Biochimica et Biophysica Acta, 352 (1974) 323—326

BBA Report

BBA 71202

CHEMICAL STIMULATION OF Na^+ CURRENT THROUGH THE OUTER SURFACE OF FROG SKIN EPITHELIUM

W. ZEISKE and B. LINDEMANN

Abteilung für Membranforschung an Epithelien, Fachbereich 3, Universität des Saarlandes, 665 Hamburg (G.F.R.)

(Received April 9th, 1974)

Summary

A number of organic molecules were found to increase the Na^+ permeability of the Na^+-selective membrane in frog skin epithelium quickly and reversibly when added to the outer bathing solution. The most effective was benzoylimidazole-guanidine. This substance stimulates the Na^+ current by preventing the decrease of Na^+ permeability which is normally caused by Na^+ at the outer surface of the Na^+-selective membrane.

The aminopyrazine-guanidine compound Amiloride is known to be a very effective competitive inhibitor of the passive Na^+ flux through the Na^+-selective (outer) membrane into frog skin epithelium [1—3]. In an attempt to evaluate the relative importance of parts of the Amiloride molecule for the inhibitory action, we have tested a number of Amiloride analogs and other related compounds. The main results of this investigation will be described elsewhere, but we should like to report now that some of the guanidine compounds tested had stimulatory rather than inhibitory effects on Na^+ current.

Technique: At the outer surface of the abdominal skins of *Rana ridibunda* or *Rana esculenta*, K^+—Na^+ substitutions were made with our fast-flow chamber [4]. This instrument allows a change between two outer solutions within 20 ms under short-circuit conditions [5, 6]. The solutions will be called the pre-equilibration solution (P solution) and the test solution (T solution). Their composition was identical except for the Na^+/K^+ ratio and/or the presence of drugs in the T solution. Each flow cycle consisted of one P—T—P sequence, exposing the Na^+-selective membrane of frog skin briefly and under optimal

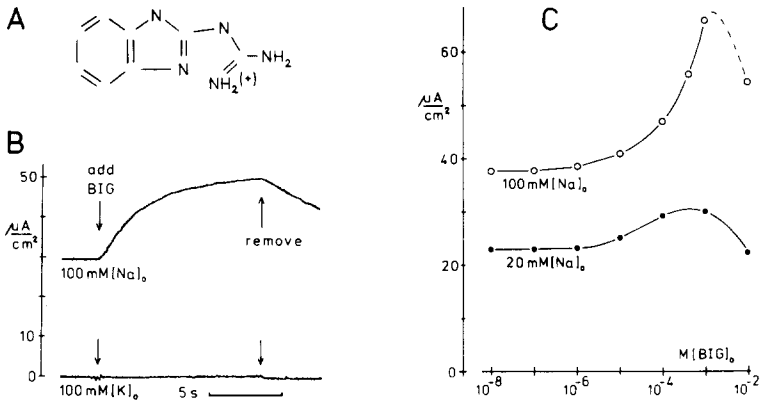

Fig. 1. A, structure of benzoylimidazole-2-guanidine. B, response of Na^+ current (short-circuit current) to addition and removal of benzoylimidazole-guanidine (BIG) (4 mM). Upper trace: 100 mM sodium gluconate in outer solution, with 1 mM calcium gluconate and 5 mM Tris—sulfate, pH 7.2. Lower trace: all $[Na^+]_0$ replaced by K^+. C, benzoylimidazole-guanidine dose response curves. Readings were taken 10 s after stepping a stepwise increase of $[Na^+]_0$ from 0 to 20 or 100 mM (substituting K^+, which was 100 mM in both P solutions).

stirring to Na^+ or drugs contained in the T solution. All solutions contained 1 mM calcium gluconate, 5 mM Tris—sulfate, pH 7.2, and 100 mM potassium gluconate or sodium gluconate or a mixture of both, the sum always being 100 mM. The inside bathing solution was sodium gluconate Ringer.

A number of guanidine compounds were found to stimulate rather than inhibit the Na^+ current. Most effective was benzoylimidazole-2-guanidine (Fig. 1A) and benzoylthiazole-2-guanidine. Introduction of an NO_2 group at carbon atom 5 of the benzene ring rendered the molecule ineffective. Substitution of the guanidino group by OH (2-hydroxybenzimidazole) decreased the effect considerably. Mere omission of the guanidino group and simultaneous introduction of one or two methyl groups on the benzene ring decreased the stimulatory effect very little. Apparently, the stimulatory effect requires a molecule with a hydrophobic end (benzene ring) and a positively charged end (guanidine or imidazole). The first may be important for fixing the molecule to the membrane, the latter for water solubility and/or the stimulatory effect itself.

Experiments were performed to test the specificity and to investigate the mechanism of the stimulatory response caused by benzoylimidazole-guanidine. Fig. 1B shows that the current increase is fast, reversible and specific for Na^+. A 60% increase in Na^+ current was obtained with a halftime of 2.6 s under fast-flow conditions. The dose—response curves of Fig. 1C show that, besides the stimulatory effect at low concentrations of benzoylimidazole-guanidine, an inhibitory effect develops at high concentrations. For this reason it is difficult to determine the dose of half-maximal stimulation which, however, appears to

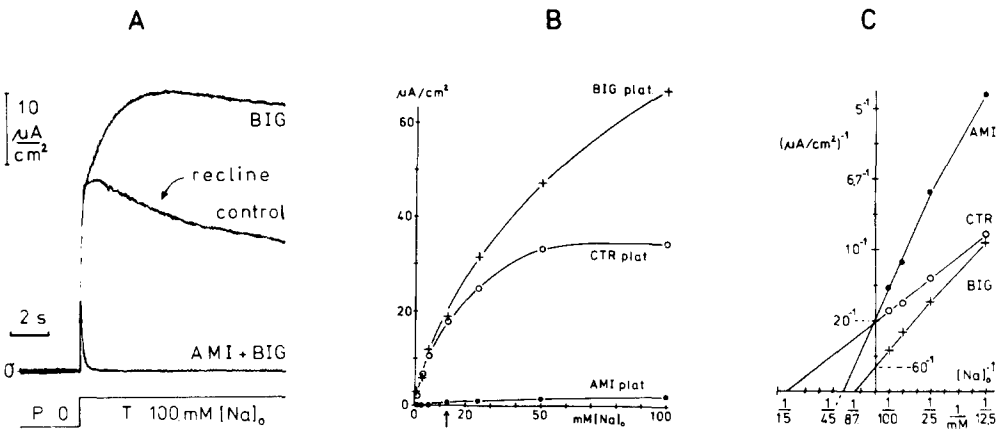

Fig. 2. A, response of short-circuit current (Na^+ current) to a stepwise increase of $[Na^+]_0$ from 0 to 100 mM (replacing K^+). The control curve shows a peak followed by a recline to a plateau. After adding 1.5 mM benzoylimidazole-guanidine (BIG) to both P and T solutions the recline becomes much smaller (upper curve). 30 μM Amiloride (AMI) introduced together with the increasing Na^+ step overrules the benzoylimidazole-guanidine response (lower curve). B, plateau-currents as a function of $[Na^+]_0$ in the test solution. Plateau readings were taken 15 s after increasing $[Na]_0$ from zero to the values plotted on the abscissa (substituting K^+). Benzoylimidazole-guanidine, 3 mM; AMI, 30 μM; CTR (control): no drugs. C, double-reciprocal plot of Na^+ current versus $[Na]_0$, showing the competitive inhibitory effect of Amiloride (0.3 μM), and the stimulating effect of benzoylimidazole-guanidine (3 mM) with a three-fold increase of both K_m and the maximal Na^+ current. Readings were taken as in B (plateaus).

decrease when $[Na^+]_0$ (the Na^+ concentration of the outside solution) is lowered.

The control curve of Fig. 2A shows the normal response of the Na^+-selective membrane to a sudden replacement of $[K^+]_0$ (in the P solution) by Na^+ (in the T solution). The initially large inward Na^+ current reclines to a smaller plateau current with a halftime of several seconds. This recline is paralleled by a decrease of Na^+ conductance and was recognized as a decrease of Na^+ permeability in response to the increase of $[Na^+]_0$ [6]. It is independent of the Na^+ current itself and therefore due to an effect of Na^+ at the outer surface of the membrane [6]. When benzoylimidazole-guanidine is present in both outer solutions (P and T) the increasing Na^+ step causes a larger increase of current with a much smaller secondary recline (upper curve). Apparently, at least a large part of the stimulatory effect of benzoylimidazole-guanidine is due to an inhibition of the recline mechanism. This is in contrast to the current increase caused by the vasopressin—cyclic AMP mechanism, which does not show a diminution of the recline [7]. The lower curve demonstrates that the benzoylimidazole-guanidine stimulation can be overcome with Amiloride (added to the Na^+-containing T solution only). Clearly, therefore, the additional current observed in the presence of benzoylimidazole-guanidine is a Na^+-current, passing the same Amiloride-blockable channels as the control Na^+ current. Benzoylimidazole-guanidine modifies the saturation properties of these channels as shown below.

In Fig. 2B plateau currents obtained 15 s after an increasing step of $[Na^+]_o$ are plotted versus $[Na^+]_o$. The control curve was obtained in the absence of benzoylimidazole and Amiloride, and shows the well-known saturation which was also described for the unidirectional Na^+ inward flux [8—11]. It is drastically changed in the presence of benzoylimidazole-guanidine: saturation occurs at about 3 times larger maximal currents, and with an apparent K_m value also about 3 times larger than that of the control (Fig. 2C). The benzoylimidazole-guanidine curve of Fig. 2B is close to the curve obtained by plotting peak Na^+ currents (preceding the recline, see Fig. 2A) of the control versus $[Na^+]_o$. This means that in the presence of benzoylimidazole-guanidine the recline mechanism, which decreases maximal current and K_m in response to an increase of $[Na^+]_o$, is partially or completely inhibited: the Na^+ channels remain in the state of high permeability — which is normally found only at low $[Na^+]_o$ — even when $[Na^+]_o$ is high.

In contrast to benzoylimidazole-guanidine in stimulatory doses, Amiloride increases K_m without changing the maximal Na^+ current (Fig. 2C). This indicates that Amiloride acts by competitive inhibition, as proposed by others [3].

Probably, the molecule competes with Na^+ for the entrance of the Na^+ channel. Like Amiloride, benzoylimidazole-guanidine contains a guanidino group attached to an organic ring system, and like Amiloride, it shows an inhibitory effect on Na^+ current, although much larger concentrations are needed. We might speculate, therefore, that the inhibitory effect of benzoylimidazole-guanidine is due to competition with Na^+ as in the case of Amiloride, but our data do not yet provide evidence for this.

At lower concentrations benzoylimidazole-guanidine stimulates Na^+ current by increasing the maximal capacity and decreasing the Na^+ affinity of the Na^+ transport mechanism. The mechanism of stimulation seems to involve an inhibition of the secondary decrease of Na^+ conductance (recline), which normally follows an increasing step of Na^+ concentration at the outer surface of the Na^+-selective membrane.

Supported by the D.F.G. through Sonderforschungsbereich 38, project C1. This work is part of a thesis by W. Zeiske.

References

1 Ehrlich, E.N. and Crabbé, J. (1968) Pflügers Arch. 302, 79—96
2 Nagel, W. and Dörge, A. (1970) Pflügers Arch. 317, 84—92
3 Cuthbert, A.W. and Shum, W.K. (1974) Naunyn-Schmiedeberg's Arch. Pharmacol. 281, 261—269
4 Lindemann, B., Gebhardt, U. and Fuchs, W. (1972) TIT J. Life Sci. 2, 15—26
5 Gebhardt, U. and Lindemann, B. (1974) Pflügers Arch. 347, 9—18
6 Lindemann, B. and Gebhardt, U. (1973) Transport Mechanisms in Epithelia, (Ussing, H.M. and Thorn, N.A., eds), pp. 115—130, Academic Press, New York
7 Brennecke, R. and Lindemann, B. (1974) Rev. Sci. Instr. 45, in the press
8 Ussing, H.M. (1948) Acta Physiol. Scand. 17, 1—37
9 Kirschner, L.B. (1955) J. Cell. Comp. Physiol. 45, 61—87
10 Cereijido, M., Herrera, F.C., Flanigan, W.J. and Curran, P.E. (1964) J. Gen. Physiol. 47, 879—893
11 Biber, T.U.L. and Curran, P.F. (1970) J. Gen. Physiol. 56, 83—99