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Physiology of Transport Regulation

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Summary. The regulation of biological transport is discussed on the basis of studies on sodium transport through amphibian skin. The following types of regulation are briefly considered:

1) Hormonal regulation

2) Regulation of Na entry by apparent or real saturation of entry path by outside Na

3) Regulation of Na transport by changes in resistance to the counter ion (mostly chloride)

4) Role of cellular Na concentration which may act both by controlling the passive entry of Na and by influencing the pumping rate.

5) Dependence of Na entry upon cell volume. It is shown that a moderate osmotic swelling of ouabain-poisoned skins leads to excessive swelling of the whole epithelium when NaCl is present on the outside. This indicates that cell swelling leads to opening of the Na channels, but it also indicates coupling between the different layers of the epithelium.

1. Hormones and Sodium Transport

The organizers of this symposium have asked me to talk about "Physiology of transport regulation". Nobody will expect me to cover this huge subject in 20 minutes, so I have chosen to illustrate some of its most important aspects through examples of which I have firsthand knowledge, stemming almost exclusively from work with my favorite test object, the amphibian skin.

In 1945, induced by my teacher, the great physiologist August Krogh, who had just retired, I started a program aiming at developing the use of isotopes in the study of active and passive ion transport.

During the planning phase of this study we discussed the possible mechanisms for the regulation of the rate of active ion transport. Krogh had demonstrated that fresh water animals like frogs would take up sodium chloride from exceedingly dilute solutions if they were in need of salt. Krogh suggested that it was the drop in plasma concentration which directly induced the epithelial cells of the skin to increase their

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active salt transport. Of course I then had to take the opposite view: *viz.*, that low NaCl concentration activated the formation of some hormonal factor which in turn triggered the increase in salt uptake. To prove my point, I went into our storeroom to find a likely candidate for the transport-stimulating factor. I looked first for some adrenal cortical preparation, but we had none. Then I found a sample of antidiuretic hormone. Luckily forgetting the dogma of the high priests of kidney physiology that this hormone has no effect on salt transport, we injected it in axolotls and found that it stimulated the NaCl uptake from dilute solutions quite dramatically (Jørgensen *et al.*, 1946). Later we repeated the experiment with adrenocorticotropic hormone (Koefoed-Johnsen & Ussing, 1949). Even this preparation induced greatly increased active Na uptake in axolotls, thus giving strong suggestive evidence for a role of adrenal cortical hormones in the regulation of sodium transport in the skin of these animals.

A detailed biophysical analysis of the events could not be undertaken on whole animals, however, and we switched to the isolated frog skin for our subsequent work. Quite early in the game we succeeded in demonstrating that antidiuretic hormone and related factors stimulated the active Na transport of the isolated frog skin (Fuhrman & Ussing, 1951) and with the development of the short-circuiting technique (Ussing & Zerahn, 1951) a convenient method for following the kinetics of the transport stimulation was at hand.

Incidentally, the interest in the stimulation of Na transport by ADH was greatly increased when, during his stay in our institute, Alex Leaf developed his toad urinary bladder preparation and found that it transported sodium like the frog skin and that this transport was also stimulated by ADH. I shall only mention in passing that the isolated toad urinary bladder has rendered enormous services in the studies of the action mechanism of aldosterone on Na transport (*see* reviews by Crabbé, 1964; Leaf, 1965; Edelman & Fimognari, 1968).

In the course of this symposium we shall hear much more about the mechanisms underlying both the effects of neurohypophyseal hormone and the aldosterone effect. Therefore, I shall say nothing more about these things but go on to other factors regulating transport.

2. Apparent Saturation Kinetics Regulating Sodium Entry

It is well known that many biological transport systems exhibit some kind of saturation kinetics. For example, the rate of transport of K

into a red cell depends on the outside K concentration in a manner which can be described satisfactorily by Michaelis-Menten kinetics.

Even the uptake of Na from the outside by a frog skin follows such a kinetic pattern (*compare* Ussing, 1949). The phenomenon was first described in detail by Kirschner (1955), and later many others have studied aspects of the phenomenon. In contrast to the K transport into red cells, however, the Na uptake by frog skin must involve passage through at least two membranes (*compare* Koefoed-Johnsen & Ussing, 1958) and a simple interpretation of the K_m and V_{max} values may not be meaningful.

In the Koefoed-Johnsen-Ussing two-membrane theory the entry step for Na is assumed to be a passive but selective diffusion process. This assumption has been under vivid debate ever since it was advanced, but the consensus today probably is that the assumption is phenomenologically correct, although there are differences in opinion with respect to the molecular nature of the entry process. Some workers assume (*see,* for instance, Biber *et al.,* 1966) that the entry of Na takes place by way of a passive but saturable carrier system, whereas others find evidence for a Na-selective electrodiffusion channel similar to the transient Na channel of excitable cells (*see* Fuchs *et al.,* 1977). In the case of the latter hypothesis, the apparent saturation of the Na entry with increasing outside concentration is brought about by a Na-sensitive inhibitor site at the outside end of the Na channel. Both hypotheses then assume an automatic regulation of Na entry.

3. Chloride Permeability Regulating Sodium Transport

This apparent or real saturation of the Na entry is perhaps not the most important factor regulating sodium uptake. Only during short circuiting can Na transport be studied separately. Otherwise, at least with reasonably high outside NaCl concentrations, Na and Cl are transported inward in almost equal amounts. This again means that the rate at which Cl can follow Na (at least in skins of *R. temporaria*) may determine the net transport rate of sodium. This was clearly demonstrated by Koefoed-Johnsen, Levi and Ussing (1952), who found an inverse relationship between the Cl permeability and the electrical potential developed under equal conditions. This is exactly what one would expect from a Na transport battery, shunted by a variable but passive Cl pathway (*compare also* Ussing & Zerahn, 1951).

The question may now be asked: Do the animals make any use of the device of changing the Cl permeability in a systematic way. The answer is that they seem to do so, but before we consider this point any further it is necessary to discuss the possible transport pathways for Cl and other passive ions.

4. Chloride Pathways

Based on an extensive study of the frog skin potential under different functional states, it was proposed (Ussing & Windhager, 1964) that there are two passive transport pathways through the frog skin, *viz.*, one through the epithelial cells and one via the "tight seals". Variations in the properties of these two pathways might account for the fact that the skin potential varies so dramatically even though the charging device is always the Na pump.

This concept has proven quite useful both in relation to amphibian skin and other epithelia. Often it is assumed that the extracellular pathway is the all important one, both for passive sodium efflux and especially for chloride fluxes (Mandel & Curran, 1972).

A few years ago we found, however, (Koefoed-Johnsen, Lyon & Ussing, 1973; Koefoed-Johnsen & Ussing, 1974) that skins from frogs adapted to room temperature exhibited a pronounced cellular pathway for Cl, whereas this path was closed in the cold-adapted animals. In all likelihood, then, the mechanism plays a biological role also in the intact animals, but experiments on whole animals are needed to substantiate this assumption.

Summing up, we can now say that the NaCl uptake by the animal can be regulated by the Cl permeabilities of the two shunts; active Cl transport may aid the passive transport under certain circumstances (Zadunaisky *et al.*, 1963; Erlij, 1971; Kristensen, 1972), Na transport is automatically regulated by the apparent or real saturation of the Na entry path. Furthermore, the number, and possibly the properties, of the sodium entry units seem to be under hormonal control, depending on both neurohypophyseal hormones and aldosterone.

Very likely, the cellular Na concentration is a factor of importance. In the first place the Na pump seems to show saturation kinetics (*see* Fuchs *et al.*, 1977). Furthermore, it has been suggested that an increase in cellular Na inhibits the entry of Na over and above the effect expected

from the reduction in driving force acting on outside Na (Erlij & Smith, 1973).

But there seems to be more factors regulating Na entry. Thus, it was shown several years ago (Ussing, 1965) that all procedures which tend to increase the volume of the epithelium cells stimulate the short-circuit current and thus the rate of Na entry (assuming for the moment that this process is the rate limiting one).

5. Dependence of Na Transport on Cell Volume

For the time being, the nature of such a permeability change, depending on the cell volume, can only be guessed at, but it has an interesting corollary in the potassium permeability of the inward facing membranes of the epithelium cells. Together with MacRobbie (MacRobbie & Ussing, 1961), I studied the volume regulation of the frog epithelium cells in response to changes in osmolarity for the bathing solutions. We found that when the Ringer's solution bathing the inside of the skin was diluted to half, the epithelium first swelled and then shrunk partly back. When the normal Ringer's solution was again introduced, the reverse process was observed: First shrinkage and then some secondary swelling, bringing the volume back to the volume it had before the application of half Ringer's. A detailed study of the phenomenon showed that during swelling the cells lose some KCl which is taken up again when the cells are brought back to the Ringer's medium. The implication is that in their normal state the cells contain K over and above the concentration expected from electrochemical equilibrium, but on swelling their K permeability increases and the ion leaks out until electrochemical equilibrium is attained. Brought back to normal volume the K permeability of the epithelium again drops and the Na/K pump or a separate K pump of the cells then restores the original situation.

A similar regulation of K permeability and cell volume has later been described for several other cell types (Hendil & Hoffmann, 1974; Roti Roti & Rothstein, 1973). The mechanism underlying the changes in K permeability possibly depends on release of Ca during cell swelling. I shall not go deeper into that problem, but only point out that, at first sight, this dependency of the K permeability on cell volume might offer a simple explanation for the above mentioned increase in short-circuit current which frog skins show during swelling of the epithelial cells. Assuming the correctness of the two membrane theory for the

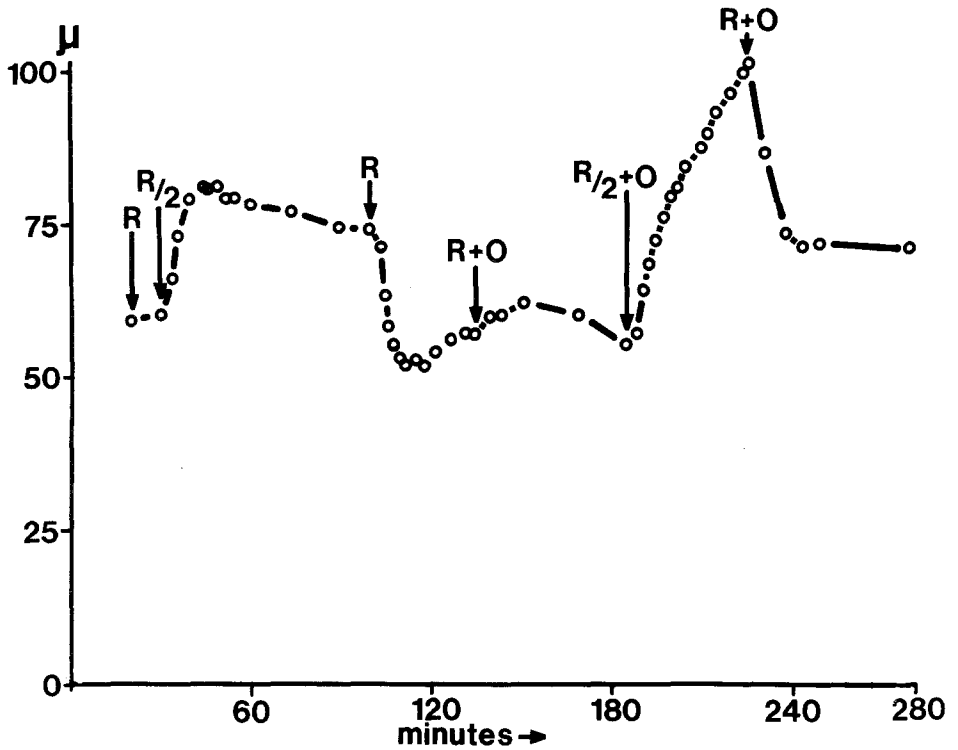

Fig. 1. Volume changes of epithelium of skin of *Rana temporaria* in response to changes in inside osmolarity and to ouabain poisoning. Outside medium: NaCl Ringer's throughout. Inside medium: initially also NaCl-Ringer's (first arrow, marked *R*). At second arrow (*R/2*), inside medium is diluted to half Ringer's. At third arrow (*R*), NaCl-Ringer's is again applied. At arrow marked *R+O* ouabain is added to the inside solution to give a 10^{-5} M solution. At arrow marked *R/2+0*, half Ringer's, made 10^{-5} M with respect to ouabain, is introduced and finally at last arrow Ringer's with ouabain is applied. *Abscissa:* time in min. *Ordinate:* thickness of epithelium in μm

potential development of the frog skin, an increase in K permeability of the inward facing membrane would give a reduction in the overall resistance to the short-circuit current. But, furthermore, if K is normally present over and above its equilibrium potential, a reduction of the K resistance would raise the K potential-step to that of a K-electrode, thus increasing the effective voltage of the battery.

This cannot be the whole truth, however. In the first place, skins in sulphate Ringer's, where cellular K must be close to its equilibrium potential, also show the stimulation of Na transport when the cells are swollen. But, furthermore, we have just been able to show that skins, poisoned with ouabain, can be forced by a modest osmotic swelling

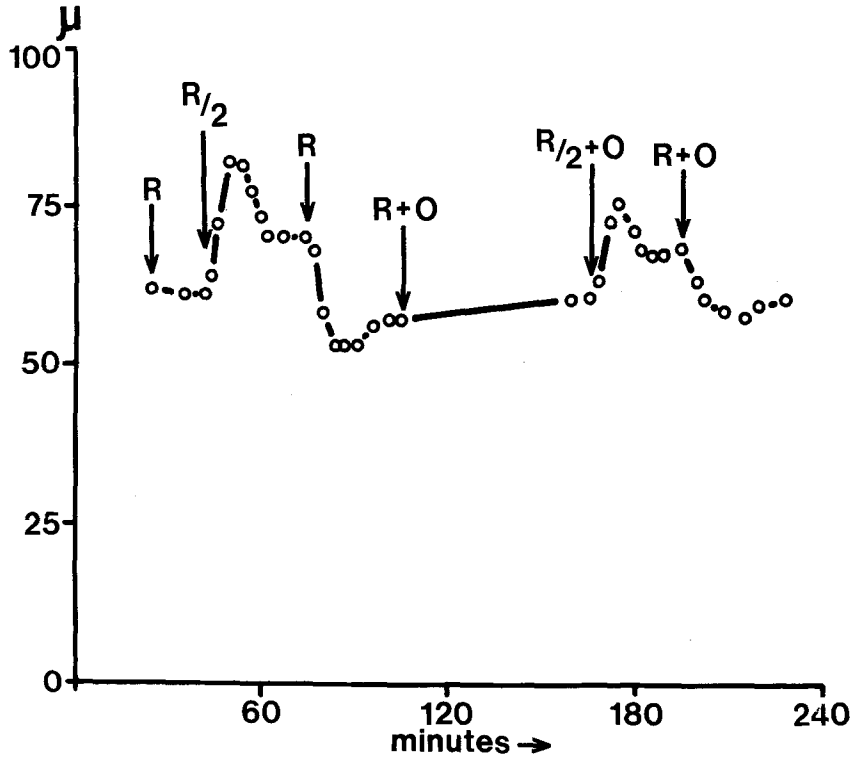

Fig. 2. Volume changes of epithelium of *Rana temporaria* in response to changes in inside osmolarity and to ouabain poisoning. Outside medium: KCl Ringer's throughout. Composition and sequence of changes in inside medium like in Fig. 1

to swell excessively when NaCl is present in the outside medium, whereas the swelling remains modest if the outside medium is KCl. Thus it seems that the excessive epithelial swelling is due to the fact that the outward facing, sodium-selective membrane, becomes more permeable when a modest cell swelling is produced osmotically.

The fact that the above mentioned excessive swelling is measurable with the MacRobbie-Ussing technique poses another important question, however. The swelling is so large that it must involve more than one cell layer. This indicates then that there is a direct Na path (i.e., coupling; *compare* Loewenstein, 1966) from one cell layer to the next like it was originally proposed by Ussing & Windhager (1964) and Farquhar & Palade (1964).

Such cell connections so far have been very elusive. Thus, in experiments where the epithelium cells were rapidly fixed (Voûte & Ussing, 1968) or rapidly frozen (Voûte & Hänni, 1973) in different functional

states, only the outermost living cell layer showed a clear correlation between volume and rate of Na transport. Also only this cell layer showed expansion of the endoplasmic reticulum in a fashion which was correlated to the rate of Na transport (Voûte *et al.*, 1975). On the other hand, the fact that outside lithium accumulates in all cell layers (Hansen & Zerahn, 1964; Morel & Leblanc, 1973) speaks in favor of coupling.

Perhaps one might conclude that the cells are coupled but that one cell layer usually is able to handle all the Na offered to it. Only in case of the poorly transported Li or in the case of Na in a system where the pump is stopped by ouabain would one then notice the coupling.

The role of the endoplasmic reticulum in the handling of Na is still undecided. Does the correlation between endoplasmic volume and the rate of Na transport indicate that a major part of the Na entering the cells is handled by the reticulum, or is the latter only a cul-de-sac acting as a transient sink for Na when the cell is overloaded. We still do not know. Neither do we know with certainty whether the rate of Na entry through the outer membrane is the rate-limiting step under normal function of the system or whether the variations in the number of pump sites and or their efficiency are factors of equal importance.

6. Other Regulating Factors

The capacity of the cellular metabolism might also be a limiting factor, but except for situations where the metabolism is excessively inhibited by poisoning (*compare* Kristensen & Schousboe, 1969), the metabolic apparatus mostly seems adequate. There is ample evidence, however, that the Na transport is extremely sensitive to the CO_2 tension, probably acting via the intracellular pH (Ussing, 1949; Funder *et al.*, 1967).

In conclusion, I should like to say that only lack of time keeps me from mentioning other factors which might play a role in the regulation of transport, even if we limit our considerations to the frog skin. One should, however, bear in mind that most of the factors discussed above have been described only for isolated systems. Their actual role in normal physiological regulations will require extensive whole animal experiments.

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