BBA 75524

THE EFFECTS OF HIGH HYDROSTATIC PRESSURE ON THE PERMEABILITY CHARACTERISTICS OF THE ISOLATED FROG SKIN*

A. BROUHA, A. PEQUEUX, E. SCHOFFENIELS** AND A. DISTECHE

Laboratory of General Biology and ***Department of Biochemistry, University of Liège (Belgium)*

(Received June 1st, 1970)

SUMMARY

We have studied the effects of high hydrostatic pressures on the electrical potential difference existing spontaneously across the isolated abdominal skin of the frog *Rana temporaria* L.

A pressure of 100 kg/cm^2 produces first an instantaneous and short depolarization, followed by a much larger hyperpolarization phase. The decompression is accompanied by a short positive or negative variation before the electrical potential reaches its control value.

This phenomenon seems to involve the outer barrier of the skin and results from changes in the permeability characteristics to Na^+; moreover, the active transport of Na^+ does not seem to be stimulated directly by the hydrostatic pressure. The effect of pressure seems rather specific, since the permeabilities of other ionic species such as Cl^- and K^+ are not modified.

The hydrostatic pressure seems to be a valid tool with which to approach the study of the structure of the cell membrane in relation to function. If pressure acts specifically on some permeability characteristics of the cell membrane, we may conclude that the different ionic species move through structures spatially separated in the membrane and far more complex than the ones postulated in the simple theory of the porous membrane.

INTRODUCTION

Although several properties of biological membranes have become well known after numerous morphological and functional studies, the mode of transfer of ions still remains very obscure when considered at the molecular level.

The case of water is also far from being elucidated. When biological or artificial membranes are subjected to an osmotic gradient, the flux of water is mainly non-diffusional. This has led some authors to postulate the existence of holes in the membrane, thus giving a renewal of interest to the theory of the sieve-membrane[4–6]. The theory of the porous membrane has had some success among the physiologists, though it is generally admitted that it gives an oversimplified picture of the situation.

* Most of the results presented in this paper have been submitted in 1965 by BROUHA[1] for the obtention of the degree of "Licenciée en Sciences zoologiques", University of Liège. Short communications on the subject have been given by SCHOFFENIELS[2,3].

Hydrostatic pressure applied from all sides on an isolated frog skin may help to throw some light on the problem. If we are dealing with inert fixed pores in the cell membranes, it is clear that pressure, acting on the whole structure of the skin, will have no influence on the permeability characteristics. On the other hand, since pressure is known to enhance the dissociation of weak electrolytes[7,8], it can be expected to influence the state of ionization of the membrane components.

Moreover, by changing the ionization of proteins, pressure may act on the structure and activity of enzymes involved in transport mechanisms, and then affect the physiological properties of membranes.

Our experiments do show that pressure induces variations in the electrical potential difference existing across the frog skin. This paper presents some conclusions as to the origin of these changes.

METHODS

Experiments are performed on isolated abdominal skins from *Rana temporaria* L.

The skins are mounted between plexiglass chambers, containing 5 ml of saline. 30 min before the beginning of the experiment, air is bubbled through the saline on both sides of the skin. The exposed skin area is 3.78 cm^2.

The apparatus used for compressing the skin is described by DISTECHE[7].

The skin potential is measured with Ag–AgCl electrodes connected in opposition to a precision potentiometer (Cambridge); the output of this circuit is fed to an electrometer (603-Keithley Electrometer). Potential variations during the compression are read from a pen recorder (Varian G.14).

The reference saline is NaCl Ringer's soln. (115 mM NaCl, 2 mM KCl, 0.45 mM $CaCl_2$, buffered at pH 7.8 with phosphate buffer). The other salines used are Na_2SO_4 Ringer's soln., where Cl^- is replaced by the nonpenetrating SO_4^{2-}; and KCl Ringer's soln., LiCl Ringer's soln., $MgCl_2$ Ringer's soln., choline chloride Ringer's soln., where Na^+ is replaced by K^+, Li^+, Mg^{2+} and choline ions, respectively. Sucrose is added to distilled water and Na_2SO_4 Ringer's soln. to adjust the osmotic pressure. In some experiments, skins were treated with $5 \cdot 10^{-5}$ M ouabain or with 10 munits/ml ocytocin.

RESULTS

A. Skin bathed on both sides with ordinary saline (NaCl Ringer's soln.)

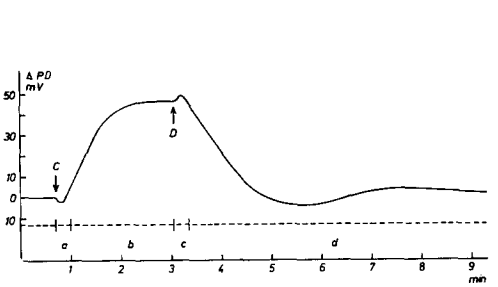
When a pressure step of 100 kg/cm^2 is applied to the skin, its potential immediately changes, as shown in Fig. 1. We first see an instantaneous and short depolarization phase (a) from 0 to 10 mV, followed by a hyperpolarization phase (b) that may reach 60 mV and lasts as long as the pressure is maintained. Decompression is generally followed by a short positive or negative potential variation (c) up to 10 mV, and then the skin potential slowly decreases to reach its first value. As shown in Fig. 2, it seems that there is no relation between the initial potential difference, the values of the depolarization (a) and the hyperpolarization (b). For larger pressure steps, we have observed that the skin potential variations reach a maximum value between 100 and 500 kg/cm^2; the recordings are similar to the one shown in Fig. 1. Above these values, pressure seems to damage the skin since it induces a slow and irreversible depolarization.

B. Skin bathed on both sides with a sulfate saline (Na_2SO_4 Ringer's soln.)

It is well known that the frog skin is a little permeable to SO_4^{2-}. As a consequence, the spontaneous potential difference recorded across the skin may increase to values close to 100 mV or even more. In Na_2SO_4 Ringer's soln., the depolarization phase (a) seems to be unchanged but the hyperpolarization phase (b) is always reduced and generally abolished (Fig. 3). If we plot the value of the hyperpolarization (b) recorded when the skin is compressed in NaCl Ringer's soln. as a function of the increase of potential observed when the skin, first in NaCl Ringer's soln., is brought in Na_2SO_4 Ringer's soln. at atmospheric pressure, we see (Fig. 4) that the large potential variation, when the skin is brought into contact with Na_2SO_4 Ringer's soln., corresponds to a large hyperpolarization phase (b).

C. Skin bathed with isotonic sucrose

When the inside of the skin is bathed with distilled water made isotonic by addition of sucrose, and the outside with NaCl Ringer's soln., the response recorded under the influence of pressure is not much different from the response of the control. Table I shows that the potential is somewhat higher and that the hyperpolarization (b) and depolarization (a) have decreased a little. It may be noted that if the potential



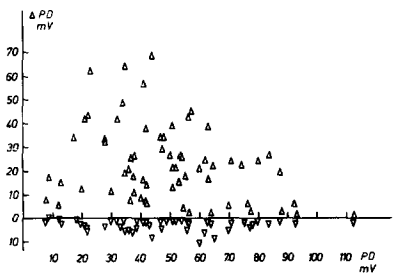
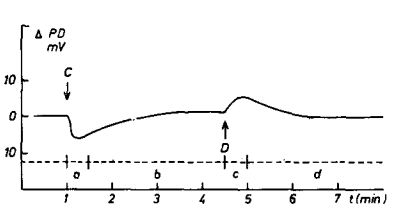


Fig. 1. Effect of a pressure step of 100 kg/cm² on the potential difference of a skin bathed, on both sides, by NaCl Ringer's soln. (pressure is applied at C, removed at D). Abscissa: time in minutes. Ordinate: variations of the potential difference in mV.

Fig. 2. Potential variations observed during a compression cycle as a function of the initial potential difference of the skins (in NaCl Ringer's soln.). Abscissa: initial potential difference in mV. Ordinate: potential variations in mV. △, hyperpolarization (b) values; ▽, depolarization (a) values.



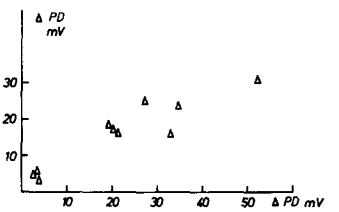


Fig. 3. Effect of a pressure step of 100 kg/cm² on the potential difference of a skin bathed, on both sides, by Na_2SO_4 Ringer's soln. (pressure applied at C, removed at D). Abscissa: time in minutes. Ordinate: variations of the potential difference in mV.

Fig. 4. Magnitude of the hyperpolarization phase (b) obtained when the skin is compressed in NaCl Ringer's soln. (ordinate), as a function of the increase of potential (abscissa) observed when the same skin is bathed by a Na_2SO_4 Ringer's soln. at atmospheric pressure.

TABLE I

EVOLUTION OF THE SKIN POTENTIAL DIFFERENCE (PD) AND OF THE POTENTIAL DIFFERENCE VARIATIONS RECORDED UNDER THE EFFECT OF PRESSURE, WHEN ONE SIDE OF THE SKIN IS BATHED WITH DISTILLED WATER MADE ISOTONIC BY ADDITION OF SUCROSE

Results in mV.

	NaCl Ringer's soln. on both sides	*Distilled water and sucrose on the side facing*	
		Outwards	*Inwards*
PD	41	20	50
Depolarization (a)	4	1	1
Hyperpolarization (b)	40	0	25

TABLE II

EVOLUTION OF THE SKIN POTENTIAL DIFFERENCE (PD) AND OF THE POTENTIAL DIFFERENCE VARIATIONS RECORDED UNDER THE EFFECT OF PRESSURE, WHEN THE OUTSIDE OF THE SKIN IS BATHED WITH A SALINE IN WHICH Na^+ IS SUBSTITUTED

Results in mV.

Expt.	*External saline*	*PD*	*Depolarization (a)*	*Hyperpolarization (b)*
1	NaCl Ringer's soln.	65.6	4.3	9.7
	KCl Ringer's soln.	48.8	8	3
2	NaCl Ringer's soln.	55.9	2	0
	KCl Ringer's soln.	22.6	2.5	0
3	NaCl Ringer's soln.	58.4	0.6	10
	KCl Ringer's soln.	35.9	1.5	5.5
4	NaCl Ringer's soln.	50	4	30
	KCl Ringer's soln.	29	1	9
1	NaCl Ringer's soln.	76.2	0.6	28
	$MgCl_2$ Ringer's soln.	41.5	0	4.7
2	NaCl Ringer's soln.	63.4	2	22
	$MgCl_2$ Ringer's soln.	28.2	3.7	0
3	NaCl Ringer's soln.	46	1	28
	$MgCl_2$ Ringer's soln.	30	1	9
1	NaCl Ringer's soln.	46.1	0	40
	Choline chloride Ringer's soln.	35	0	4.7
2	NaCl Ringer's soln.	64.2	0.7	15.3
	Choline chloride Ringer's soln.	31	0.7	6.3
3	NaCl Ringer's soln.	57	2.5	35
	Choline chloride Ringer's soln.	28	1	19
1	NaCl Ringer's soln.	44.4	2	17
	LiCl Ringer's soln.	39.1	2.2	1.7
2	NaCl Ringer's soln.	42	3.3	6
	LiCl Ringer's soln.	40	1.5	0.7
3	NaCl Ringer's soln.	75	1.5	19
	LiCl Ringer's soln.	66	2	2

first increases when the inside saline is replaced by distilled water and sucrose, it later decreases and may reach negative values after 45 min of treatment.

When the outside of the skin is bathed with isotonic sucrose, the record obtained under the influence of pressure is quite different from the control. The skin potential difference immediately decreases and the hyperpolarization phase (b) is always abolished (Table I).

D. Outside of the skin bathed with a saline in which Na^+ is substituted

When the outside of the skin is bathed with a saline in which Na^+ is substituted by K^+, Mg^{2+} or choline ions, the spontaneous potential difference, as well as the hyperpolarization (b) observed under the influence of a pressure step, decreases, while the magnitude of the depolarization (a) remains small though variable (Table II).

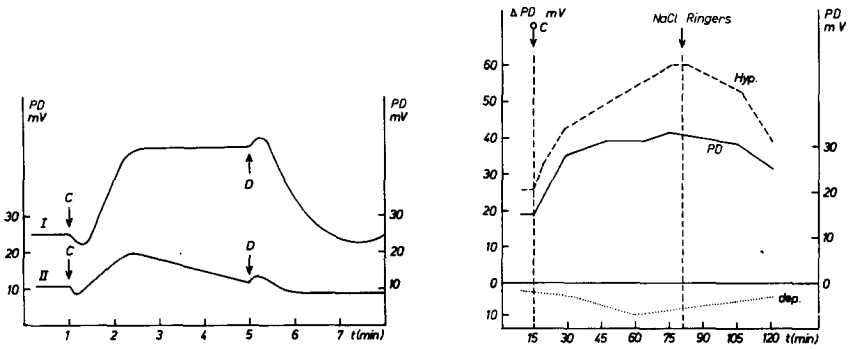


Fig. 5. Effect of $5 \cdot 10^{-5}$ M ouabain on the initial potential difference and on the potential variations produced by pressure. C = pressure step of 100 kg/cm^2; D = release of pressure. I. Control in NaCl Ringer's soln. II. 50 min after addition of $5 \cdot 10^{-5}$ M ouabain in the solution bathing the inside of the skin. Ordinate: time (min). Abscissa: potential difference (mV).

Fig. 6. Effect of 10 munits/ml ocytocin on the spontaneous skin potential difference and on the potential variations produced by a pressure step of 100 kg/cm^2. Ocytocin 10 munits/ml is added in the inside medium at 15 min, and NaCl Ringer's soln. is replaced on both sides at 80 min. ———, PD = spontaneous potential difference; ·····, dep. = depolarization (a); -------, Hyp. = hyperpolarization (b). Left ordinate: potential variations (mV) produced by pressure. Right ordinate: spontaneous potential difference (mV). Abscissa: time (min).

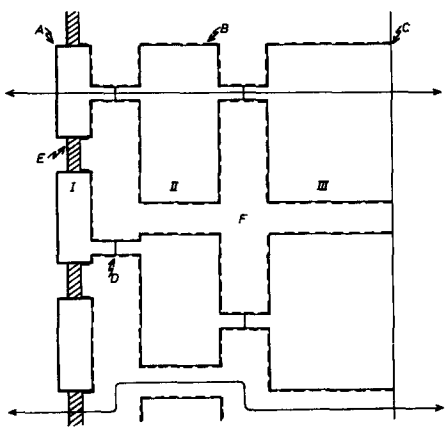


Fig. 7. Schematic representation of the frog epidermis. Explanations in the text (after FARQUHAR AND PALADE[10]).

When Na^+ is substituted by Li^+, the potential difference decreases less, but the hyperpolarization (b) is very much reduced (see Table II).

E. Effect of ouabain and ocytocin

Fig. 5 shows the records obtained when the frog skin is compressed before (I) and 50 min after the addition of $5 \cdot 10^{-5}$ M ouabain in the inside medium (II). We see that in Fig. 5, II, the skin potential difference, the depolarization (a) and the hyperpolarization (b) are reduced. It is worth noting that after application of ouabain, the hyperpolarization (b) decreases rather rapidly.

The effects of ocytocin on the Na^+ fluxes are generally explained as resulting from an increase of the passive permeability to Na^+ of the outer border of the skin. When added to the saline bathing the inside of an isolated skin subjected to a pressure step of 100 kg/cm^2, 10 munits/ml ocytocin acts as follows: the skin potential, the depolarization (a) and the hyperpolarization (b) increase quickly in the first 50 min and then more slowly in the following hour (Fig. 6). The effect is reversible.

DISCUSSION

In the analysis of the experimental results, we will refer to the schematic representation of the epidermis given in Fig. 7, established according to findings of KOEFOED-JOHNSEN AND USSING[9], and the morphological study of FARQUHAR AND PALADE[10].

The cells of the *Stratum corneum* (I) constitute the only continuous structure of the skin, since they are united by *zonulae occludentes* (E). Their external faces (A) are specifically permeable to Na^+, which induces at this level a Na^+ diffusion potential. The cells of the *stratum corneum* (I), *stratum granulosum* (II) and *stratum germinativum* (III) are in relation to the others by junction bridges called desmosomes (D). They are separated by an extracellular space which forms a continuous network (F). The cellular membranes (B) limiting this intercellular lake are generally believed to be the site of the active transport for Na^+. On the other hand, the membranes of the *stratum germinativum* in contact with the basement membrane (C) have no ATPase activity, and they would correspond to the site of the specific permeability to K^+ described by KOEFOED-JOHNSEN AND USSING[9], inducing at this level a K^+ diffusion potential. The electrical potential difference existing across the isolated frog skin is then the sum of both Na^+ and K^+ diffusion potentials in series, but as the skin is permeable to Cl^-, both diffusion potentials are short-circuited by a Cl^- diffusion potential. Finally, the expression of the frog skin total potential difference E is rather similar to that used by HODGKIN AND KATZ[11] to explain the membrane potential of the giant squid axon.

According to this formulation, we may consider that the variations in the skin potential occur either when the permeability coefficients or the concentration gradients vary.

We have shown that a pressure step of 100 kg/cm^2 produces a short depolarization phase (a), followed by a much larger hyperpolarization phase (b). Decompression is accompanied by a short positive or negative variation and then the skin potential slowly decreases to reach its initial value.

In order to test these possibilities, we have modified the ionic composition of the saline solutions bathing the skin.

When isotonic sucrose is placed on the inside of the skin, no great changes can be noticed. On the contrary, if the same solution replaces the outside medium, the hyperpolarization phase (b) is abolished. Consequently, we may consider that the hyperpolarization results from the effects of pressure on the cellular membranes facing outwards.

When Cl^- is substituted by SO_4^{2-} in the saline, the hyperpolarization phase (b) is reduced or abolished. Moreover, there is a relation between the hyperpolarization (b) obtained in NaCl Ringer's soln. and the increase of potential observed when the skin, first in NaCl Ringer's soln., is brought in contact with Na_2SO_4 Ringer's soln. Since this increase represents the shunt of the Cl^- through the skin, it may be considered as measuring the actual permeability of the skin to Cl^-. Thus, the higher the initial permeability to Cl^-, the larger the hyperpolarization (b). Those results bring us to assume that Cl^- could be responsible for the hyperpolarization phase which would then result from a decrease of the skin permeability to Cl^-.

However, if we consider the Goldman equation (ref. 11), we see that a change in Na^+ permeability could also produce a potential variation but only if Cl^- permeability is not zero. In the latter case, one obtains the Nernst's equation, and the potential difference at the mucosal border would only depend on Na^+ activities in each compartment. It is thus equally reasonable to explain the hyperpolarization (b) by an increase of the Na^+ permeability under the effect of pressure. Our other results are consistent with this interpretation. If Na^+ is missing in the external medium, the gradient at the outer barrier of the skin is considerably reduced thus leading to a low value of potential at this level. Any increase of Na^+ permeability will not much affect the value of this electrical potential. On the other hand, the increase of the hyperpolarization phase (b), obtained when ocytocin is added in the inside medium, is also in favor of this view. As already mentioned, ocytocin is generally assumed to increase the Na^+ permeability of the outer side of the skin. As ocytocin and pressure give identical and additive effects on the potential difference, it seems justifiable to think that they act on the same structures.

If, when applied, a high hydrostatic pressure increases the permeability to Na^+ of the outer membrane of the skin, the cellular Na^+ concentration will also increase, stimulating, in turn, the Na^+ active transport. This could well explain the enhancement of active transport of Na^+ observed (unpublished results). Experiments made in the presence of ouabain show that the hyperpolarization (b) decreases progressively when the Na^+ active transport is inhibited, but in this case the Na^+ concentration gradient at the outer face also decreases. As a consequence, the number of Na^+ penetrating the cell during the compression is reduced and decreases continually, since the cellular Na^+ concentration keeps increasing. Therefore, the progressive fall of hyperpolarization during the experiment would correspond to a slow abolition of the Na^+ gradient.

Finally, it is apparent from our results that pressure does not seem to affect greatly the permeability of the skin to K^+.

To conclude briefly, our results show that changes in the ionic composition of the saline in contact with the external face of the skin modify the electrical response of the skin to an application of a high hydrostatic pressure. Compounds able to affect

the permeability characteristics of the skin and the active transport of Na^+ also influence the response of the skin to pressure.

It thus seems logical to propose that hydrostatic pressure affects the permeability characteristics, and this conclusion is of importance. If ions move across the skin through structures that may be modified under the influence of a high hydrostatic pressure, those structures cannot be inert pores. Moreover, pressure seems to act selectively on the permeability of the skin to a given ionic species.

So far little can be said as to the molecular aspects of the changes in electrical potential observed when a high hydrostatic pressure is applied on the isolated frog skin. It is certainly reasonable to assume that the specific permeability properties of the skin to Na^+ are related to chemical groups, the dissociation of which control the transfer of Na^+ at the outer border of the skin. A high hydrostatic pressure is known to favor the dissociation of weak electrolytes[8], and one could well explain our observations by assuming that the configuration of the molecular architecture responsible for the specific permeability to Na^+ of the outer cellular membranes of the skin is related to the number of ionized functions.

ACKNOWLEDGEMENT

A.P. is an Aspirant du Fonds National de la Recherche Scientifique.

REFERENCES

1 A. BROUHA, *Action de la Pression sur la Perméabilité de la Peau Isolée de Grenouille*, Thèse, Université de Liège, 1965.
2 E. SCHOFFENIELS, *23 Intern. Kongr. Pharmazeut. Wissensch., Münster*, 1963.
3 E. SCHOFFENIELS, *Cellular Aspects of Membrane Permeability*, Pergamon Press, Oxford, 1967, p. 284.
4 B. ANDERSEN AND H. H. USSING, *Acta Physiol. Scand.*, 39 (1957) 228.
5 H. H. USSING, *Proc. 7th Symp. of Colston Res. Soc.*, Vol. VII, 1954, p. 33.
6 H. H. USSING AND B. ANDERSEN, *Proc. 3rd Intern. Congr. Biochem.*, Brussels, 1955, p. 434.
7 A. DISTECHE, *Rev. Sci. Instr.*, 30, No. 6 (1959) 474.
8 A. DISTECHE AND S. DISTECHE, *J. Electrochem. Soc.*, 114, No. 4 (1967) 330.
9 V. KOEFOED-JOHNSEN AND H. H. USSING, *Acta Physiol. Scand.*, 42 (1958) 298.
10 M. G. FARQUHAR AND G. E. PALADE, *Proc. Natl. Acad. Sci. U.S.*, 51 (1964) 569.
11 A. L. HODGKIN AND B. KATZ, *J. Physiol.*, 108 (1949) 37.