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Letters to the Editor

Protocol-Dependence of Equivalent Circuit Parameters

In a recent article, Wolff and Essig (1980) analyzed the nonlinear behavior of current-voltage relationships in the toad urinary bladder. The protocol dependence of this nonlinearity may be more apparent than real. I noticed that three factors in the study appeared to be time-dependent. First, the degree of nonlinearity correlated with the length of time that each potential was applied (30 sec, 5 min or 15 min). Second, in the longer protocols the curve was concave when the potentials were applied in the $100 \rightarrow 0$ mV order but was convex for the $0 \rightarrow 100$ mV order. Third, all reduced data in the paper were calculated using the initial short-circuit current ($i^a_{o,i}$). In the shortest protocol the initial and final short-circuit current ($i^a_{o,f}$) were equal, but in the longer protocols the final short-circuit current is significantly less than the initial value. It appeared that a reevaluation of the data which took into account the time-dependent fall in short-circuit current might be fruitful.

The data from Wolff and Essig's Table 1 was recalculated by dividing their i^a at any potential by the $i^a_{o,t}$ assumed for the appropriate time. For this purpose, the assumption was made that the short-circuit current declined linearly with time. This assumption is at least partially supported by the observation that the fall in short-circuit current in the 5-min protocol was one-third of that in the 15-min protocol (*see* their Table 1). The attached figure compares the data of the top portion of their Fig. 4 and the same data renormalized as described. The current-voltage relationship of the data normalized by either procedure (if linear) is constrained to the line shown in the figure. The renormalized current is nearly linear while the current calculated by Wolff and Essig appears to saturate at large negative potentials. The positive deviation of the renormalized current at -100 mV is to be expected since Wolff and Essig reported an increase in passive conductance at this potential. For the longer protocols (5 and 15 min/potential), renormalization led to as close or closer fit of the data to the expected line at positive or small negative potentials, and consistent, more than linear increases in current at -100 mV. Since the current at $+100$ mV (another factor used to calculate i^a) is also likely to be time-dependent, further improvement in the fit of the data might be possible.

In a simple equivalent circuit model, the current equals the product of a conductance and an electromotive force. The observed decline of the short-circuit current during the experiment would therefore indicate that the conductance and/or the electromotive force were reduced as a function of time. (I suggest time-dependence rather than voltage-dependence because the final short-circuit current was similar whether the tissue was previously clamped at a potential of -25 or -100 mV.)

The fact that correction for time-dependent changes in only the i^a_o led to significant linearization of the data suggests that at least a portion of the protocol dependence of equivalent circuit parameters reported by Wolff and Essig was due to the method of data analysis rather than voltage-dependent changes in transport parameters. Any interpretation of voltage-dependence of transport parameters should probably be limited to circumstances where other factors (such as time) are not having significant effects on transport.

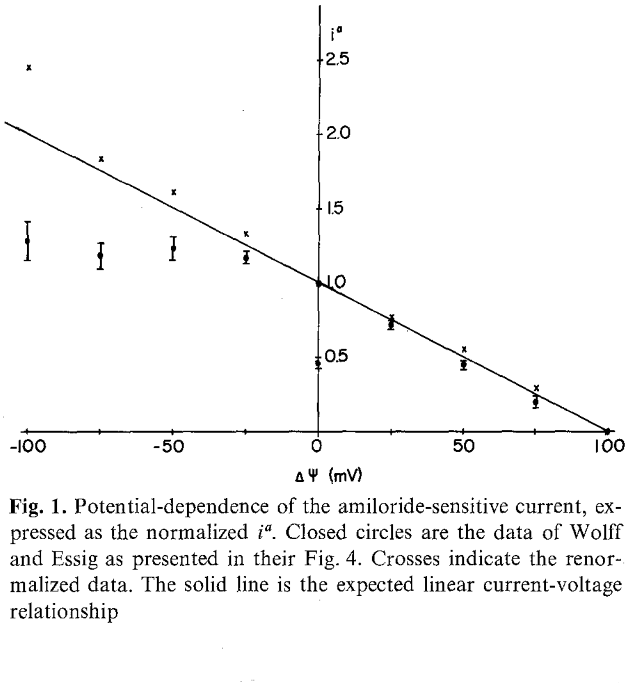

Fig. 1. Potential-dependence of the amiloride-sensitive current, expressed as the normalized i^a. Closed circles are the data of Wolff and Essig as presented in their Fig. 4. Crosses indicate the renormalized data. The solid line is the expected linear current-voltage relationship

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Reference

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Reply to: Protocol-Dependence of Equivalent Circuit Parameters

Dr. Husted is correct in pointing out that decline of function attributable simply to the passage of time could in principle result in current-voltage relationships qualitatively similar to those observed by us [4]. Our reasons for feeling that this is not the complete explanation are that (i) near-constancy of transport was usually observed both during initial control periods at short-circuit and during subsequent periods at positive settings of $\Delta\psi$; (ii) marked changes of short-circuit current are commonly observed following perturbations of $\Delta\psi$ for extended periods (*see*, e.g., the early demonstration of "memory effects" in frog skin [2, Fig. 6]); and (iii) morphological effects of serosal negativity have been described previously: Voûte and Ussing found that doubling inward current with respect to the short-circuit level for 20–30 min caused marked swelling or islets of cell necrosis in the outer layer of the stratum granulosum of frog skin [3], and Bobrycki et al. found that setting $\Delta\psi$ at -50 mV caused pronounced swelling of granular cells in toad urinary bladders in which I fell during 30 min of voltage clamping [1]. Admittedly, since we did not carry out control studies on paired tissues clamped at short-circuit, we cannot state precisely to what extent our findings reflected effects of voltage-perturbations *per se* as against effects of time.

In any case, effects on the conductance κ^a and the apparent electromotive force "E_{Na}" were uninfluenced by the passage of time, since they were much the same irrespective of the order of perturbation of $\Delta\psi$.

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