their suggestion is at variance with the measurements of unidirectional fluxes of chloride ions under these conditions (Bastide & Jard, 1968), whereas the latter alternative is compatible with these chloride flux measurements. On the other hand, Salee & Vidrequin-Deliege (1967) claimed that the hyperpolarization was produced by the activation of an active influx of potassium ions.

This paper presents experimental evidence about the nature of the hyperpolarizing phase of the response of toad skin to noradrenaline.

METHODS

Experiments were performed on the isolated skin of the toad, *Bufo bufo*, and of the South African clawed toad, *Xenopus laevis*. Animals were killed by cutting the spine and pithing; abdominal skin was excised, cleaned of adherences and washed in a volume of Ringer solution. Table 1 gives the composition of Ringer (saline A) and of the other solutions used in this investigation. All of the solutions were buffered with tris at pH 7·6. Pieces of skin were mounted between Perspex half-chambers of the type described by Ussing & Zerahn (1951) and the exposed area of skin was 5·1 cm^2. Approximately 30 ml. of solution bathed each surface and these volumes were continuously aerated. The potential difference (p.d.) across the skin was monitored through polyethylene cannulae, filled with 3% agar in 3 M-KCl, which were connected to reservoirs containing Ag–AgCl electrodes placed in 3M-KCl. The Ag–AgCl electrodes were connected to a Vibron electrometer (33B-2) whose output was coupled to a recording oscillograph (type 5-124, Consolidated Electrodynamics Corp., California). During each experiment the skin was periodically short-circuited

TABLE 1. Composition of solutions (mM). The pH of each saline was 7·6

	A	B	C	D	E	F	G
NaCl	100·0	100·0	10·0	6·0	—	—	—
KCl	2·5	0·25	2·5	2·5	102·5	—	—
$CaCl_2$	1·0	1·0	1·0	1·0	1·0	—	—
Na propionate	—	—	—	94·0	—	—	—
Choline Cl	—	2·25	90·0	—	—	—	—
Na_2SO_4	—	—	—	—	—	50·0	—
K_2SO_4	—	—	—	—	—	1·25	51·25
$CaSO_4$	—	—	—	—	—	1·0	1·0
Tris	5·0	5·0	5·0	5·0	5·0	5·0	5·0

by passing current between Ag–AgCl electrodes; the current electrodes were located in separate reservoirs within each half-chamber and the reservoirs were connected to the bathing media by cylindrical channels with 3% agar in Ringer solution. Short-circuiting current pulses were applied for about 5–10 sec and the magnitude of the current was monitored in a microammeter in the external circuit.

After the skin had been mounted in the apparatus, the p.d. and short-circuit current were measured frequently. When it was observed that both p.d. and short-circuit current were constant, a small volume of a stock solution of noradrenaline was added to the inner bathing medium and the electrical response was recorded. In each experiment the final concentration of noradrenaline in the inner medium was about 4×10^{-5} M.

In a series of experiments the time course of the secretion from the glands in *Xenopus* skin was measured in a volumetric chamber which has been described previously (see Fig. 1, House, 1969).

All experiments were performed at room temperature in the range 18–21° C and, in particular, it was found unnecessary to control the environmental temperature in the experiments with the volumetric chamber since the experimental period was short.

THEORY

It has been proposed by Koefoed-Johnsen & Ussing (1958) and Ussing & Windhager (1964) that the frog skin potential, which is registered conventionally as the potential of the inner surface with respect to the outer surface, is the sum of two p.d.'s generated at two separate boundaries within the skin. The outer barrier has been identified with the outer membranes of the epithelial cells immediately underneath the cornified layer (Ussing & Windhager, 1964); it is considered to be relatively permeable to sodium and chloride ions. The inner barrier, which may be composed of the inward-facing membranes of the epithelial syncytium, is assumed to be selectively permeable to potassium and chloride ions. This model suggests that the skin p.d., V, can be described empirically by:

$$V = \frac{RT}{F}\left[\ln\left\{\frac{P^{o}_{Na}[Na_o]+P^{o}_{Cl}[Cl_c]}{P^{o}_{Na}[Na_c]+P^{o}_{Cl}[Cl_o]}\right\}+\ln\left\{\frac{P^{i}_{K}[K_c]+P^{i}_{Cl}[Cl_i]}{P^{i}_{K}[K_i]+P^{i}_{Cl}[Cl_c]}\right\}\right], \quad (1)$$

where the superscripts o and i refer to outer and inner barriers, the subscripts o, c and i refer to outer, cellular and inner compartments, P_{Na}, P_K and P_{Cl} are parameters related to the permeabilities to sodium, potassium and chloride ions, [Na], [K] and [Cl] are the respective ionic activities and R, T and F are the gas constant, the absolute temperature and the Faraday respectively. Equation (1) has been adopted as an operational starting point for the following analysis and the limitations of its use will be discussed later.

In general, a change in $[Na_o]$ produces an observable change in V and the increment or decrement in V is a function of P^{o}_{Na}. This is evident from the following argument.

If y is a function of many variables a, b, c,... then the change, Δy, in y, induced by small increments Δa, Δb, Δc,... in a, b, c,... respectively is given by:

$$\Delta y = \frac{\partial y}{\partial a}\Delta a+\frac{\partial y}{\partial b}\Delta b+\frac{\partial y}{\partial c}\Delta c+\ldots, \quad (2)$$

where $\partial y/\partial a$, $\partial y/\partial b$ and $\partial y/\partial c$ are the partial derivatives of y with respect to a, b and c respectively.

Thus if we make a small change, $\Delta[Na_o]$, in $[Na_o]$, the change, ΔV, in V will be

$$\Delta V = \frac{\partial V}{\partial[Na_o]}\Delta[Na_o], \quad (3)$$

i.e.

$$\Delta V = \frac{RT}{F}\left[\frac{P^{o}_{Na}}{P^{o}_{Na}[Na_o]+P^{o}_{Cl}[Cl_c]}\right]\Delta[Na_o]. \quad (4)$$

In this case ΔV is a function of P^o_{Na}. Equation (4) suggests a basis for examining the variations in P^o_{Na} which may occur during the response of the amphibian skin to noradrenaline. Consider that ΔV_n is the perturbation in V produced by $\Delta[Na_o]$ before the application of noradrenaline and that ΔV_r is the corresponding change in V produced by an identical $\Delta[Na_o]$ during the response to noradrenaline, then

$$\frac{\Delta V_r}{\Delta V_n} = \frac{[Na_o] + (P^o_{Cl}/P^o_{Na})_n[Cl_c]_n}{[Na_o] + (P^o_{Cl}/P^o_{Na})_r[Cl_c]_r}, \tag{5}$$

where $[Na_o]$ is the initial external sodium concentration and the subscripts n and r denote values during the control period and the response respectively. Provided that it is possible to make some assumptions about $[Cl_c]_n$ and $[Cl_c]_r$, the comparison of ΔV_r with ΔV_n might indicate how the ratio of chloride to sodium permeability of the outer membrane has altered during the experiments.

A similar treatment has been applied to the circumstances where $[K_i]$, $[Cl_o]$ and $[Cl_i]$ are changed before and during the response to noradrenaline and the respective expressions for $(\Delta V_r/\Delta V_n)$ are:

$$\frac{\Delta V_r}{\Delta V_n} = \frac{[K_i] + (P^i_{Cl}/P^i_K)_n[Cl_c]_n}{[K_i] + (P^i_{Cl}/P^i_K)_r[Cl_c]_r}, \tag{6}$$

$$\frac{\Delta V_r}{\Delta V_n} = \frac{[Cl_o] + (P^o_{Na}/P^o_{Cl})_n[Na_c]_n}{[Cl_o] + (P^o_{Na}/P^o_{Cl})_r[Na_c]_r}, \tag{7}$$

$$\frac{\Delta V_r}{\Delta V_n} = \frac{[Cl_i] + (P^i_K/P^i_{Cl})_n[K_c]_n}{[Cl_i] + (P^i_K/P^i_{Cl})_r[K_c]_r}. \tag{8}$$

The foregoing analysis is the basis of my experimental attempts to examine changes in P_{Na}, P_K and P_{Cl} for the outer and inner membranes of amphibian skin during its response to noradrenaline.

In order to apply the analysis, outlined above, with some confidence, the effects of changes in the shunt resistance of the skin must be considered. If we assume that p.d., represented by the right hand side of eqn. (1), constitutes a battery with its own internal resistance, R_i, which is in parallel with a shunt resistance, R_s, then the skin p.d. V, will be given by

$$V = \frac{RT}{F}\left[\ln\left\{\frac{P^o_{Na}[Na_o] + P^o_{Cl}[Cl_c]}{P^o_{Na}[Na_c] + P^o_{Cl}[Cl_o]}\right\} + \ln\left\{\frac{P^i_K[K_c] + P^i_{Cl}[Cl_i]}{P^i_K[K_i] + P^i_{Cl}[Cl_c]}\right\}\right]\frac{R_s}{R_i + R_s}. \tag{9}$$

If G_i and G_s are the conductances of R_i and R_s, respectively, then the skin conductance, G_{skin}, equals $(G_i + G_s)$, and eqn. (9) can be rewritten as:

$$V = \frac{RT}{F}\left[\ln\left\{\frac{P^o_{Na}[Na_o] + P^o_{Cl}[Cl_c]}{P^o_{Na}[Na_c] + P^o_{Cl}[Cl_o]}\right\} + \ln\left\{\frac{P^i_K[K_c] + P^i_{Cl}[Cl_i]}{P^i_K[K_i] + P^i_{Cl}[Cl_c]}\right\}\right]\frac{G_i}{G_{skin}}. \tag{10}$$

Eqn. (5) now becomes

$$\frac{\Delta V_r}{\Delta V_n} = \left[\frac{[Na_o]+(P^o_{Cl}/P^o_{Na})_n[Cl_c]_n}{[Na_o]+(P^o_{Cl}/P^o_{Na})_r[Cl_c]_r}\right]\left(\frac{G_l}{G_{skin}}\right)_r\left(\frac{G_{skin}}{G_l}\right)_n. \quad (11)$$

The product $(G_l/G_{skin})_r(G_{skin}/G_l)_n$ has been estimated for skins bathed in Ringer by obtaining the time courses of changes in G_{skin} (Fig. 6, House, 1969) and in G_l (Fig. 11, present study); the product equals 0·50 at the peak of the hyperpolarization. During the hyperpolarizing phase of the response to ADH the product reaches a maximal value of 1·12. Therefore, the observations of $(\Delta V_r/\Delta V_n)$ will be underestimated by a factor of 2, in the case of noradrenaline, and will be over-estimated by about 10% in the case of ADH.

The theoretical argument, which has been presented, assumes the validity of a double-membrane theory for ion transport across amphibian skin. Since the skin contains several layers of epithelial cells, all of which are involved apparently in active ion transport (Ussing & Windhager, 1964), some serious doubt exists about the ability of the double-membrane theory to describe adequately the skin potential. The experiments reported in this paper were designed to investigate the effect of noradrenaline on the skin potential not only from the standpoint of the double-membrane model but also with the use of an electrical analogue of the skin. Further, the latter approach does not rest explicitly on any particular view of the origin of the skin potential.

RESULTS

Fig. 1 shows the typical response of a toad skin to the presence of 4×10^{-5} M noradrenaline on the inner surface. The response consists of a pronounced transient rise in the short-circuit current and of a large transient decrease in skin resistance. House (1969) suggested that the sharp drop in skin resistance reflected a precipitous fall in the skin's shunt resistance which was responsible for the initial depolarization of the skin potential. The hyperpolarizing phase of the response occurs while the skin resistance is still below the initial value; thus the hyperpolarization cannot result from an increase in the shunt resistance of the skin. The latter contention will be substantiated later.

Preliminary experiments

Before employing the analytical approach, outlined in the theoretical section, to the response of toad skin to noradrenaline, the analysis was tested in circumstances where previous work had demonstrated alterations in the sodium permeability of the outer barrier of the skin. The addition of ADH to the inner bathing solution increases the outer permeability to

sodium ions (Curran, Herrera & Flanigan, 1963) whereas the elevation of external calcium concentration decreases the sodium permeability of the outer barrier (Curran & Gill, 1962; Curran *et al.* 1963). The effects of both ADH and calcium on the rate of active sodium transport by the skin have been explained in terms of these changes in the sodium permeability of the outer barrier (Curran *et al.* 1963). Fig. 2*A* shows the typical effects of

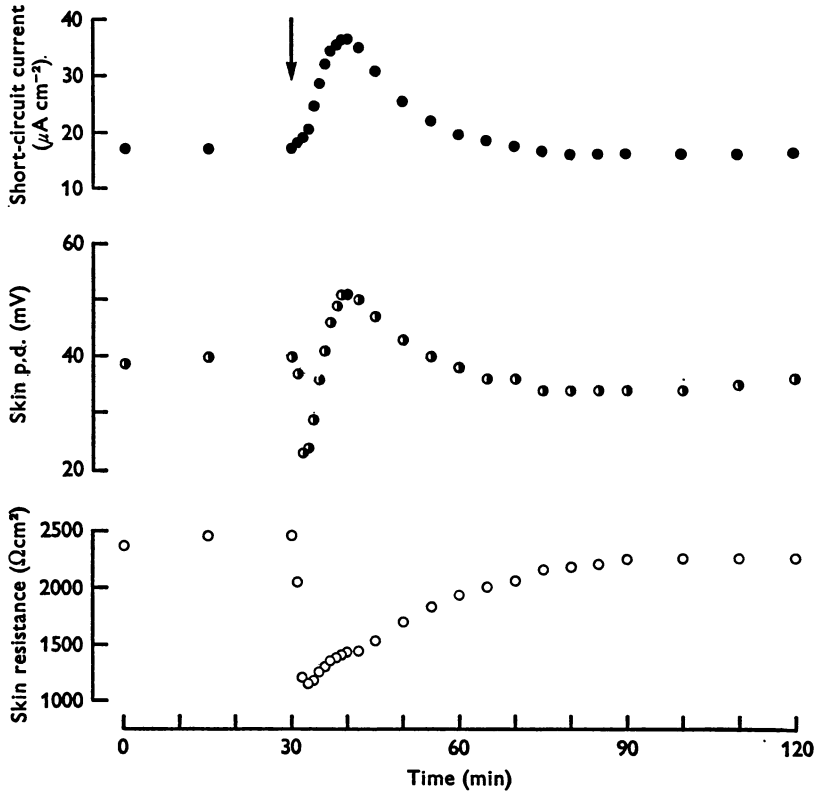

Fig. 1. Time course of the responses in the short-circuit current, p.d. and the resistance of an isolated skin of *Bufo bufo* to noradrenaline. At the time indicated by the arrow noradrenaline was added to the inner medium to give a final concentration of 4×10^{-5} M.

identical step changes, $\Delta[Na_o]$, in the external sodium concentration, $[Na_o]$, before and during the application of ADH (0·2 u./ml. Pitressin, Park, Davis & Co.) to the inner surface of the skin. Comparison of the perturbations in the skin potential, evoked by identical $\Delta[Na_o]$, in Fig. 2*A* demonstrates that ADH increases the sodium permeability of the outer barrier (see eqn. 5). Fig. 2*B* shows the corresponding effects of identical $\Delta[Na_o]$ on the skin potential before and after the elevation of external

calcium concentration from 1 to 11 mM and this evidence confirms the view that calcium decreases the sodium permeability of the outer barrier.

Thus the results of the preliminary experiments on the effects of ADH and the elevation of calcium concentration on the sodium permeability of the outer barrier are compatible with the conclusions of other workers.

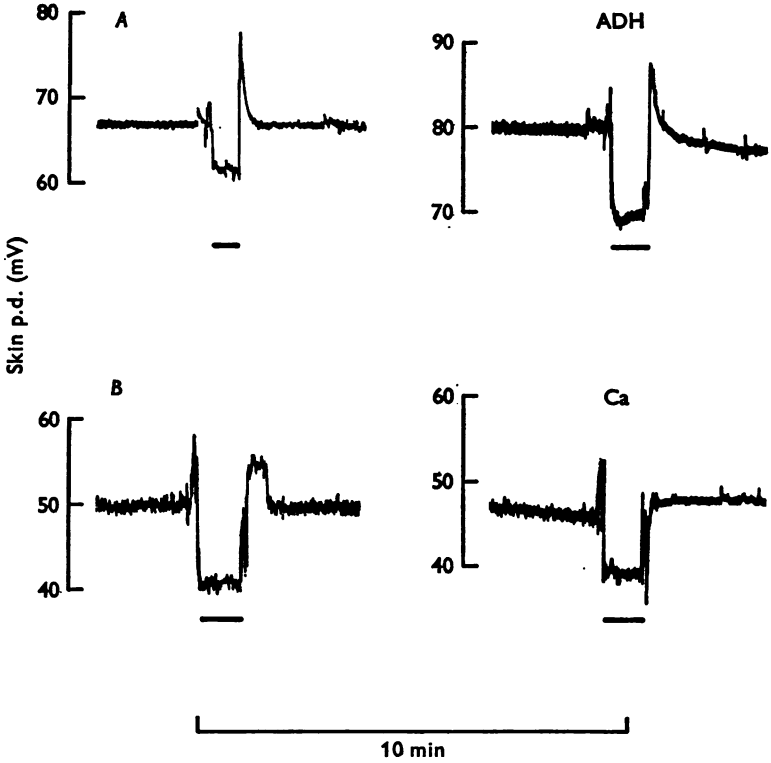

Fig. 2. The effect of changes in the external sodium concentration on the skin p.d. of *Bufo bufo*. *A* records the effects of changing [Na_o] from 100 mM (saline A) to 10 mM (saline C) before and after the addition of ADH to the internal medium (final concentration = 0·2 u./ml.). *B* records the effects of similar changes in [Na_o] before and after the elevation of the external calcium concentration from 1 to 11 mM. The horizontal bars indicate the time during which the experimental saline C was applied.

Effect of noradrenaline on the outer barrier

Fig. 3 shows the effects of step changes, $\Delta[Na_o]$, before and during typical responses of two toad skins to noradrenaline. The perturbations in the skin p.d., produced by identical $\Delta[Na_o]$, are magnified during the hyperpolarizing phase of the response; at the maximal hyperpolarizations in Fig. 3*A* and *B* the values of $(\Delta V_r/\Delta V_n)$ are 1·6 and 2·0 respectively. Subsequently the perturbations, evoked by $\Delta[Na_o]$, decreased in size and the

diminution in the perturbations seems to correlate with the rate of decline of the hyperpolarizing phase of the response (compare Fig. 3*A* and *B*). The corresponding values of ($\Delta V_r/\Delta V_n$) for the experiments reported in Fig. 3*A* and *B* are 1·6, 1·6 and 1·4 (not shown in record) and 2·0, 1·3 and 1·1 (not shown) respectively.

Since Fig. 2*A* demonstrates that the presence of ADH in the inner medium increases the perturbation in the skin p.d., evoked by $\Delta[Na_o]$, it was considered important to investigate the effect of noradrenaline on toad skins which had been treated with ADH. Fig. 4*A* shows the combined effects of ADH and noradrenaline on the perturbations in the skin

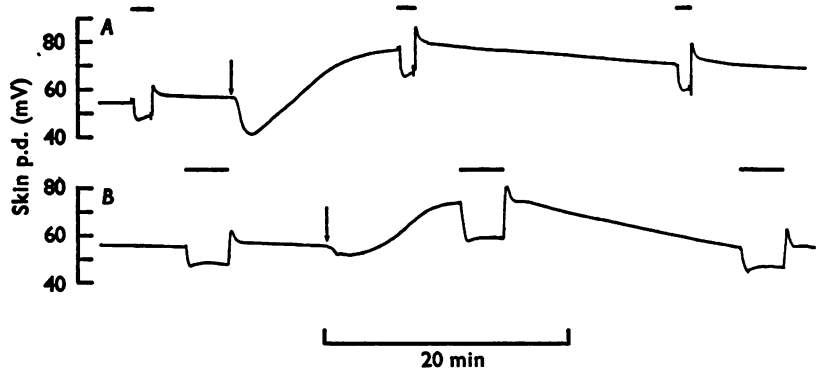

Fig. 3. The effect of changes in the external sodium concentration on the skin p.d. of *Bufo bufo* before and during the hyperpolarizing phase of the response to noradrenaline. At the time indicated by the arrows noradrenaline was added to the inner medium to give a final concentration of 4×10^{-5} M. *A* and *B* record the effects on two different skins of changing $[Na_o]$ from 100 mM (saline A) to 10 mM (saline C). The horizontal bars indicate the time during which the experimental saline C was applied.

p.d. evoked by $\Delta[Na_o]$. These substances apparently exert additive effects on the permeability of the outer barrier to sodium ions. Fig. 4*B* shows the typical effect of noradrenaline on a toad skin which had been treated with an elevated concentration of calcium in the external medium. Evidently the reduction in the perturbation in the skin p.d., evoked by $\Delta[Na_o]$, which is produced by elevated external calcium, is cancelled partially by the presence of noradrenaline in the inner medium.

Thus the different actions of ADH and elevated external calcium on the sodium permeability of the outer barrier do not interfere significantly with the influence of noradrenaline on the permeability of this barrier.

The effects of noradrenaline on the chloride permeability of the outer barrier are shown in Fig. 5. In the upper record (Fig. 5*A*) perturbations in the p.d., produced by identical $\Delta[Cl_o]$, occur before and during the depolarizing and repolarizing phases of the response to noradrenaline; there

is a significant small increase in the perturbation in p.d. during the depolarizing phase of the response to noradrenaline ($\Delta V_r/\Delta V_n = 1{\cdot}1$). During the repolarizing phase the value of ($\Delta V_r/\Delta V_n$) falls to unity. Fig. 5*B*, on the other hand, shows that the perturbations in p.d. during the hyperpolarization are smaller than the control perturbation. These records of typical experiments demonstrate that ($\Delta V_r/\Delta V_n$) (for chloride ions at the peak of the hyperpolarization) is less than or equal to unity.

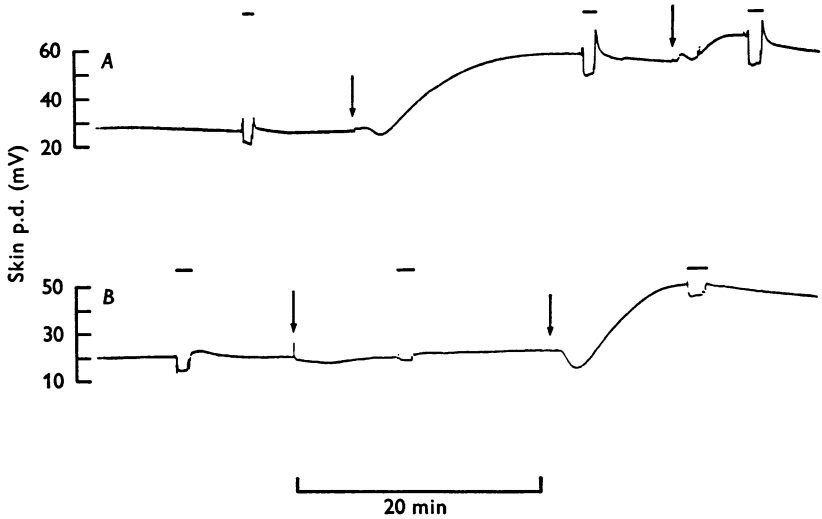

Fig. 4. The effects of ADH and calcium on the changes, evoked by identical $\Delta[Na_o]$, in the skin p.d. of *Bufo bufo* before and during the response to noradrenaline. In record *A* the first and second arrows indicate respectively the additions of ADH (final concentration = 0·2 u./ml.) and of noradrenaline (final concentration = 4×10^{-5} M) to the inner medium. In record *B* the first and second arrows indicate respectively the elevation of external calcium concentration from 1 to 11 mM and the addition of noradrenaline to the inner medium. In both experiments $[Na_o]$ was changed from 100 mM (saline A) to 10 mM (saline C) and the horizontal bars indicate the time during which the experimental saline C was applied.

The observations on the p.d. changes produced by $\Delta[Na_o]$ and $\Delta[Cl_o]$ during the response to noradrenaline will be discussed subsequently in the light of the analysis outlined in the theoretical section.

Effect of noradrenaline on the inner barrier

Since Share & Ussing (1965) have alluded to unpublished observations, which suggested that glandular secretion might be stimulated by elevated values of $[K_i]$, some care about the choice of $\Delta[K_i]$ is required. In preliminary experiments I found that raising $[K_i]$ in a series of steps of 2·5 mM did not stimulate glandular secretion from *Xenopus* skin until

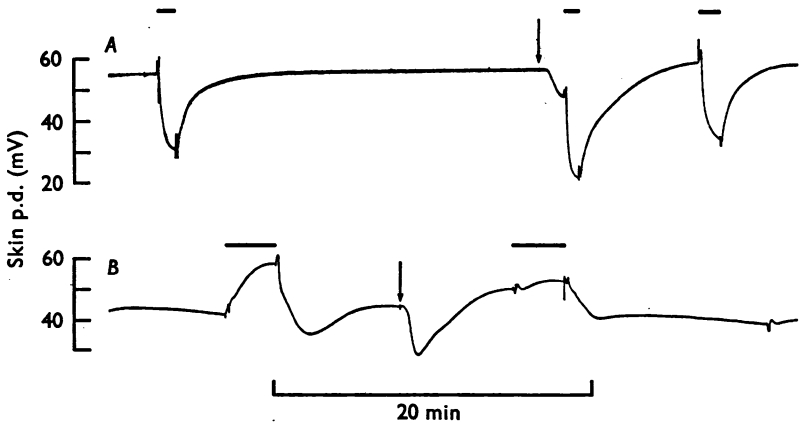

Fig. 5. The effect of changes in the external chloride concentration on the skin p.d. of *Bufo bufo* before and during the response to noradrenaline. At the time indicated by the arrow noradrenaline was added to the inner medium to give a final concentration of 4×10^{-5} M. *A* records the effects of changing $[Cl_o]$ from 10·5 mM (saline D) to 104·5 mM (saline A). *B* records the effects of changing $[Cl_o]$ from 104·5 mM (saline A) to 10·5 mM (saline D) on a different skin. The horizontal bars indicate the time during which the experimental salines A and C were applied.

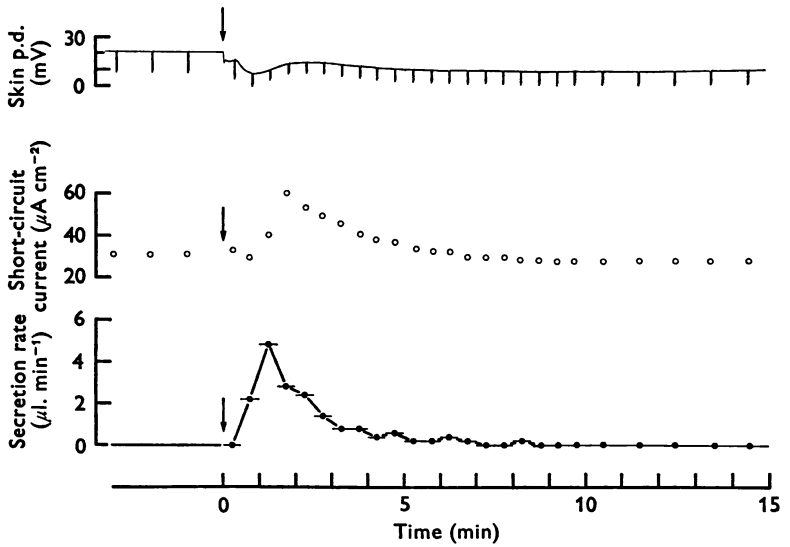

Fig. 6. The time course of the secretory responses to the stimulation of *Xenopus* skin glands by elevation of the potassium concentration in the inner medium. At the time indicated by the arrow $[K_i]$ was raised from 2·5 to 25 mM. The brief depolarizations on the potential record were produced by constant current pulses of 20 μA.

$[K_i]$ reached 15 mM. Fig. 6 shows the typical secretory response of *Xenopus* skin to an elevation of $[K_i]$ from 2·5 to 25 mM. It is evident, therefore, that a $\Delta[K_i]$ of this sort is entirely unsuitable, and I decided to produce perturbations in the skin p.d. by reducing $[K_i]$ from 2·5 to 0·25 mM.

Fig. 7 shows the effects of identical $\Delta[K_i]$ on the skin p.d. before and during the hyperpolarization. Evidently there is an increase in the response to $\Delta[K_i]$ during the hyperpolarizing phase; in Fig. 7*A* and *B* the values of $(\Delta V_r/\Delta V_n)$ are 1·8 and 1·4 respectively.

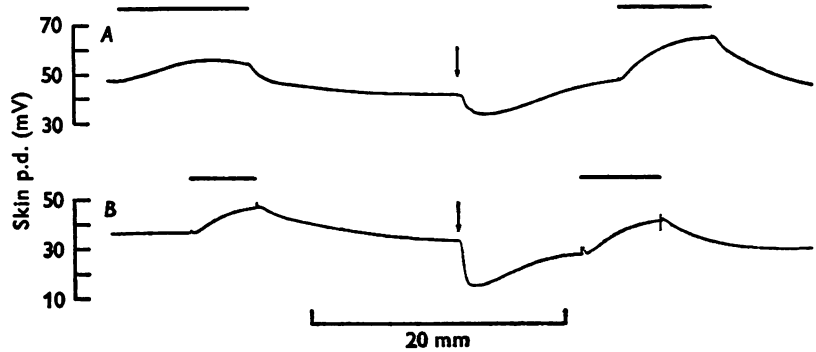

Fig. 7. The effect of changes in the internal potassium concentration on the skin p.d. of *Bufo bufo* before and during the hyperpolarizing phase of the response to noradrenaline. At the time indicated by the arrows noradrenaline was added to the inner medium to give a final concentration of 4×10^{-5} M. *A* and *B* record the effects on two different skins of changing $[K_i]$ from 2·5 mM (saline A) to 0·25 mM (saline B). The horizontal bars indicate the time during which the experimental saline B was applied.

Attempts to record the effects of $\Delta[Cl_i]$ on the skin p.d. before and during the response to noradrenaline were unsuccessful because $\Delta[Cl_i]$ did not produce reversible alterations in p.d.

Skin conductance measurements

Ussing and his collaborators have asserted frequently that the skin p.d. in the absence of permeant anions, such as chloride, is given by:

$$V = \frac{ER_s}{R_i + R_s}, \tag{12}$$

where E represents the electromotive force of the system. It is widely held that E is given by:

$$E = \frac{RT}{F}\left[\ln\left\{\frac{[Na_o]}{[Na_c]}\right\} + \ln\left\{\frac{[K_c]}{[K_i]}\right\}\right] \tag{13}$$

under these circumstances. In eqn. (12) R_i is assumed to be equivalent to the diffusional resistance of the outer membrane for sodium ions plus the

resistance of the inner membrane for potassium ions. Finally, R_s is the effective resistance of all shunt pathways in the skin. Ussing & Windhager (1964) have used this basic framework to establish ways of estimating R_s, R_i and E; the arguments which they employed are still valid even if E is generated by an 'electrogenic pump' and not by the sum of two diffusion potentials as eqn. (13) implies. Ussing & Windhager assumed that the resistance of the sodium transport pathway through the skin would greatly exceed R_s when external sodium was replaced with potassium; under these circumstances the skin resistance becomes equal to R_s. Moreover, Ussing & Windhager emphasized that the resistance measurements should be performed on skins bathed in sulphate Ringer since this avoided alterations in the volume of the epithelium; Ussing (1965) found that epithelial swelling decreased skin resistance and shrinkage increased skin resistance. In particular, Ussing & Windhager confirmed that this method of estimating R_s yielded values which were similar to the sum of the partial conductances of ions moving through the shunt pathway.

The conductance, G_{skin}, of the skin bathed in sulphate Ringer is given by (G_i+G_s), where G_i and G_s are the conductances $^1/R_i$ and $^1/R_s$. Because R_s can be determined, G_i can be computed from values of G_{skin} and $^1/R_s$. Equation (12) can be rewritten in the form

$$V = \frac{EG_i}{G_i+G_s}. \tag{14}$$

Knowing G_i, G_s and V for skins bathed in sulphate Ringer, one can estimate E. In principle, therefore, it is possible to obtain the separate time courses of G_i, G_s and E during the response of the skin to agents, such as ADH and noradrenaline.

I decided to test this approach on the electrical response of toad skin to ADH since Ussing & Zerahn (1951) had shown by sodium flux measurements that while neurohypophyseal extract produced no significant increase in E, it did increase G_i. Fig. 8 shows the average skin conductances in conditions where sulphate Ringer bathed both sides (○) and where sodium-free sulphate Ringer bathed the outside of the skin (●); each point represents the mean of ten measurements and the bars indicate the S.E. of mean. At the arrow ADH was added to the inner medium. Values of G_i (◑) were obtained by subtracting G_s from G_{skin}; throughout the time course of the response each value of G_{skin} was significantly larger than the corresponding value of G_s. Since the variances associated with the G_{skin} and G_s data did not increase as a function of time it may be taken that G_i does increase genuinely in the manner shown in Fig. 8. The values of E in this series of experiments have been computed by eqn. (14) and are shown in Fig. 9. Again each point is the mean of ten measurements and the

average electrical response to ADH consists of a rise in short-circuit current and a pronounced increase in the skin p.d. By comparison with the changes in V the changes in E are small and, in fact, the initial response in E is a small decrease. The data in Figs. 8 and 9 are in qualitative

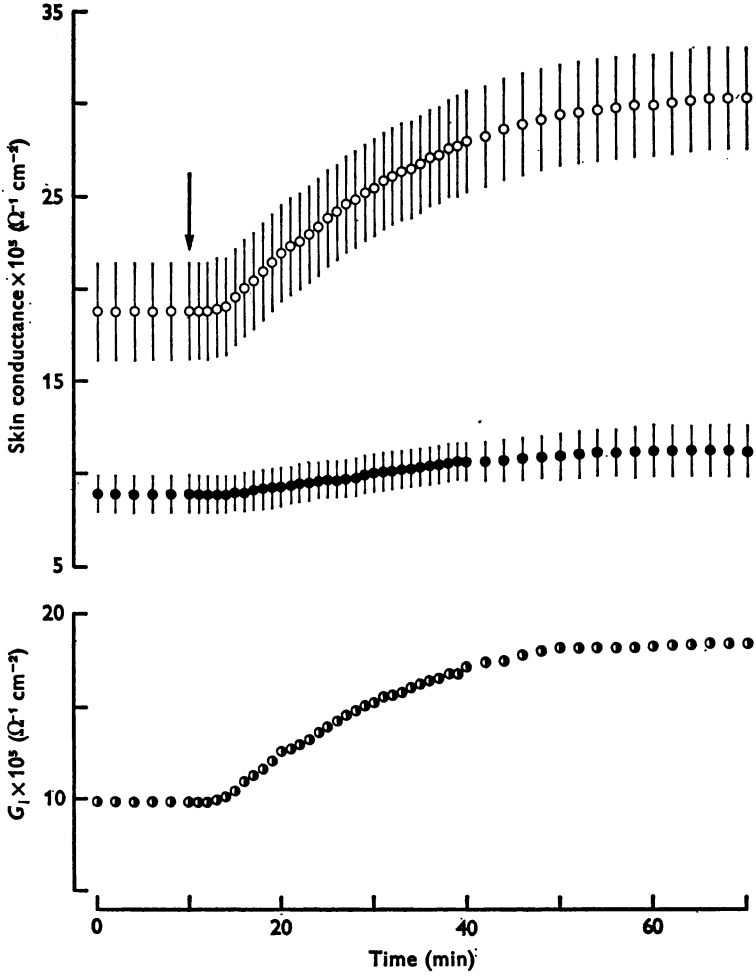

Fig. 8. The effect of ADH on the skin conductance of *Bufo bufo*. At the time indicated by the arrow ADH was added to the inner medium to give a final concentration of 0·2 u./ml. Measurements (○) were made on skins bathed on both sides by sulphate Ringer (saline F) while other measurements (●) were made on skins bathed on the outer surface by potassium sulphate Ringer (saline G) and on the inside by saline F. Each point (○ and ●) is the mean of ten measurements on ten skins, ± S.E. Estimates of G_i (◑) were obtained by subtracting the estimates of G_s (●) from the corresponding values of skin conductance (○).

agreement with those of Ussing & Zerahn (1951) and, furthermore, the present technique reveals the time course of G_s, G_i and E.

Figs. 10 and 11 show the results of a similar approach to the electrical response of the skin to noradrenaline. It was found that noradrenaline produced a transient increase in G_{skin}, and G_s and, by inference, in G_i too

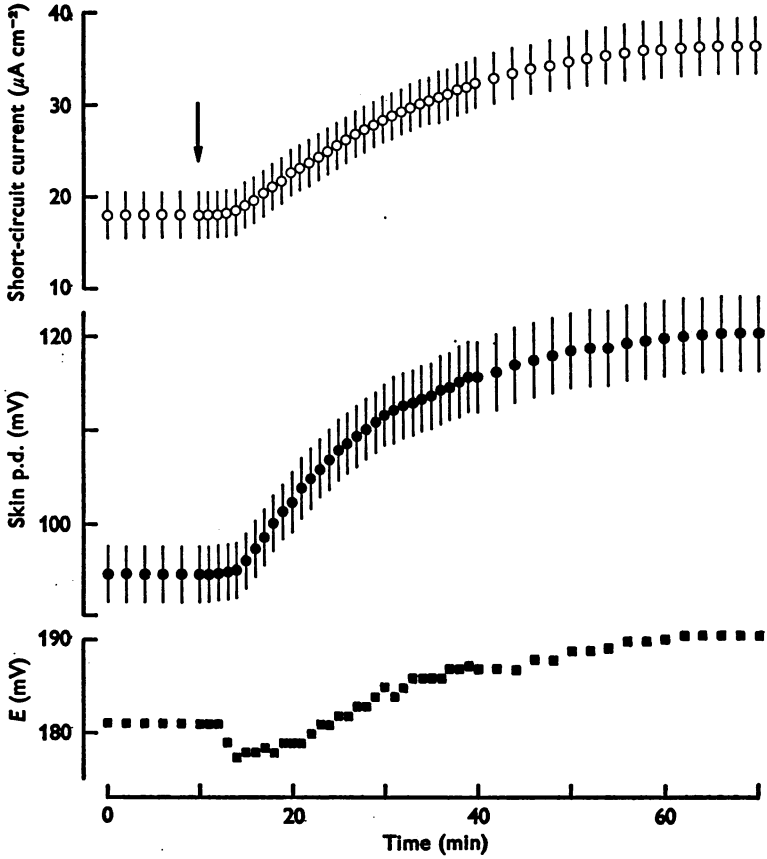

Fig. 9. The effect of ADH on the short-circuit current, p.d. and the electromotive force, E, of *Bufo bufo* skin bathed in sulphate Ringer. At the time indicated by the arrow ADH was added to the inner medium to give a final concentration of 0·2 u./ml. Each point (○ and ●) is the mean of ten measurements on ten skins, ± S.E. Estimates of E (■) were obtained by eqn. (14) since V, G_i and $(G_i + G_s)$ were known.

(Fig. 10). The transient increase in G_s is compatible with the time course of glandular secretion but no doubt the permeation of ions, such as sulphate anions, across the cell boundaries and between the cells contributes to G_s. The transient increase in G_i implies that some decrease in the diffusional resistance of the outer membranes for sodium ions has occurred; also a

similar decrease in the resistance of the inner membrane for potassium ions may have occurred. Fig. 11 shows that the short-circuit current of skins, bathed in sulphate Ringer, increases transiently in response to noradrenaline; however, the skin p.d. decreases. Under these conditions both V and E fall slowly after the addition of noradrenaline, whereas in Ringer solution the skin p.d. increases (Fig. 1).

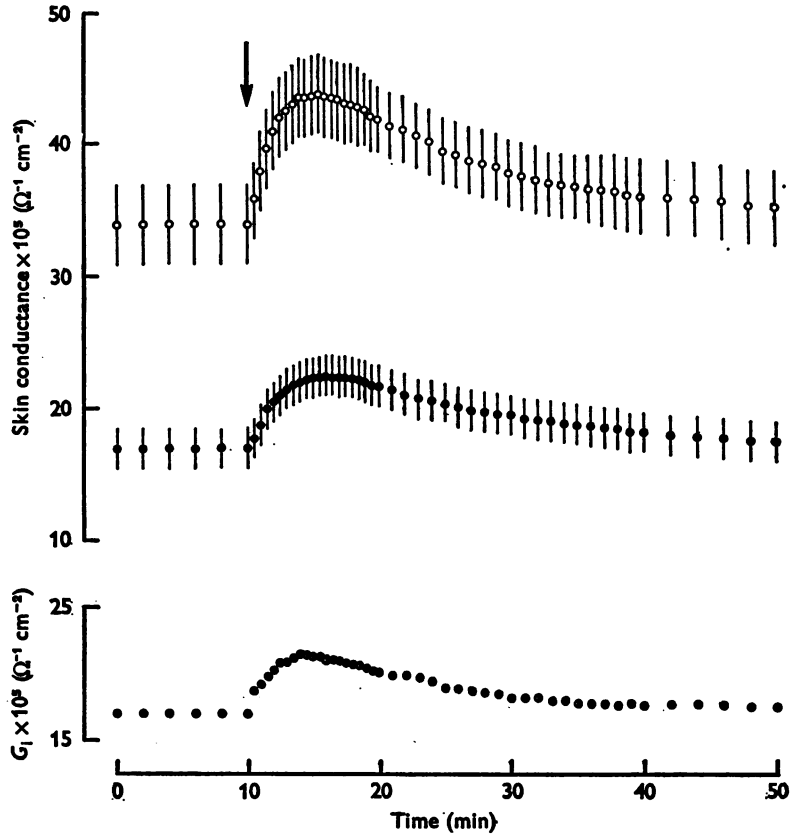

Fig. 10. The effect of noradrenaline on the skin conductance of *Bufo bufo*. At the time indicated by the arrow noradrenaline was added to the inner medium to give a final concentration of 4×10^{-5} M. Measurements (○) were made on skins bathed on both sides by sulphate Ringer (saline F) while other measurements (●) were made on skins bathed on the outer surface by potassium sulphate Ringer (saline G) and on the inside by saline F. Each point (○ and ●) is the mean of ten measurements on ten skins, ± S.E. Estimates of G_i (◑) were obtained by subtracting the estimates of G_s (●) from the corresponding values of skin conductance (○).

Effect of elevated potassium Ringers on the response to noradrenaline

Fig. 12 shows that skins, bathed on the inside by potassium sulphate Ringer (saline G) and by potassium chloride Ringer (saline E), do not respond to noradrenaline in a manner which is characteristic either of skins, bathed in sulphate Ringer (Fig. 11), or of skins bathed in Ringer

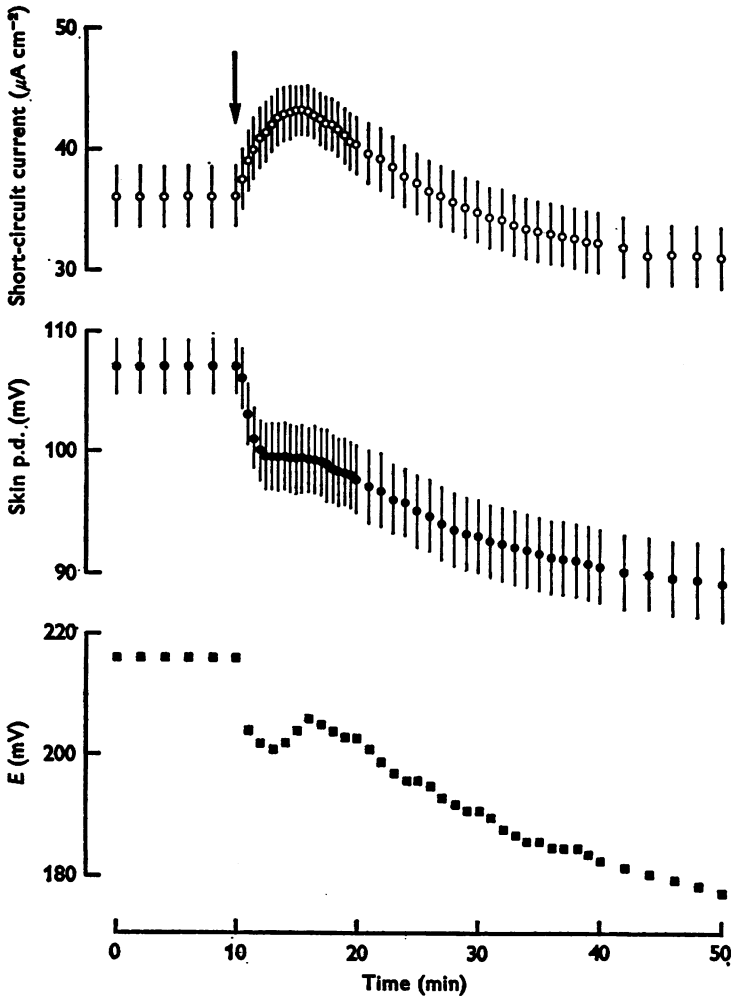

Fig. 11. The effect of noradrenaline on the short-circuit current, p.d. and the electromotive force, E, of *Bufo bufo* skin bathed in sulphate Ringer. At the time indicated by the arrow noradrenaline was added to the inner medium to give a final concentration of 4×10^{-5} M. Each point (○ and ●) is the mean of ten measurements on ten skins, ± S.E. Estimates of E (■) were obtained by eqn. (14) since V, G_1 and (G_1+G_2) were known.

(Fig. 1). Since the brief hyperpolarizations on the potential records of Fig. 12 are produced by constant current pulses, the amplitudes of these voltage deflexions are proportional to skin resistance. In both records there is evidence for an initial reduction of skin resistance following the application of noradrenaline. The absence of a pronounced initial depolarization in both records is consistent with the previous finding of House (1969) that the maximal depolarization, ΔV, was given by $V_0\ \Delta R/R_0$, where $\Delta R/R_0$ is the resistance change divided by the initial resistance and

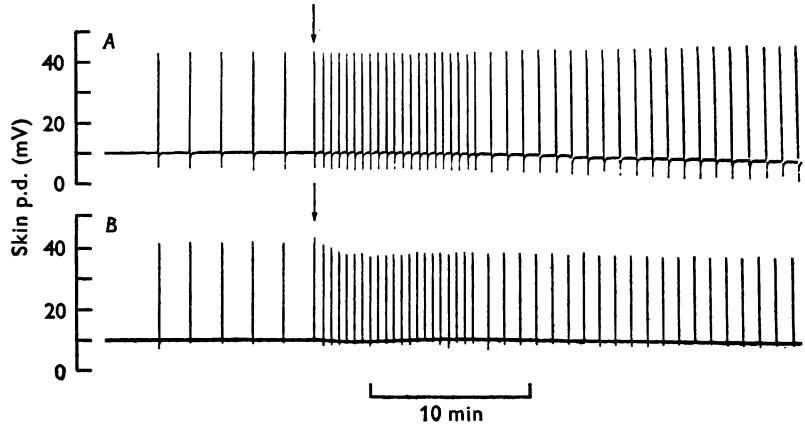

Fig. 12. Time course of the electrical responses of *Bufo bufo* skins, bathed internally with elevated potassium Ringers, to noradrenaline. At the time indicated by the arrows noradrenaline was added to the inner medium to give a final concentration of 4×10^{-5} M. *A* records the p.d. response of a skin bathed by sulphate Ringers (inside: saline G; outside: saline F). The brief hyperpolarizations on the potential record were produced by constant current pulses of 40 μA. *B* records the p.d. response of a skin bathed by chloride Ringers (inside: saline E; outside: saline A). The brief hyperpolarizations on the potential record were produced by constant current pulses of 200 μA.

V_0 is the original skin p.d. Since the relevant data for the experiment shown in Fig. 12*B* are $V_0 = 10$ mV, $\Delta R = -75\Omega$ cm^2 and $R_0 = 840\ \Omega$ cm^2, the predicted value of ΔV is $-0{\cdot}9$ mV.

The data in Fig. 12 illustrate that replacement of sodium by potassium ions in the inner medium abolishes the hyperpolarizing phase of the response in Ringer solution and the large depolarization observed in sulphate Ringer.

A similar replacement of sodium by potassium ions in the outer medium also excludes the main features of the response in both Ringer and sulphate Ringer solutions (see Fig. 13). The reason for the absence of the large

initial depolarizing phase under these circumstances is similar to that suggested for the records in Fig. 12. Actually the potential trace in Fig. 13*A* shows an initial small hyperpolarization and this will be discussed later.

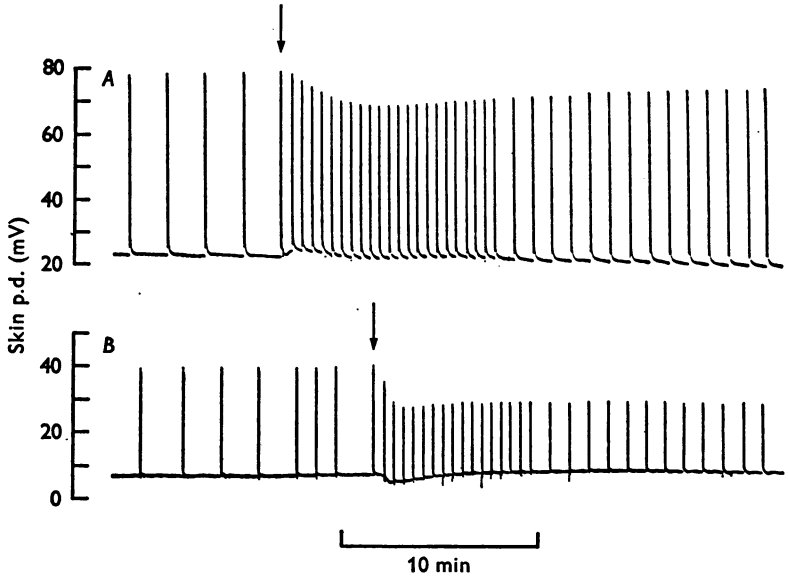

Fig. 13. Time course of the electrical responses of *Bufo bufo* skins, bathed externally with elevated potassium Ringers, to noradrenaline. At the time indicated by the arrows noradrenaline was added to the inner medium to give a final concentration of 4×10^{-5} M. *A* records the p.d. response of a skin bathed by sulphate Ringers (inside: saline F; outside: saline G). The brief hyperpolarizations on the potential record were produced by constant current pulses of 40 μA. *B* records the p.d. response of a skin bathed by chloride Ringers (inside: saline A; outside: saline E). The brief hyperpolarizations on the potential record were produced by constant current pulses of 40 μA.

DISCUSSION

My experimental records in Figs. 3, 5 and 7 reveal that during the electrical response of toad skin to noradrenaline there are significant alterations in the changes in skin p.d., evoked by identical $\Delta[Na_o]$, $\Delta[Cl_o]$ or $\Delta[K_i]$. These transient changes, which occur during the hyperpolarization of the response, may reflect corresponding alterations in the ionic permeabilities of the outer and inner barriers of the toad skin. The analysis, outlined in the theoretical section, allows some conclusions to be made about the effect of noradrenaline on the permeability barriers of the skin.

Equation (5) predicts that $(\Delta V_r/\Delta V_n)$ for identical $\Delta[Na_o]$ will be larger than unity provided that:

$$[Na_o] + (P^o_{Cl}/P^o_{Na})_n[Cl_c]_n > [Na_o] + (P^o_{Cl}/P^o_{Na})_r[Cl_c]_r, \tag{15}$$

i.e.

$$(P^o_{Cl}/P^o_{Na})_n[Cl_c]_n > (P^o_{Cl}/P^o_{Na})_r[Cl_c]_r. \tag{16}$$

During the hyperpolarizing phase of the response to noradrenaline the experimental data demonstrate that $(\Delta V_r/\Delta V_n) > 1$ for $\Delta[Na_o]$. The deviation of $(\Delta V_r/\Delta V_n)$ from unity is even larger when errors due to 'shunting' are corrected (see eqn. 11). Since $[Cl_c]_n > [Cl_c]_r$, by itself, is unlikely to be compatible with a hyperpolarization of the skin (see eqn. 1), the inequality (16) suggests that the hyperpolarizing phase results from a relative increase of P^o_{Na} over P^o_{Cl}, or mathematically

$$(P^o_{Cl}/P^o_{Na})_n > (P^o_{Cl}/P^o_{Na})_r.$$

Equation (7) predicts correspondingly that $(\Delta V_r/\Delta V_n)$ for identical $\Delta[Cl_o]$ will be smaller than unity provided that

$$[Cl_o] + (P^o_{Na}/P^o_{Cl})_r[Na_c]_r > [Cl_o] + (P^o_{Na}/P^o_{Cl})_n[Na_c]_n, \tag{17}$$

i.e.

$$(P^o_{Na}/P^o_{Cl})_r[Na_c]_r > (P^o_{Na}/P^o_{Cl})_n[Na_c]_n. \tag{18}$$

During pronounced hyperpolarizing phases of responses my experimental data demonstrate that $(\Delta V_r/\Delta V_n) \leqslant 1$ for $\Delta[Cl_o]$ even after 'shunting' errors have been corrected. Since $[Na_c]_r > [Na_c]_n$, by itself, is incompatible with a hyperpolarization of the skin, the inequality (16) suggests that the hyperpolarizing phase of the response is compatible with $(P^o_{Na}/P^o_{Cl})_r > (P^o_{Na}/P^o_{Cl})_n$. This conclusion is in accord with the foregoing analysis of the data for ΔV_r and ΔV_n, evoked by $\Delta[Na_o]$, during the hyperpolarization.

Equation (6) predicts that $(\Delta V_r/\Delta V_n)$ for identical $\Delta[K_i]$ will be larger than unity provided that:

$$[K_i] + (P^i_{Cl}/P^i_K)_n[Cl_c]_n > [K_i] + (P^i_{Cl}/P^i_K)_r[Cl_c]_r, \tag{19}$$

i.e.

$$(P^i_{Cl}/P^i_K)_n[Cl_c]_n > (P^i_{Cl}/P^i_K)_r[Cl_c]_r. \tag{20}$$

During the hyperpolarizing phase of the response the experimental data show that $(\Delta V_r/\Delta V_n) > 1$ for identical $\Delta[K_i]$. Since $[Cl_c]_n > [Cl_c]_r$, by itself, is incompatible with a hyperpolarization, the inequality (20) suggests that during the hyperpolarizing phase of the response $(P^i_{Cl}/P^i_K)_n > (P^i_{Cl}/P^i_K)_r$.

My interpretation of these experimental results is that the hyperpolarizing phase of the response of skins, bathed in Ringer, is generated by an increase in P^o_{Na} relative to P^o_{Cl}. Examination of ΔV_n for $\Delta[Na_o]$ and $\Delta[Cl_o]$ shows that the outer membrane is relatively more permeable to chloride than to sodium ions before the addition of noradrenaline (Figs. 3 and 5). Employing the same arguments to the values of $(\Delta V_r/\Delta V_n)$ for $\Delta[Cl_o]$ during the depolarizing phase of the response (Fig. 5*A*), I conclude that the initial effect of noradrenaline on the outer barrier is an increase in (P^o_{Cl}/P^o_{Na}). The view that noradrenaline increases the permeability of the

outer membranes for sodium and chloride ions is compatible with the flux measurements of Ussing & Zerahn (1951) and of Bastide & Jard (1968). Thus, the outer barrier of skins treated with noradrenaline becomes temporarily leaky first to chloride ions and then to sodium ions in such a manner that a cyclic variation in skin p.d. occurs. Of course, the initial depolarization is augmented possibly by a glandular shunt pathway.

The analysis raises the possibility that a contributory factor to the hyperpolarizing phase of the response may be a relative increase in P^i_K over P^i_{Cl}. Unfortunately it was not possible to obtain confirmatory evidence of an increase in (P^i_K/P^i_{Cl}) from experiments where changes in $[Cl_l]$ were made. Moreover, two arguments seem to rule out the suggestion that P^i_K has increased relative to P^i_{Cl}. First, the micro-electrode studies of Cereijido & Curran (1965) reveal that the change in skin p.d., which is produced by a decrease in $[K_l]$, is actually generated at the outer membrane; Cereijido & Curran concluded that a decrease in $[K_l]$ increased P^o_{Na}. The apparent increase in (P^i_K/P^i_{Cl}), which is reported in this paper, may be taken, therefore, as further evidence for a relative increase in P^o_{Na} relative to P^o_{Cl} during the hyperpolarization. Secondly, in some experiments, where the effect of changes in P^o_{Na} was excluded (Fig. 13), no pronounced hyperpolarization resulted from the application of noradrenaline. The small hyperpolarization in Fig. 13*A* may result from an increase in P^i_K; however, the hyperpolarizations, observed in nine similar experiments, were significantly smaller than that in Fig. 13*A*.

The foregoing analysis of the effects of noradrenaline on the permeability characteristics of the outer and inner barriers of toad skin has assumed that there are no significant changes in the cellular ionic concentrations. Measurements of unidirectional ionic fluxes (Ussing & Zerahn, 1951; Bastide & Jard, 1968) and of skin conductance (Figs. 1 and 10) indicate that the tissue becomes temporarily leaky to ions. This evidence points possibly to an increase in $[Na_c]$ and $[Cl_c]$ and a fall in $[K_c]$. These tenuous predictions are actually compatible with the response of skins, bathed in sulphate Ringer; in particular, the estimated amplitude of E falls significantly and this might indicate a rise in $[Na_c]$ and/or a fall in $[K_c]$ (see eqn. (13)). The contention that E is generated by the operation of an 'electrogenic pump' rather than by diffusion potentials will be discussed later. All of the available evidence indicates that during the hyperpolarizing phase of the response $[Na_c]_r > [Na_c]_n$, $[Cl_c]_r > [Cl_c]_n$ and possibly $[K_c]_r < [K_c]_n$. Since these changes would lead to a depolarization, which is actually observed only in sulphate Ringer, the conclusion must be that the relative increase in P^o_{Na} over P^o_{Cl} is actually larger than the analysis suggests for skins bathed in Ringer.

The results of the skin conductance measurements (Fig. 10) demonstrate

an increase in the conductance of the shunt pathway and also an increase in the conductance of pathways for the passive flows of actively transported ions. The latter pathways are assumed to be essentially the outer barrier for sodium movement and the inner barrier for potassium movement (Ussing & Windhager, 1964). Since the micro-electrode experiments of Cereijido & Curran (1965) have shown that the resistance of the outer barrier in frog skin constitutes about 70% of the over-all skin resistance, G_1 may be predominantly an index of the sodium permeability of the outer membranes. Because the skin conductance measurements do not rest upon any assumptions about the origin of the toad skin potential, they offer stronger evidence about the effect of noradrenaline on the skin than the results of the analysis outlined in the theoretical section. Nevertheless, both methods yield the convergent conclusion that noradrenaline increases the permeability of the outer barrier to sodium ions (G_1) and to chloride ions (G_s). Moreover, the separate time courses of the ionic permeability changes, inferred from the analysis, apparently agree with the transient behaviour of G_1 and G_s.

All of the above discussion has ignored the possibility that active ion transport across the skin is electrogenic. Schoffeniels & Salee (1965) claimed that an active chloride efflux might be responsible for the hyperpolarizing phase of the response, whereas Salee & Vidrequin-Deliege (1967) suggested that an electrogenic chloride 'pump' (inwards) produced the initial depolarization while an electrogenic potassium 'pump' (inwards) generated the subsequent hyperpolarization. These particular hypotheses must be abandoned since the potential response to noradrenaline can be easily abolished in the presence of these ions (Figs. 12 and 13). Not only is the concept too simple that *only* electrogenic ion 'pumps' generate the response of the skin to noradrenaline but also this view is in serious conflict with certain experimental evidence. For example, if the hyperpolarizing phase of the response is to be attributed to the active electrogenic influx of sodium ions and active electrogenic efflux of chloride ions, then *prima facie* there should be an equivalence between the time, T_I, after the application of noradrenaline at which short-circuit current is maximal and the corresponding time, T_V, for the maximal hyperpolarization. Fig. 14 shows the result of such comparisons for thirty-seven separate experiments and there is no obvious correlation between T_I and T_V. Furthermore, there is another serious objection to the electrogenic 'pump' explanation for this response; this study has shown that the electrical response of toad skin to noradrenaline can have several forms. First, in Ringer solution the short-circuit current increases while the skin p.d. decreases initially and subsequently increases. Secondly, in sulphate Ringer solution the short-circuit current increases whereas the skin p.d.

decreases. Finally, in certain circumstances the short-circuit current increases but there is no significant change in skin p.d. (Figs. 12 and 13). These data exclude the possibility that electrogenic ion 'pumps' play an important role in the response of toad skin to noradrenaline.

When the amphibian skin is bathed on the inner surface by elevated potassium Ringer, the outer membranes of the epithelial cells become very permeable to water and ions (Ussing, Biber & Bricker, 1965; Share & Ussing, 1965) and there is a brief phase of glandular activity (Fig. 6). It is not surprising, therefore, that noradrenaline fails to elicit

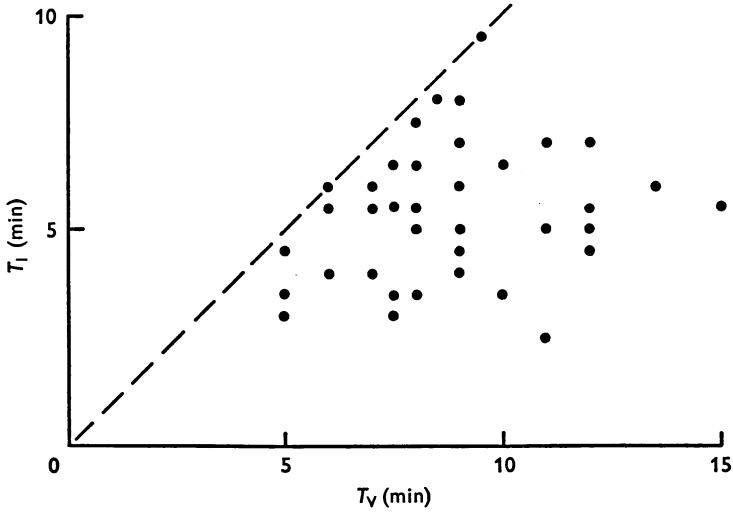

Fig. 14. The relation between T_I and T_V. Each point represents the result of a single experiment performed on an isolated skin of *Bufo bufo*, bathed in Ringer solution. T_I is the interval between the application of noradrenaline and the time at which short-circuit current is maximal and T_V is the corresponding interval for the response in p.d. The interrupted line is the line of equality between T_I and T_V.

a hyperpolarization in the presence of elevated [K_I]. When the sodium in the outer medium is replaced by potassium, noradrenaline again fails to produce an electrical response. In this case the absence of external sodium ions impedes the generation of a hyperpolarization and this failure is consistent with the origin of the response which has been proposed in this paper.

There are several conclusions from this investigation. Antidiuretic hormone generates a hyperpolarization of the toad skin p.d. by increasing almost exclusively the sodium permeability of the outer membranes; the absence of a pronounced rise in shunt conductance suggests that there is no

increase in anion permeability of the outer barrier. These results are in agreement with the work of numerous investigators and this confirmation vindicates the experimental methods employed in this study. These techniques demonstrate that noradrenaline produces an increase in the sodium to chloride permeability ratio for the outer barrier in toad skin. Apparently the outer membranes are relatively more permeable to chloride than to sodium ions under control conditions and, therefore, noradrenaline tends to hyperpolarize the toad skin. The initial phase of the response is a depolarization resulting from a transient increase in the shunt conductance. Skins, bathed in sulphate Ringer, also show a pronounced increase in shunt conductance which must be attributed partly to permeation of sulphate anions into the epithelial cells. This increase in anion permeability, which is normally very low in sulphate Ringer, in addition to the increment in sodium permeability of the outer barrier produces a depolarizing response to noradrenaline.

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REFERENCES

BASTIDE, F. & JARD, S. (1968). Actions de la noradrenaline et de l'ocytocine sur le transport actif de sodium et à la perméabilité à l'eau de la peau de grenouille. Rôle du 3′, 5′-AMP cyclique. *Biochim. biophys. Acta* **150**, 113–123.

CEREIJIDO, M. & CURRAN, P. F. (1965). Intracellular electrical potentials in frog skin. *J. gen. Physiol.* **48**, 543–557.

CURRAN, P. F. & GILL, J. R. (1962). The effect of calcium on sodium transport by frog skin. *J. gen. Physiol.* **45**, 625–641.

CURRAN, P. F., HERRERA, F. C. & FLANIGAN, W. J. (1963). The effect of Ca and antidiuretic hormone on Na transport across frog skin. II. Sites and mechanisms of action. *J. gen. Physiol.* **46**, 1011–1027.

HOUSE, C. R. (1969). The role of glandular activity in the electrical response of amphibian skin to noradrenaline. *J. Physiol.* **202**, 631–644.

KOEFOED-JOHNSEN, V. & USSING, H. H. (1958). The nature of the frog skin potential. *Acta physiol. scand.* **42**, 298–308.

LINDLEY, B. D. (1969). Nerve stimulation and electrical properties of frog skin. *J. gen. Physiol.* **53**, 427–449.

SALEE, M. L. & VIDREQUIN-DELIEGE, M. (1967). Nervous control of the permeability characteristics of the isolated skin of the toad, *Bufo bufo* L. *Comp. Biochem. Physiol.* **23**, 583–597.

SCHOFFENIELS, E. & SALEE, M. L. (1965). The effects of the electrical stimulation of the brachial plexus on the potential difference of frog skin. *Comp. Biochem. Physiol.* **14**, 587–602.

SHARE, L. & USSING, H. H. (1965). Effect of potassium on the movement of water across the isolated amphibian skin. *Acta physiol. scand.* **64**, 109–118.

USSING, H. H. (1965). Relationship between osmotic reactions and active sodium transport in the frog skin epithelium. *Acta physiol. scand.* **63**, 141–155.

USSING, H. H., BIBER, T. U. L. & BRICKER, N. S. (1965). Exposure of the isolated frog skin to high potassium concentrations at the internal surface. II. Changes in epithelial cell volume, resistance, and response to antidiuretic hormone. *J. gen. Physiol.* **48**, 425–433.

USSING, H. H. & WINDHAGER, E. E. (1964). Nature of shunt path and active sodium transport path through frog skin epithelium. *Acta physiol. scand.* **61**, 484–504.

USSING, H. H. & ZERAHN, K. (1951). Active transport of sodium as the source of electric current in the short-circuited isolated frog skin. *Acta physiol. scand.* **23**, 110–127.