J. Physiol. (1971), **218**, *pp.* 305–324
With 8 *text-figures*
Printed in Great Britain

THE EFFECT OF DIFFERENT IONIC LEVELS ON THE ELECTRICAL RESPONSE OF TOAD SKIN TO NORADRENALINE

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(*Received* 6 *April* 1971)

SUMMARY

1. After the addition of noradrenaline (final concentration 4×10^{-5} M) to the inner medium of isolated toad skins, they underwent a depolarization (inner surface becoming less positive) followed by a hyperpolarization.

2. The dependence of the minimal and maximal levels of the depolarizing and hyperpolarizing phases of the response upon the external concentrations of sodium and chloride ions was examined.

3. The experimental data were considered to support the view that the hyperpolarization was generated by an increase in the sodium to chloride permeability ratio for the outer barrier of the skin and that the depolarization resulted from a transient increase in the conductance of transient shunt pathways in the skin.

4. When the external sodium and chloride ions were replaced by magnesium (or potassium) and sulphate ions, noradrenaline initiated a transient hyperpolarization. During this hyperpolarizing response the time course of the change in the skin's conductance resembled that of the skin potential. The polarity of the response was changed by reversing the chloride concentration gradient across the skin.

5. The dependence of the maximal level of the hyperpolarizing response upon the internal potassium concentration was examined.

6. It was concluded that the hyperpolarizing response was generated partially by an increase in the potassium to chloride permeability ratio for the inner barrier of the skin and predominantly by the movement of chloride and accompanying cations through a transient shunt pathway, probably the active glands.

INTRODUCTION

Several workers (Schoffeniels & Salee, 1965; Salee & Vidrequin-Deliege, 1967; Lindley, 1969) have shown that noradrenaline accurately mimics the effect of nervous stimulation on the amphibian skin potential. In particular, either cutaneous nerve stimulation or the presence of noradrenaline at the inner surface of the toad skin bathed by normal Ringer solutions produces a cyclic disturbance of the skin potential. The response consists of an initial depolarization followed by a hyperpolarization. Moreover, each stimulus initiates a period of glandular secretion and a transient decrease in the skin resistance.

House (1969*b*, 1970) has suggested that the initial depolarizing phase of the response is produced partially by an increase in the chloride permeability of the outer membranes of the epithelial cells and predominantly by ionic movements through a transient shunt pathway in the active skin glands. In his account of the response the hyperpolarization is generated by an increase in the sodium to chloride permeability ratio for the outer membranes. The alternative views of Schoffeniels & Salee (1965) and Salee & Vidrequin-Deliege (1967) about the nature of the electrical response to noradrenaline and nerve stimulation are largely inconsistent with some of the experimental evidence of House (1969*b*, 1970) and Lindley (1969), and this divergence of opinion has been discussed in detail by House (1970).

The experiments reported in this paper have been designed to examine the nature of the electrical response to noradrenaline under a variety of circumstances where the ionic levels in the bathing media have been altered. The aim of this work was to establish whether or not the responses recorded in those different conditions were in accord with my previous accounts of the response to noradrenaline.

METHODS

Experiments were performed on the isolated skin of the toad, *Bufo bufo*. After the animals had been killed by cutting the spine and pithing, the 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; an appropriate volume of acid (HCl for A, B, C, D, E, I, J, K, L, M, N, O; H_2SO_4 for F, G, H, P, Q, R) was added to each solution. 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. The volumes of solution bathing each surface of the skin 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 3 M-KCl. The skin p.d. was registered conventionally as the potential of the inner surface with respect to the outer surface. The Ag/AgCl electrodes were connected to a Vibron electrometer

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

	NaCl	KCl	$CaCl_2$	NaBs*	KBs†	ChCl‡	$MgCl_2$	TEACl§	Na_2SO_4	K_2SO_4	$CaSO_4$	$MgSO_4$	Tris	Sucrose
A	100·0	2·5	1·0	—	—	—	—	—	—	—	—	—	5·0	—
B	20·0	82·5	1·0	—	—	—	—	—	—	—	—	—	5·0	—
C	5·0	97·5	1·0	—	—	—	—	—	—	—	—	—	5·0	—
D	1·0	101·5	1·0	—	—	—	—	—	—	—	—	—	5·0	—
E	—	102·5	1·0	—	—	—	—	—	—	—	—	—	5·0	—
F	25·0	—	1·0	75·0	2·5	—	—	—	—	—	—	—	5·0	—
G	5·0	—	1·0	95·0	2·5	—	—	—	—	—	—	—	5·0	—
H	—	—	1·0	100·0	2·5	—	—	—	—	—	—	—	5·0	—
I	92·5	10·0	1·0	—	—	—	—	—	—	—	—	—	5·0	—
J	102·0	0·5	1·0	—	—	—	—	—	—	—	—	—	5·0	—
K	102·4	0·1	1·0	—	—	—	—	—	—	—	—	—	5·0	—
L	102·5	—	1·0	—	—	—	—	—	—	—	—	—	5·0	—
M	—	2·5	1·0	—	—	100·0	—	—	—	—	—	—	5·0	—
N	—	2·5	1·0	—	—	—	50·0	—	—	—	—	—	5·0	50·0
O	—	2.5	1·0	—	—	—	—	100·0	—	—	—	—	5·0	—
P	—	—	—	—	—	—	—	—	50·0	1·25	1·0	—	5·0	50·0
Q	—	—	—	—	—	—	—	—	—	51·25	1·0	—	5·0	50·0
R	—	—	—	—	—	—	—	—	—	—	1·0	102·5	5·0	—

* Sodium benzenesulphonate. † Potassium benzenesulphonate. ‡ Choline chloride. § Tetra-ethyl ammonium chloride.

(33 B-2) whose output was coupled to a Devices M2 recorder. During an equilibration period each skin was periodically short-circuited by passing current between Ag/AgCl electrodes which were located in separate reservoirs within each half-chamber; the reservoirs were connected to the bathing media by cylindrical channels filled 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 micro-ammeter in the external circuit.

When it was observed that 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. All experiments were performed at room temperature in the range 18–21 °C.

RESULTS

Previous work (House, 1969*b*) demonstrated that the magnitude, ΔV, of the skin depolarization evoked by noradrenaline was given by $\Delta V = V_0 \Delta R/R_0$, where V_0, R_0 and ΔR are the original skin p.d., original skin resistance and the change in resistance at the minimal level of the depolarization respectively. Other experimental data (House, 1970) showed that the subsequent hyperpolarizing phase of the response was generated by an increase in the sodium to chloride permeability ratio for the outer barrier of the skin. If that view of the temporal characteristics of the response is correct then the skin p.d. at the peak of the hyperpolarization should be more dependent on the external sodium concentration, $[Na_o]$, than the original skin p.d. A further expectation is that the skin p.d. at the minimal level of the depolarizing phase should be less dependent on $[Na_o]$ than the original skin p.d.

Fig. 1 shows the effect of $[Na_o]$ on the response to noradrenaline. It is evident that the dependence of the resting skin p.d. (○) on $[Na_o]$ is quite weak and that the peak values (●) of the skin p.d. after noradrenaline show a more pronounced dependence on $[Na_o]$. Thus the data in Fig. 1 substantiate the claim (House, 1970) that the outer barrier of toad skin becomes preferentially more permeable to sodium ions during the hyperpolarizing phase of the response.

On the basis of the double-membrane model of the frog skin potential (Koefoed-Johnsen & Ussing, 1958; Ussing & Windhager, 1964) the skin p.d., V, can be described 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,

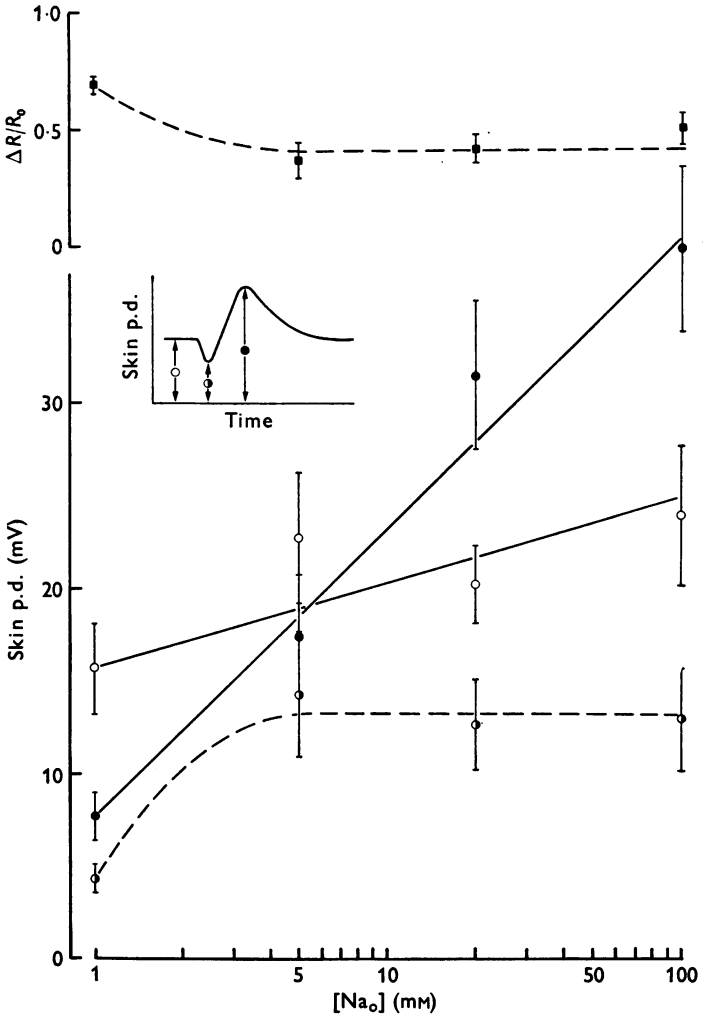

Fig. 1. The effect of external sodium concentration on the response to noradrenaline. Each point is the mean of ten measurements on ten skins, ± S.E. Measurements (○, ◑, ●) of the skin p.d. were made before the addition of noradrenaline and during the depolarizing and hyperpolarizing phases of the response (see inset diagram). Estimates (■) of ($\Delta R/R_0$) were obtained at the minimum of the depolarizing phase. In each experiment the skin was bathed internally by saline A and externally by saline A, B, C or D.

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. It can be shown that

$$\frac{\partial V}{\partial [Na_o]} = \frac{RT}{F} \frac{P^o_{Na}}{P^o_{Na}[Na_o] + P^o_{Cl}[Cl_c]} \tag{2}$$

and since

$$\frac{\partial V}{\partial [Na_o]} = \frac{\partial V}{\partial (\ln [Na_o])} \frac{\partial (\ln [Na_o])}{\partial [Na_o]} \tag{3}$$

it follows that

$$\frac{\partial V}{\partial (\ln [Na_o])} = \frac{RT}{F} \frac{P^o_{Na}[Na_o]}{P^o_{Na}[Na_o] + P^o_{Cl}[Cl_c]}. \tag{4}$$

Provided that $P_{Na}\,[Na_o] \ll P_{Cl}\,[Cl_c]$, eq. (4) can be rewritten as

$$\frac{\partial V}{\partial (\ln [Na_o])} = \frac{RT}{F} \frac{P^o_{Na}[Na_o]}{P^o_{Cl}[Cl_c]}. \tag{5}$$

Inspection of eqn. (5) reveals that a plot of V against log $[Na_o]$ should not yield obligatorily a linear relationship and for this reason no attempt has been made to compute regression lines for the data in Fig. 1. Because $[Cl_c]$ is an unknown variable it is not possible to use eqn. (5) for quantitative estimations of (P_{Na}/P^o_{Cl}). The significant increase in $\partial V/\partial(\log [Na_o])$, which occurs during the hyperpolarization, over that for the original skin p.d. can be attributed to an increase in (P^o_{Na}/P^o_{Cl}). Alternatively a reduction in $[Cl_c]$ would produce an increase in the slope but this seems an unlikely possibility since that change in $[Cl_c]$, by itself, would not hyperpolarize the skin significantly.

In contrast to the results on the hyperpolarizing phase of the response in Fig. 1 the data on the depolarizing phase (◑) show a relatively weak dependence on $[Na_o]$ above $[Na_o] = 5$ mM. At lower values of $[Na_o]$, however, the depolarized potential level becomes *apparently* more dependent on $[Na_o]$ than the original potential. The explanation for this discrepancy is related to the magnitude of $(\Delta R/R_o)$ at low values of $[Na_o]$. Throughout the entire range of $[Na_o]$ employed in this study it was observed that the skin resistance decreased to a minimal value of about 1000 Ω cm² during the depolarization whereas R_o ranged from 1900 Ω cm² at $[Na_o] = 100$ mM to 3400 Ω cm² at $[Na_o] = 1$ mM. Fig. 1 shows the mean values of $(\Delta R/R_o)$ found in this experiment and it is clear that the magnitude of $(\Delta R/R_o)$ increases significantly at $[Na_o] = 1$ mM above the mean values for the other values of $[Na_o]$. Thus the relatively larger depolarizing phase at $[Na_o] = 1$ mM is caused by a more effective shunt than that found at higher values of $[Na_o]$.

The corresponding study of the effect of $[Cl_o]$ on the response is shown in Fig. 2. Evidently the toad skin is relatively more permeable to chloride ions than to the sodium ions under normal conditions, but during the hyperpolarizing phase (●) of the response the dependence of the skin p.d. on $[Cl_o]$ is significantly reduced below that of the original potential. By performing a similar analysis for $[Cl_o]$ to that for $[Na_o]$ it can be shown that

$$\frac{\partial V}{\partial(\ln [Cl_o])} = -\frac{RT}{F}\left[1 - \frac{P^o_{Na}[Na_c]}{P^o_{Cl}[Cl_o]}\right] \tag{6}$$

provided that $P^o_{Cl}[Cl_o] \gg P^o_{Na}[Na_c]$. Again no attempt has been made to compute regression lines for the data in Fig. 2 since the slope $\partial V/\partial(\log [Cl_o])$ is not independent of $[Cl_o]$. The significant decrease in $\partial V/\partial(\log [Cl_o])$, which occurs during the hyperpolarization, from that for the original skin p.d. can be explained by an increase in (P^o_{Na}/P^o_{Cl}) which is consistent with the description of the hyperpolarization recorded at different values of $[Na_o]$. It is possible, however, that the decrease in $\partial V/\partial(\log [Cl_o])$ might result from a decrease in $[Na_c]$.

Below $[Cl_o] = 110$ mM in Fig. 2 the dependence of the skin p.d. on $[Cl_o]$ during the depolarization phase (◑) is not significantly different from the original skin p.d. The relative depolarization, $(\Delta V/V_o)$, observed at

$$[Cl_o] = 110 \text{ mM}$$

is significantly larger than the relative depolarizations occurring at other values of $[Cl_o]$. The skin resistance measurements, which were made in this series of experiments, revealed that the minimal resistance in the depolarizing phase was dependent upon $[Cl_o]$. It was found that R_o ranged from 1900 Ω cm² at $[Cl_o] = 110$ mM to 3600 Ω cm² at $[Cl_o] = 2$ mM and Fig. 2 shows the mean values of $(\Delta R/R_o)$ and, in particular, the increase in $(\Delta R/R_o)$ which occurs at $[Cl_o] = 110$ mM. Thus the relatively larger depolarizing phase at $[Cl_o] = 110$ mM is consistent with the existence of a more effective shunt relative to that at lower values of $[Cl_o]$.

The description of the depolarizing phase of the response to noradrenaline at different levels of $[Cl_o]$ and $[Na_o]$ is based on the concept that noradrenaline generates a transient shunt in the skin; presumably, the ionic movements in this shunt pathway are composed of a net influx of chloride ions and a net efflux of sodium and (or) potassium ions. The separate experimental determinations of $(\Delta V/V_o)$ associated with $(\Delta R/R_o)$ are displayed in Fig. 3 where the open symbols (○, △, ▽, □) and the filled symbols (●, ▼, ■) refer to the $[Na_o]$ and $[Cl_o]$ experiments of Figs. 1 and 2 respectively. Thus it seems that the relation between $(\Delta V/V_o)$ and $(\Delta R/R_o)$ is linear provided that $[Cl_o] = 110$ mM; however, when

$$[Cl_o] < 110 \text{ mM}$$

the values of $(\Delta V/V_0)$ deviate from the line of equality. A similar deviation from linearity was found in skins bathed in sulphate Ringer solution (Fig. 3, ×). The source of the deviations from linearity between $(\Delta V/V_0)$ and $(\Delta R/R_0)$ will be discussed later.

The evidence, which has been presented in this paper, does not indicate to what extent either the secretion of ions by the skin glands or changes in the ionic permeabilities of the inner membranes of the epithelial cells

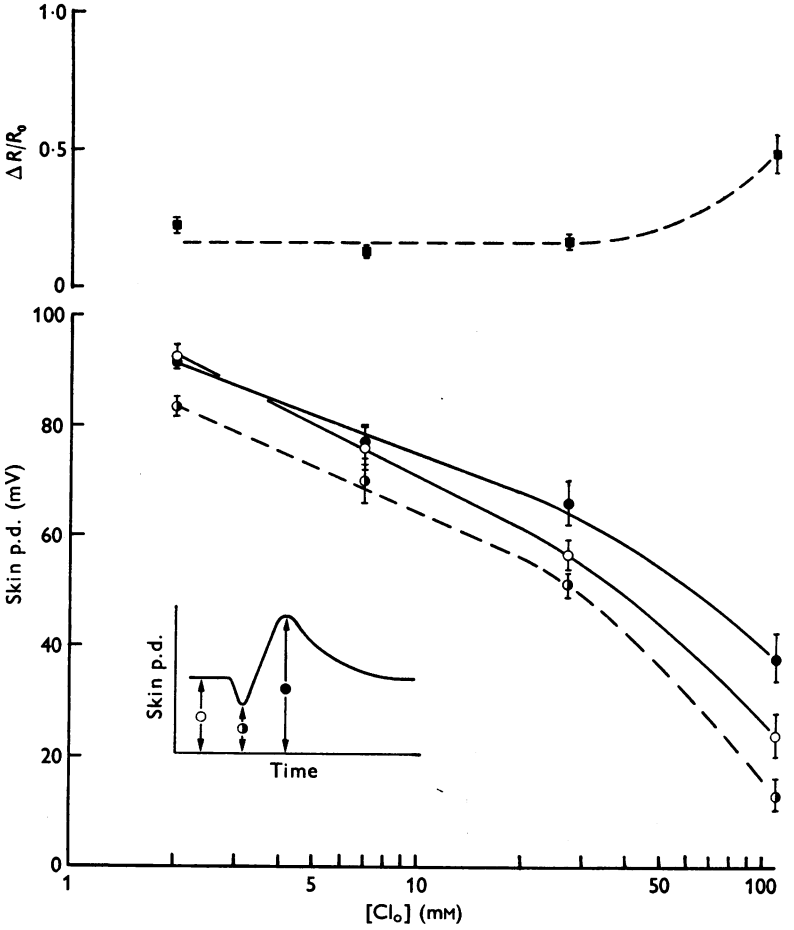

Fig. 2. The effect of external chloride concentration on the response to noradrenaline. Each point is the mean of ten measurements on ten skins, ± S.E. Measurements (○, ◑, ●) of the skin p.d. were made before the addition of noradrenaline and during the depolarizing and hyperpolarizing phases of the response (see inset diagram). Estimates (■) of $(\Delta R/R_0)$ were obtained at the minimum of the depolarizing phase. In each experiment the skin was bathed internally by saline A and externally by saline A, F, G or H.

might contribute directly to the response to noradrenaline. For example, it has been suggested by Koefoed-Johnsen, Levi & Ussing (1952) and Koefoed-Johnsen, Ussing & Zerahn (1953) that the skin glands actively secrete chloride ions and if this transport were electrogenic it ought to add a hyperpolarizing component to the response to noradrenaline. It appears,

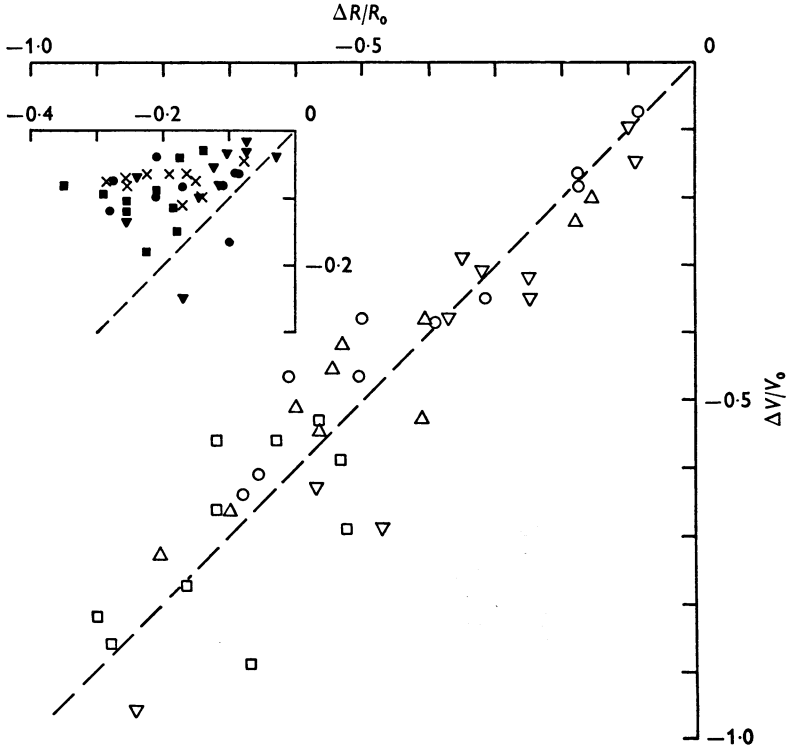

Fig. 3. Relation between the skin p.d. the resistance changes at the minimum of the depolarizing phase of the response to noradrenaline. Each point represents the results of a single experiment; □, ▽, ○, △ refer to experiments where $[Na_o]$ had the values 1, 5, 20 and 100 mM respectively while, ■, ▼, ●, △ refer to experiments where $[Cl_o]$ had the values 2, 7, 27 and 110 mM respectively. In all of these experiments the internal medium was saline A. Experiments, where the skin was bathed on both sides by sulphate Ringer (saline P), are denoted by ×. The interrupted lines are the lines of equality between $\Delta V/V_o$ and $\Delta R/R_o$.

however, that the peak of the secretory activity occurs during the depolarizing phase of the response (Seldin & Hoshiko, 1966; House, 1969*a*, *b*; Lindley, 1969). To establish whether or not the active chloride transport occurring during glandular activity does generate a hyperpolarization it is necessary to eliminate external chloride and sodium ions since the influx of the former is responsible for the initial depolarization while the influx of the

latter generates the subsequent hyperpolarization. These conditions have already been studied in earlier work (House, 1970) where the skin was bathed externally by potassium sulphate Ringer and internally by sodium sulphate Ringer solutions. Fig. 4*A* shows the mean results of such an experiment performed on ten skins; in nine of the ten experiments there was an initial hyperpolarization (range 0·5–3·0 mV) whereas in the tenth experiment no initial hyperpolarization occurred. Such a hyperpolarizing phase might result from an active electrogenic efflux of chloride ions from the skin glands. I decided to test this view by replacing the internal sulphate Ringer with normal Ringer to encourage optimal glandular secretion since Seldin & Hoshiko (1966) have shown that the secretory response is relatively poor in sulphate Ringer. Fig. 4*B* shows the results of those experiments and the initial hyperpolarizing phase of the response is markedly increased. Unfortunately these data are not clear proof of electrogenic chloride transport because the hyperpolarization may be generated by several sources. It might result from an active electrogenic influx of potassium ions and this view would be compatible with the mechanism proposed by Salee & Vidrequin-Deliege (1967) for the later hyperpolarization found in normal Ringer solutions. There are two objections to that hypothesis. First, if an active influx of potassium is responsible for the hyperpolarization it is difficult to see why the magnitudes of the responses are so distinctly different in Fig. 4*A* and *B*. Secondly, it is still possible to record the hyperpolarizing response when the external potassium sulphate Ringer has been replaced by magnesium sulphate Ringer solution (Fig. 4*C*); the latter Ringer solution (saline R) is potassium-free.

An alternative description of the hyperpolarizing responses shown in Fig. 4 is that they are generated by an increase in the potassium permeability of the inner membranes of the epithelial cells. Since no satisfactory evidence for such a change in potassium permeability was found in earlier work (House, 1970) it was important to establish whether or not the level of the hyperpolarization was affected by alternations in $[K_I]$. Therefore, the hyperpolarizing response to noradrenaline was recorded in skins bathed externally by magnesium sulphate Ringer and internally by salines A, I, J and K (see Fig. 5). Under these conditions the dependence of both the original skin p.d. (○) and the peak level of the hyperpolarization (●) on $[K_I]$ is relatively weak, but similar. At first glance there is no evidence, therefore, for an increase in (P^i_K/P^i_{Cl}); however, if the hyperpolarization is generated solely by some other mechanism then the apparent dependence on $[K_I]$ ought to have been reduced during the response. Thus, it seems that a certain component of the response stems from an increase in (P^i_K/P^i_{Cl}). That $[K_I]$ is implicated in the response is also evident from the time courses of the responses of both skin p.d. and

conductance under the conditions of Fig. 5. The average responses at different levels of $[K_1]$ have been plotted in Fig. 6. Both the skin p.d. and the conductance responses decay with similar time courses at each value of $[K_1]$ and the rates of decay are increased at low values of $[K_1]$. When the skin is bathed internally by potassium-free Ringer (saline L) the hyperpolarizing response again decays relatively rapidly to a final value less than the original skin p.d., whereas the conductance asymptotes towards

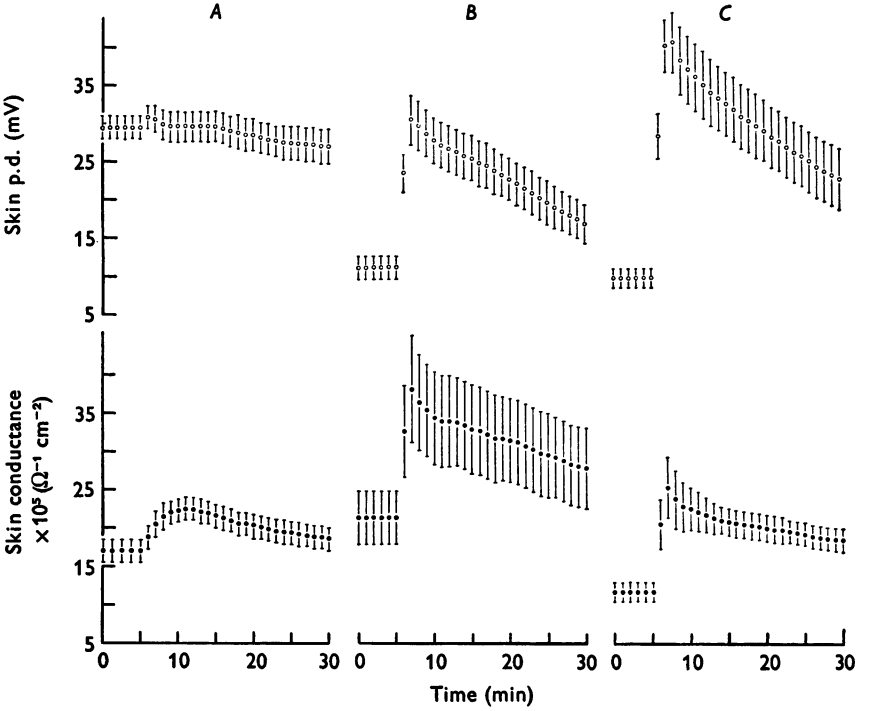

Fig. 4. The electrical responses to noradrenaline in the absence of external sodium and chloride ions. Each point is the mean of ten experiments on ten skins, ± S.E. Noradrenaline was added to the inner medium at the end of the fifth minute of the experimental period. *A* records the effect of bathing the skins internally by saline P and externally by saline Q. *B* records the effect of saline A (internal) and saline Q (external). *C* records the effect of saline A (internal) and saline R (external).

its original value (Fig. 7). The relatively low levels of the skin p.d. during the final phase of the responses in Fig. 7 and Fig. 6 ($[K_1]$ = 0·1 mM) may indicate a decrease in $[K_c]$ which has arisen from an increase in (P^i_K/P^i_{Cl}).

Finally, it might be contended that the hyperpolarizations in Fig. 4*B* and *C* are transient diffusion potentials generated by a passive efflux of chloride ions and their accompanying cations through the active skin glands. This view is compatible with the observed identity between the

time courses of the responses in the skin p.d. and the conductance in Fig. 6. In order to test whether or not the hyperpolarizing response was caused by the movement of chloride ions down their concentration gradient, different chloride gradients were applied across the skin. Fig. 8 shows the typical results of experiments where skins were bathed internally by several kinds of chloride Ringers and externally by magnesium sulphate

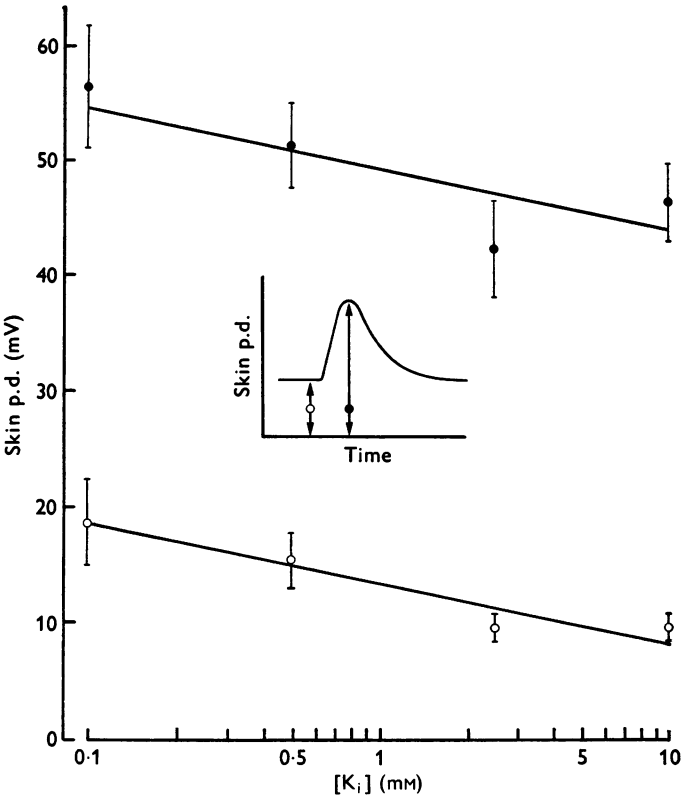

Fig. 5. The effect of internal potassium concentration on the hyperpolarizing response to noradrenaline. Each point is the mean of ten experiments on ten skins, ± S.E. Measurements (○, ●) were made before and at the maximum of the hyperpolarizing response (see inset diagram). In each experiment the skin was bathed externally by saline R and internally by saline A, I, J or K.

Ringer and vice versa. Hyperpolarizing responses were always observed in the former experiments. When potassium chloride Ringer bathed the inner surface, however, the response induced by noradrenaline was small probably because of the similar actions of high [K_i] and noradrenaline as stimulants of glandular secretion (Seldin & Hoshiko, 1966; House, 1970). In the experiments where the chloride Ringer bathed the skin's external

surface, noradrenaline generated an increase in internal negativity probably due to an influx of chloride ions through the active skin glands. Under these conditions (sulphate Ringer inside) glandular secretion is somewhat impaired but certainly not absent (Seldin & Hoshiko, 1966).

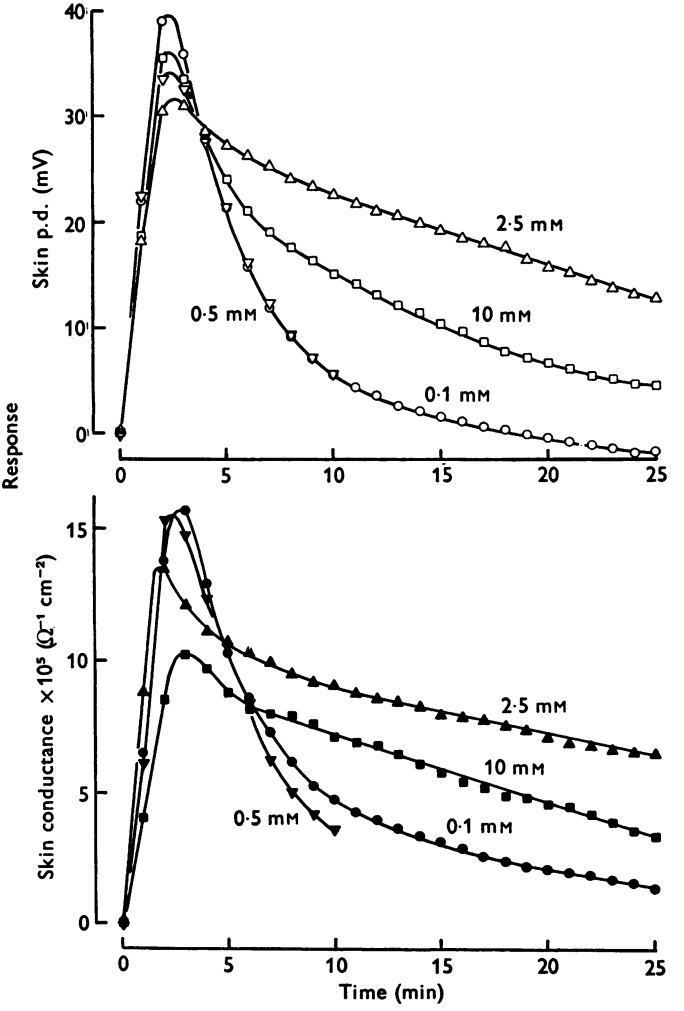

Fig. 6. The effect of internal potassium concentration on the magnitude and time course of the hyperpolarizing response to noradrenaline. The response of the skin p.d. (and the skin conductance) is expressed as the size of the *increase* in the p.d. (and conductance) above the resting value. Each point is the mean of ten experiments on ten skins and the data were obtained during the experiments reported in Fig. 5. The values of $[K_i]$ are shown beside the corresponding curves (○, ●; 0·1 mM), (▽, ▼; 0·5 mM), (△, ▲; 2·5 mM) and (□, ■; 10 mM).

Certain experiments were performed on the hyperpolarizing response to distinguish between possible active (electrogenic) and passive effluxes of chloride ions through the skin. It was found that 10^{-4} M-2, 4-dinitrophenol abolished the hyperpolarizing response whereas 10^{-4} M acetazolamide

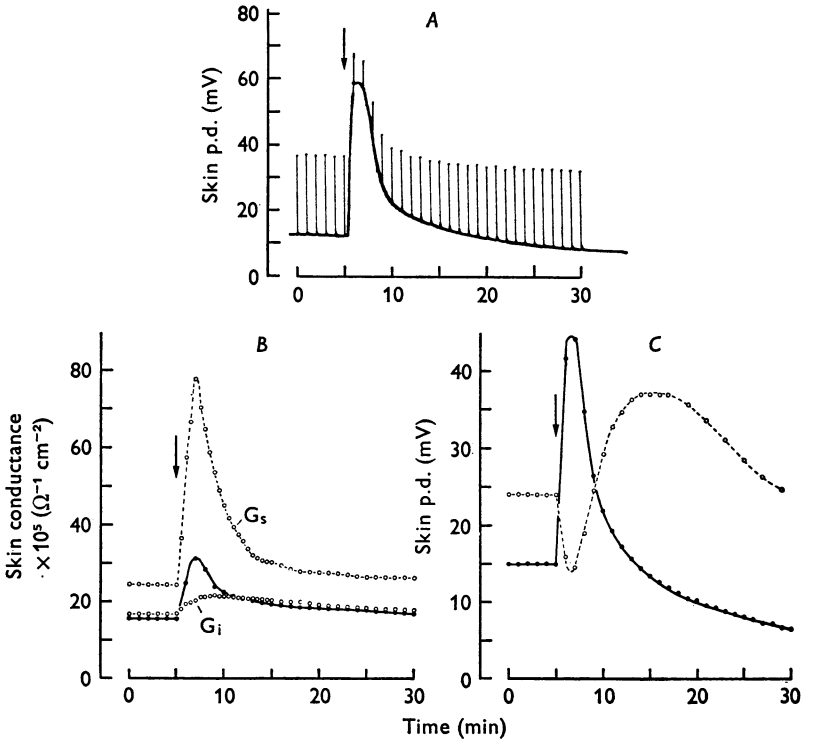

Fig. 7. A comparison between the hyperpolarizing response and the normal response to noradrenaline. At the time indicated by the arrows noradrenaline was added to the inner medium. *A* records a typical hyperpolarizing response in a skin bathed internally by potassium-free Ringer (saline L) and externally by saline R. The brief hyperpolarizations on the potential record were produced by constant current pulses of 20 μA. *B* records the mean values (●) of the skin conductance during hyperpolarizing responses in ten skins (saline L inside; saline R outside). The average values (○) of G_s and G_i during the normal response to noradrenaline are also shown. *C* records the mean values (●) of the skin p.d. during the hyperpolarizing responses and also those (○) of the normal response. In *B* and *C* each point is the mean of ten determinations on ten skins.

applied to the inner surface had no effect on the response. Since acetazolamide is a relatively specific inhibitor of active chloride transport in amphibian skin (Erlij, 1971), it is unlikely that the response is due to an active efflux of chloride ions. It is tempting to conclude, therefore, that the

hyperpolarization is a transient diffusion potential generated by a passive efflux of chloride through a shunt element whose activation is dependent upon the metabolic activity of the tissue. It is difficult to ascribe to any structure in the skin, other than the glands, such a set of active and passive properties.

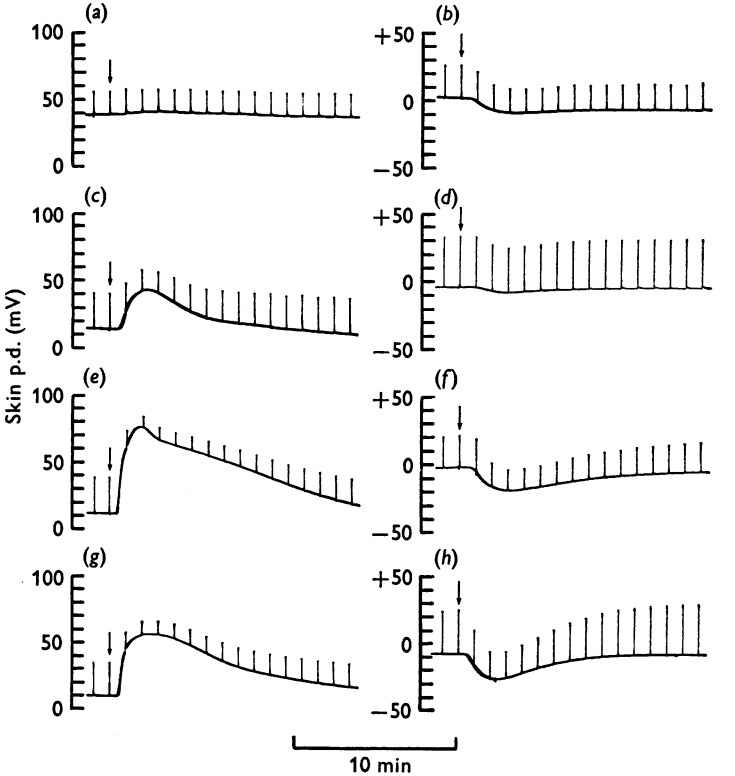

Fig. 8. The hyperpolarizing responses in toad skins bathed by a variety of chloride Ringer. In each record noradrenaline was added to the internal medium at the time indicated by the arrow. In *a*, *c*, *e* and *g* the external medium was saline R and the internal media were salines E, O, M and N respectively. In *b*, *d*, *f* and *h* the internal medium was saline R and the external media were salines E, O, M and N respectively. The brief deflexions on the potential records of *a–h* were produced by constant current pulses of 100, 100, 20, 40, 20, 40, 10 and 40 μA respectively.

DISCUSSION

The action of noradrenaline on the toad skin potential may be interpreted in the light of the separate effects which it may exert on the epithelial cells and on the glands.

Epithelial cells

Certain experimental evidence suggests that noradrenaline increases not only the permeability of the outer membranes to sodium but also to chloride ions. Unidirectional fluxes of sodium and chloride ions increase after treatment with noradrenaline (Bastide & Jard, 1968). Moreover, certain electrophysiological evidence (House, 1969*b*, 1970) points to an initial increase in P^o_{Cl} during the depolarizing phase of the response followed by an increase in P^o_{Na} during the hyperpolarizing phase. The work reported here strongly supports the view that (P^o_{Na}/P^o_{Cl}) increases to a peak value which is associated with the hyperpolarizing phase of the response when the skin is bathed by normal Ringer. With the techniques used in this study, however, it has not been possible to substantiate the earlier indication (House, 1969*b*) that an increase in P^o_{Cl} occurs during the depolarizing phase simply because it is difficult to differentiate between the depolarizing actions of chloride movement through the epithelial cells as opposed to that through the glands. Nevertheless, since the unidirectional fluxes of chloride (Bastide & Jard, 1968) remain larger than normal for periods much longer than the phase of glandular secretion (House, 1969*a*, *b*) it seems necessary to accept the previous evidence (House, 1969*b*, 1970) that noradrenaline increases P^o_{Cl}.

If this picture of the changes in P^o_{Na} and P^o_{Cl} evoked by noradrenaline is correct, then we might expect that the changes in those ionic permeabilities ought to be reflected in the changes in the conductance, G_i, for actively transported sodium ions and in the shunt conductance, G_s, respectively. Fig. 7*B* displays the estimated time courses of G_i and G_s for skins bathed in normal Ringer solutions; House (1970) obtained G_i by subtracting the shunt conductance from the total skin conductance when the skin was bathed in sulphate media. G_s has been obtained currently by subtracting the values of G_i from the corresponding values of $(G_i + G_s)$ for skins bathed in normal Ringer on the assumption that G_i is identical in both normal and sulphate Ringer. The values of G_s obtained are larger than the corresponding shunt conductances in sulphate Ringer and G_s reaches a peak value during the depolarizing phase of the response. The fact that G_s is larger than the shunt conductance in sulphate Ringer is compatible with the belief that chloride ions carry the major portion of the depolarizing current across skin bathed in normal Ringer. In contrast to the time course of G_s, it is seen in Fig. 7*B* that G_i does not reach its maximum until a later stage during the onset of the hyperpolarizing phase (○) of the normal response (Fig. 7*C*). The temporal dispersion of G_s and G_i, therefore, is compatible with the changes in P^o_{Cl} and P^o_{Na} which underlie the electrical response to noradrenaline. Since G_s includes a contribution from the

glandular shunt pathway, however, it cannot be regarded as a satisfactory measure of P^{o}_{Cl} alone.

In a previous paper (House, 1969*b*) the depolarizing phase was described by the relation, $\Delta V/V_o = \Delta R/R_o$ and it was assumed that during depolarization no change occurred in either the electromotive force, E, of the active transport system or in G_l. A detailed analysis of the original simple analogue of the skin p.d., where again E is assumed to remain constant initially, reveals that the depolarization, ΔV, will be given by $V_o\Delta R/R_o$ provided that the change in G_s is much larger than G_l. Since the latter condition is met in skins bathed in normal Ringer but not in sulphate Ringer, it is clear why the former data (○) in Fig. 3 obey $\Delta V/V_o = \Delta R/R_o$ while the latter values (×) do not. Indeed, for skins bathed in sulphate Ringer it is to be expected that $\Delta V/V_o < \Delta R/R_o$ since the maximal increase in shunt conductance is similar to the increase in G_l (House, 1970).

Apart from the alterations in P^{o}_{Na} and P^{o}_{Cl} which have been proposed in this paper and in previous work, no other changes in the ionic permeabilities of the outer barrier of the skin seem to be elicited by noradrenaline. In particular, no evidence has been found for an increase in (P^{o}_{K}/P^{o}_{Cl}) (House, 1969*b*). Some data in this investigation (Figs. 5 and 6) suggest that noradrenaline produces an increase in (P^{i}_{K}/P^{i}_{Cl}); however, more work is required to clarify the effects of noradrenaline on the inner barrier.

Skin glands

Noradrenaline evokes a brief period of glandular activity and the peak of this secretory phase is contemporaneous with the depolarizing phase of the electrical record when the skin is bathed by normal Ringer. At least part of the depolarization must be attributed to the influx of chloride and the efflux of sodium and potassium ions through the glandular pathway. In order to analyse what role the ionic movements through the glands may play in the electrical response it is necessary to eliminate the effects of increases in P^{o}_{Na} and P^{o}_{Cl} on the skin p.d. In the present work the possible influxes of chloride (depolarizing phase) and of sodium ions (hyperpolarizing phase) stemming from increases in P^{o}_{Cl} and P^{o}_{Na} have been abolished by replacing the external Ringer with either potassium sulphate or magnesium sulphate Ringer. Under these circumstances the response is transformed into a transient phase of hyperpolarization and the peak of this new response occurs at the same time as the minimum of the depolarizing phase of the original response (Fig. 7*C*). Therefore, when the component response of the epithelial cells to noradrenaline has been removed, the remaining glandular activity is associated with a relatively rapid hyperpolarization.

The link between the hyperpolarization recorded in Fig. 7*A* and glandular secretion might be that there is an active electrogenic efflux of chloride ions. Although no

direct measurements of ionic effluxes from the skin glands have been made, it is possible to estimate the upper magnitude of the secretory flux. Seldin & Hoshiko (1966) have estimated that there may be as many as $7{\cdot}6 \times 10^3$ glands per cm^2 in frog skin and that each gland may produce a maximal secretory volume of 4×10^{-6} μl. Thus the maximal secretory volume from unit area of frog skin will be about 3×10^{-2} μl. and, if this secretion is isotonic, then the quantity of anion, say chloride, secreted will be 3×10^{-6} m-equiv. According to Seldin & Hoshiko (1966) the secretory volume from the glands does not increase significantly after 120 sec, and, therefore, the maximal efflux of chloride would be 0·09 μ-equiv. cm^{-2} hr^{-1} (equivalent to a short-circuit current of 2·4 μA cm^{-2}). Such a secretion of chloride, if it were purely electrogenic and if the shunting introduced by the accompanying cation were insignificant, would produce a hyperpolarization of 2·4 mV across a skin resistance of 1000 Ω cm^2. Therefore, provided that secretion is primarily an electrogenic efflux of chloride ions, then glandular activity might generate under optimal conditions a hyperpolarization of a few millivolts. The relatively large hyperpolarizations recorded in Figs. 4, 6, 7 and 8 are apparently well above the upper limit which is likely to stem from an active electrogenic transport of chloride ions by the glands. Lindley (1969) has reported that the lumen of skin glands becomes positive with respect to the inner surface of the skin during glandular secretion. Until more evidence becomes available on this point and about the ionic components of the secretion it is necessary to express considerable doubt about not only the expected size but also the direction of the glandular electromotive force.

The electrogenesis of the hyperpolarizing response (Figs. 4, 6, 7 and 8) is not resolved definitely although certain conclusions can be drawn about its nature. First, the polarity of the response can be altered by reversing the direction of the chloride gradient across the skin (Fig. 8). Secondly, although the response is dependent upon the metabolism of the tissue it is not reduced in magnitude by a specific inhibitor of active chloride transport. Thirdly, the time course of the p.d. is closely matched by that of the conductance (Fig. 6); indeed, both are similar to the time course of glandular secretion recorded volumetrically in *Bufo bufo* skin (unpublished work) and in *Xenopus* skin (House, 1969*a*, *b*). Also the estimated time course of the changes in G_s resembles that of the skin conductance during the response (Fig. 7*B*). Finally, the response is not dependent directly upon the presence of external potassium or internal sodium ions; however, it does require the presence of chloride ions. Thus I envisage that, when the glands are activated by noradrenaline, they offer a transient leak pathway which is occupied predominantly by chloride ions. The efflux of chloride and concomitant cations generates a diffusion potential across the skin such that the inside becomes positive with respect to the outside.

The existence of the hyperpolarizing response probably explains the origin of the discrepancy between $(\Delta V/V_o)$ and $(\Delta R/R_o)$ which was observed in Fig. 3 for the experiments where $[Cl_o]$ was altered. Invariably it was found that $\Delta V/V_o < \Delta R/R_o$ when $[Cl_o] < [Cl_i]$. Under the circumstances, where a chloride concentration gradient exists across the tissue, the level

of depolarization will depend not only on the efflux of sodium and potassium down the electrical gradient, but also on the movement of chloride down its electrochemical gradient. For example, consider the experiments where $[Cl_o]$ = 2 mM (Fig. 2). If the skin becomes very permeable to chloride ions during the initial phase of the response, then the skin p.d. will move towards a higher value which will oppose partially the shunting effect of sodium and potassium movements on the p.d. Therefore, the actual change in skin p.d. will be less than $V_o \Delta R/R_o$.

My experimental evidence indicates that the active skin glands themselves do not generate an observable component in the potential response to noradrenaline. Despite the absence of an unambiguous signal of ionic secretion from the glands, an electrical index of glandular activity is apparent when a chloride concentration gradient exists across the skin and external sodium ions are absent. That response may prove to be a useful electrical tool for analysing some of the characteristics of glandular secretion in amphibian skin.

I thank Mr G. Allan for technical assistance and Mr C. Warwick for preparing the diagrams. I also gratefully thank the Science Research Council for financial support during this investigation.

REFERENCES

BASTIDE, F. & JARD, S. (1968). Actions de la noradrénaline 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.

ERLIJ, D. (1971). Salt transport across isolated frog skin. *Proc. R. Soc.* B (in the Press).

HOUSE, C. R. (1969*a*). Secretory response to the stimulation of amphibian skin glands. *Biochim. biophys. Acta* **173**, 344–347.

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

HOUSE, C. R. (1970). The effect of noradrenaline on the toad skin potential. *J. Physiol.* **209**, 513–537.

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

KOEFOED-JOHNSEN, V., LEVI, H. & USSING, H. H. (1952). The mode of passage of chloride ions through the isolated frog skin. *Acta physiol. scand.* **25**, 150–163.

KOEFOED-JOHNSEN, V., USSING, H. H. & ZERAHN, K. (1953). The origin of the short-circuit current in the adrenaline stimulated frog skin. *Acta physiol. scand.* **27**, 38–48.

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

SELDIN, J. P. & HOSHIKO, T. (1966). Ionic requirements for epinephrine stimulation of frog skin gland secretion. *J. exp. Zool.* **163**, 111–114.

USSING, H. H. & WINDHAGER, E. E. (1964). Nature of shunt pathway 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.