Inhibitory and Stimulatory Effects of Amiloride Analogues on Sodium Transport in Frog Skin

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Received 24 July 1978; revised 5 December 1978

Summary. Effects of amiloride analogues on Na transport were studied in isolated skins of the frog Rana ridibunda. The pattern of structure-activity relationship of these compounds showed that both the –NH$_2$ group at position 5 and Cl at position 6 of the pyrazine ring of the amiloride molecule were important for their biological activity. The paramount role of the groups at position 5 was further demonstrated by the striking properties of an analogue resulting from dimethylation of that –NH$_2$ group. A stimulation of Na transport, opposite to the effect of amiloride itself, was observed in this instance. The increase in Na transport could already be seen at 10$^{-6}$ M and was equivalent to the measured increase in Na influx, reversible, dose-dependent, and additive to the natriiferic action of oxytocin. Such characteristics resemble those reported with “external” agents like propranolol and La$^{3+}$. Furthermore, mutual inhibition was observed between the stimulatory effects of this analogue and those of propranolol or La$^{3+}$. These results suggest that the analogue may be considered as another “external” agent acting at sites of the external membrane distinct from those activated by cAMP but similar to the Ca sites described by Herrera and Curran (Herrera, F.C., Curran, P.F. 1963. J. Gen. Physiol. 46:999).

In recent years, intensive work from several laboratories has been focused on the permeation processes occurring at the apical border of amphibian epithelia. According to current concepts, this portion of the plasma membrane is the main barrier to transepithelial Na and water movement and one of the loci where hormones exert their natriiferic and hydrosomatic effects.

Amiloride, first presented as a potassium-sparing diuretic (Baer et al., 1967), became a drug of choice for the study of Na transport in a variety of epithelia (Cuthbert, 1974; de Sousa, 1975). In view of its highly specific interaction with membrane Na-entry sites, much effort

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has been devoted to elucidate the mode of action of amiloride and to gain insight into the molecular structure of amiloride-sensitive pathways involved in the translocation of Na across biological membranes. In this endeavor, a variety of sophisticated approaches has been employed, including fluctuation analysis (Lindemann & Van Driessche, 1977), microelectrodes (Nagel, 1976; Helman & Fisher, 1977; Sudou & Hoshi, 1977) and C\(^{14}\)-amiloride binding studies (Cuthbert, 1973; Cuthbert & Shum, 1974).

The availability of structural analogues of amiloride provided an opportunity to join in such effort with the help of standard techniques utilized for the study of epithelial transport. In this report, effects of some amiloride analogues on Na transport in frog skin are presented. Particular emphasis was given to an analogue obtained by dimethylation of the amino group at position 5 of the pyrazine ring of amiloride. In contrast with the phenomenological inhibitory effect of the parent species, this compound exerted a stimulatory effect on Na transport. To investigate further the nature of the observed natriferic action, we also analyzed the interaction between the effects of this compound and those of three reference substances—oxytocin, propranolol, and La\(^{3+}\)—the natriferic action of which has been documented (de Sousa, 1975; Marguerat, 1975). A preliminary report of this work was presented elsewhere in abstract form (Li & de Sousa, 1977b).

Materials and Methods

The abdominal skin of *Rana ridibunda* was mounted as a flat sheet between Lucite chambers. Two types of chamber were used: a double and a single chamber.

The double chamber, with rectangular windows of 2.0 cm\(^2\) and a volume of 5 ml, had been described previously (de Sousa & Grosso, 1973). With this device, two equal areas of the same skin were exposed to Ringer solutions contained in adjacent compartments. One area of the skin was used as control of the other, thus facilitating the execution of “cross experiment” protocols. At the right and the left extremities of the chamber, rectangular plates of Ag-AgCl were positioned for passing electrical current. Through the center of these electrodes, 3% agar in 3 M KCl bridges were introduced into the solutions bathing the skin. The other end of the bridges was connected, via 3 M KCl solution, to calomel electrodes (Metrohm), for monitoring the electrical potential difference across the skin.

The single chamber, developed mainly for studying tracer flows and for eliminating Ag\(^+\) contamination from the electrodes, was a modification of the set up previously used in the study of ion transport through artificial membranes (Li, de Sousa & Essig, 1974). It consisted of four cylindrical Lucite compartments having approximately a cross sectional area of 6 cm\(^2\) and a volume of 16 ml. The skin was first mounted on rubber gaskets 0.6 cm in thickness and with a circular hole of 3.14 cm\(^2\). This assembly was then clamped.