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The effect of membrane stabilizers and ouabain on the transport of Na^+ and K^+ in rat soleus muscle

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SUMMARY

In the isolated rat soleus muscle, tetracaine was found to produce a marked inhibition of K^+ efflux, K^+ influx and Na^+ influx. The ouabain-sensitive components of K^+ uptake and Na^+ efflux were not inhibited, indicating that the local anesthetic has a rather selective effect on passive Na^+–K^+ fluxes.

It could be demonstrated that this relative rise in pump/leak ratio leads to a more than 2-fold increase in the intracellular K^+/Na^+ concentration ratio.

Other membrane stabilizers (lidocaine, thiomebumal, chlorpromazine and propranolol) were found to induce qualitatively similar changes in Na^+–K^+ exchange.

It is generally assumed that the distribution of Na^+ and K^+ across the plasma membrane is determined by the rates of net transport of these ions against and along their electrochemical gradients. Cardiac glycosides and metabolic inhibitors have been widely used for the identification and determination of the active component of Na^+–K^+ transport, but in mammalian muscle the effects of compounds primarily affecting the passive fluxes of these ions have rarely been characterized in detail[1].

The possibility of obtaining selective inhibition of passive fluxes was suggested by the observation that certain membrane stabilizers produce a prompt and marked suppression of K^+ efflux and Na^+ influx in rat soleus muscle[2]. The present investigation was undertaken with the purpose of obtaining a quantitative analysis of the consequences of membrane stabilization for the fluxes and the distribution of Na^+ and K^+.

Intact soleus muscles were prepared from fed Wistar rats (60–70 g) and incubated in Krebs–Ringer bicarbonate buffer at 30 °C as previously described[3]. ^{42}K and ^{22}Na activity was determined using an Auto-gamma spectrometer. Further experimental details are described in the legends to figures and tables.

TABLE I

EFFECT OF OUABAIN AND MEMBRANE STABILIZERS ON ^{42}K UPTAKE IN RAT SOLEUS MUSCLE

Soleus muscles were washed and preincubated for 15 min in Krebs–Ringer bicarbonate buffer containing 1 mM pyruvate without or with the additions indicated. They were then incubated for 20 min in the same media containing ^{42}K (0.5 μCi/ml), blotted on dry filter paper, weighed and counted. The amount of ^{42}K taken up in the space not available to [^{14}C] sucrose is expressed as μmoles/g wet wt per min ± S.E. The number of observations is given in parentheses.

Additions	*Concn (mM)*	*^{42}K influx (μmoles/g wet wt per min)*	*P*
Control		0.545 ± 0.014 (23)	
Ouabain	0.001	0.462 ± 0.023 (4)	< 0.05
	0.01	0.416 ± 0.006 (4)	< 0.005
	0.1	0.341 ± 0.020 (4)	< 0.001
	1.0	0.312 ± 0.014 (4)	< 0.001
Tetracaine	0.01	0.484 ± 0.018 (4)	> 0.05
	0.1	0.388 ± 0.018 (8)	< 0.001
	0.5	0.302 ± 0.027 (4)	< 0.001
	1.0	0.362 ± 0.013 (4)	< 0.001
Tetracaine + ouabain	0.5 1.0	0.066 ± 0.004 (4)	< 0.001
Tetracaine + ouabain	1.0 1.0	0.064 ± 0.004 (7)	< 0.001
Lidocaine	10	0.375 ± 0.014 (4)	< 0.001
Thiomebumal	0.5	0.301 ± 0.011 (4)	< 0.001
Chlorpromazine	0.1	0.401 ± 0.027 (4)	< 0.05
	0.2	0.378 ± 0.008 (4)	< 0.005

The uptake of ^{42}K was found to proceed with a linear time course for 30 min, and 20 min was selected as an appropriate incubation period for the measurement of initial rates of ^{42}K influx. Ouabain was found to suppress ^{42}K influx in a concentration-dependent fashion, maximum effect being obtained with 10^{-3} M (Table I). Within a certain concentration range, various categories of membrane stabilizers (tetracaine, lidocaine, thiomebumal and chlorpromazine) produced up to 45% decrease in ^{42}K influx. The inhibitory effects of maximal doses of ouabain and tetracaine were strictly additive, indicating that the two compounds act on different transport processes (Fig. 1). This relationship could be demonstrated over an extracellular K^+ concentration range from 0.5 to 30 mM.

Under basal conditions, the value determined for ^{42}K efflux did not differ significantly from that obtained for influx (Table II). This is in agreement with the earlier observation that under these experimental conditions, the K^+ content remains constant throughout an efflux period of up to 90 min duration[3]. The addition of tetracaine,

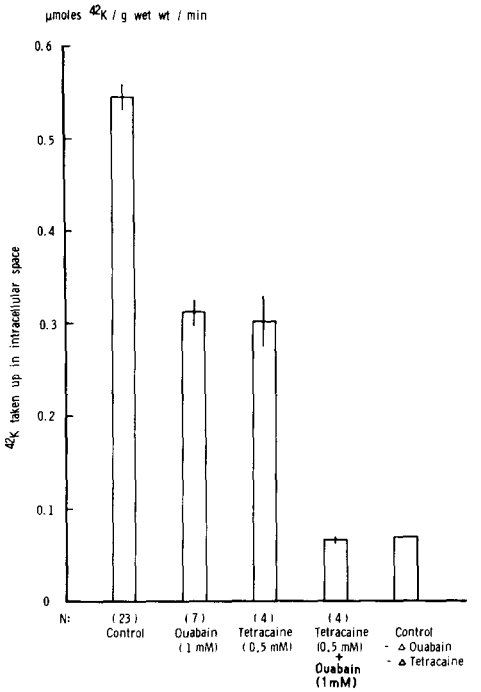

Fig. 1. Effect of ouabain and tetracaine on ^{42}K influx in rat soleus muscle. Experimental conditions as described for Table I. The vertical bars denote 2 x S.E. The last column indicates the difference between the control and the sum of the decreases induced by ouabain and tetracaine, respectively.

TABLE II

EFFECT OF MEMBRANE STABILIZERS ON ^{42}K EFFLUX FROM RAT SOLEUS MUSCLE

Soleus muscles were loaded with ^{42}K, and the washout of isotope followed by transferring the muscles through a series of tubes containing unlabelled buffer without or with the additions indicated[2]. The rate of ^{42}K efflux was determined by multiplying the intracellular K^+ content by the fraction of ^{42}K lost from the tissue per min. The values given have been measured 20–40 min after the addition of the drugs and represent the maximum inhibition obtained.

Additions	*Concn (mM)*	*^{42}K efflux (μmoles/g wet wt per min)*	*P*
Control		0.601 ± 0.027 (3)	
Tetracaine	0.1	0.324 ± 0.021 (3)	< 0.001
	0.5	0.125 ± 0.013 (4)	< 0.001
	1.0	0.101 (2)	
Lidocaine	10.0	0.253 ± 0.033 (3)	< 0.001
Thiomebumal	0.5	0.183 ± 0.015 (3)	< 0.001
	1.0	0.147 (2)	
Chlorpromazine	0.1	0.366 ± 0.030 (3)	< 0.001

lidocaine, thiomebumal or chlorpromazine produced a suppression of ^{42}K efflux which was maximal after 20–40 min[2]. Table II presents the values for ^{42}K efflux determined when the inhibitory effect of the membrane stabilizers was fully developed. None of the compounds or concentrations tested produced more than 83% decrease, indicating that a small component of the K^+ efflux may take place *via* a separate route.

In the presence of tetracaine at a concentration (0.5 mM) giving maximal inhibition of both influx and efflux, the muscles can be calculated to gain K^+ at a rate of 0.177 μmole/g wet wt per min (Tables I and II). In muscles which had been exposed to tetracaine (0.5 mM) for 60 min, flame photometric determination of the K^+ content showed a net increase of 6.8 ± 1.7 μmoles/g wet wt (above the level of the untreated controls; $P < 0.005$). This is lower than the change predicted by the flux balance (10.6 ± 1.8 μmoles/g wet wt), possibly because the action of tetracaine has a certain time-lag (see ref. 2).

The observation that the ouabain-sensitive component of K^+ influx was the same

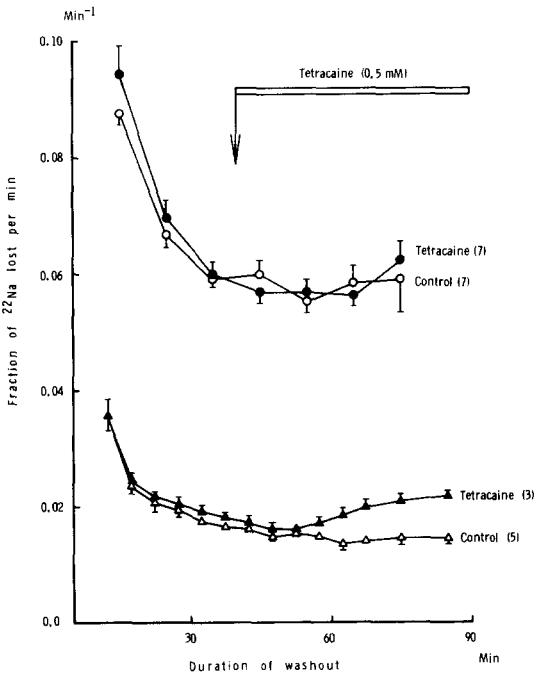

Fig. 2. Effect of ouabain and tetracaine on ^{22}Na efflux from rat soleus muscle. Soleus muscles were incubated for 60 min in Krebs–Ringer bicarbonate buffer containing ^{22}Na (6.7 μCi/ml), 1 mM pyruvate, without or with ouabain (1 mM). The washout of isotope was then followed by transferring the muscles through a series of tubes containing unlabelled buffer without or with the additions indicated[2]. Each curve represents the mean of 3–7 observations with bars denoting S.E. Tetracaine (0.5 mM) was present in the efflux medium during the interval indicated by the horizontal bar. ○——○, control; ●——●, tetracaine (0.5 mM) added at 40 min; △——△, ouabain (1 mM) present during loading and washout; ▲——▲, ouabain (1 mM) present during loading and washout, tetracaine (0.5 mM) added at 40 min.

in the absence and the presence of tetracaine (0.233 and 0.235 μmole/g wet wt per min, respectively; Fig. 1), argues that the active uptake of K^+ is not modified by the local anesthetic. Therefore, the rise in K^+ content induced by tetracaine may be due to an effect exerted solely on the passive component of K^+ transport.

In view of the coupling between the active components of Na^+–K^+ transport, it was of interest to determine whether membrane stabilizers had any effect on the active component of Na^+ transport.

The efflux of Na^+ was determined by following the washout of ^{22}Na from pre-loaded muscles, and Fig. 2 shows the time-course of this process. The addition of tetracaine (0.5 mM) to the efflux medium caused no decrease in the rate coefficient of ^{22}Na efflux, neither in the absence nor in the presence of ouabain at a concentration (1 mM) found to produce maximal (75%) inhibition of ^{22}Na efflux. On the contrary, in the presence of ouabain the local anesthetic produced a small progressive rise, which was statistically significant ($P < 0.05$) after 25 min.

The absence of any immediate effect of tetracaine on the ouabain-sensitive component of K^+ influx and Na^+ efflux indicates that this compound does not interfere directly with the operation of the Na^+–K^+ pump in rat soleus muscle. A complete analysis of the effect of membrane stabilizers on Na^+–K^+ distribution required information about the initial rate of Na^+ influx. Since the intracellular Na^+ pool is relatively small and shows a rapid turnover, this parameter could not be determined accurately under basal conditions. In order to reduce the error caused by the return of labelled Na^+ into the incubation medium, ouabain (1 mM) was added. Furthermore, following the incubation in ^{22}Na-containing medium, the label retained in the extracellular pool was washed out by repeated rinsing in ice-cold unlabelled buffer containing ouabain (1 mM). Separate experiments had shown that at 0 °C and in the presence of ouabain, the rate coefficient of ^{22}Na release was only 1.7% of the control level, and it is reasonable to assume that only a small fraction of the ^{22}Na which had gained access to the intracellular pool during the incubation at 30 °C, could be lost during the wash at 0 °C. Using this procedure, the uptake of ^{22}Na was found to proceed with a linear time-course for 20 min. With an incubation period of 10 min, reproducible values for the ^{22}Na uptake could be obtained (Table III), and it seemed justified to adopt this method for the assessment of relative changes in the initial rate of Na^+ influx.

A variety of membrane stabilizers were found to suppress the ^{22}Na uptake, and the effective concentrations corresponded to those which had been shown to inhibit the influx and efflux of ^{42}K.

These observations would predict that membrane stabilizers produce a decrease in the intracellular Na^+ concentration. This parameter was determined by measuring the amount of ^{22}Na taken up in the space not available to [^{14}C] sucrose. After 90 min of incubation, the intracellular accumulation of ^{22}Na was found to remain constant at a value of 8.4 ± 0.4 μmoles/g wet wt. In muscles, which had been incubated in the presence of tetracaine (0.5 mM) for the same length of time, an intracellular concentration of 4.1 ± 0.7 μmoles/g wet wt was found ($N = 5$, $P < 0.001$). The intracellular pool of Na^+ was also

TABLE III

EFFECT OF MEMBRANE STABILIZERS ON ^{22}Na UPTAKE IN RAT SOLEUS MUSCLE

Soleus muscles were washed and preincubated for 15 min in Krebs–Ringer bicarbonate buffer containing 1 mM pyruvate and 1 mM ouabain without or with the additions indicated. A trace quantity of ^{22}Na was then added, and following a further incubation of 10 min duration, the muscles were quickly blotted on ice-cold wet filter paper, washed 3 x 10 min in ice-cold buffer containing 1 mM ouabain, blotted on dry filter paper, weighed and counted. On the basis of the specific activity of the ^{22}Na present during the 10 min incubation, the amount of ^{22}Na retained in the muscles was expressed as μmoles/g wet wt per min ± S.E. The number of observations is given in parentheses.

Additions	*Concn (mM)*	*^{22}Na uptake (μmoles/g wet wt per min)*	*P*
Control		0.762 ± 0.036 (8)	
Tetracaine	0.01	0.574 ± 0.017 (5)	< 0.01
	0.1	0.469 ± 0.027 (6)	< 0.01
	0.5	0.452 ± 0.036 (3)	< 0.01
	1.0	0.446 ± 0.022 (3)	< 0.01
Control		0.725 ± 0.043 (4)	
Lidocaine	10	0.474 ± 0.025 (4)	< 0.005
Thiomebumal	0.5	0.474 ± 0.024 (4)	< 0.005
Chlorpromazine	0.2	0.554 ± 0.015 (4)	< 0.01
Control		0.790 ± 0.014 (4)	
Propranolol	0.1	0.554 ± 0.011 (3)	< 0.001
	0.5	0.530 ± 0.040 (4)	< 0.001
Preincubation omitted:			
Control		0.790 ± 0.014 (4)	
Tetracaine	0.5	0.442 ± 0.032 (4)	< 0.001

estimated by extrapolating the straight portion of the washout curve for ^{22}Na back to the onset of washout. This gave a value of 9.6 ± 1.9 μmoles/g wet wt in control muscles, whereas in muscles which had been loaded and washed in the presence of tetracaine (0.5 mM), a pool of 4.8 ± 0.7 μmoles/g wet wt was found. Thus, from both estimates, it would appear that the exposure to tetracaine leads to the establishment of a new steady state where the intracellular Na^+ concentration is maintained at roughly half the control level.

This implies that the increase in the rate coefficient of ^{22}Na efflux produced by tetracaine (Fig. 2) may not represent a real increase in the amount of Na^+ extruded per unit time. It seems reasonable to assume that the delayed rise in the rate coefficient of ^{22}Na efflux is secondary to an inhibition of the influx of unlabelled Na^+, and ensuing maintenance of a higher specific activity of ^{22}Na in the pool from which washout is measured.

It is concluded that tetracaine has no direct effect on the active transport of Na^+ and K^+. The membrane stabilizers tested appear to produce a rather selective inhibition of

the passive fluxes of Na^+ and K^+. Although by far the major part of the K^+ efflux and the ouabain-resistant influx of K^+ were suppressible by these compounds, it has not been possible to obtain a complete inhibition of ^{22}Na influx. The tetracaine-resistant component of Na^+ influx may be mediated by a separate transport system, the nature of which is under further investigation.

The category of membrane stabilizers comprise a wide variety of compounds, among which several are frequently used in clinical and experimental work. Some of their actions may be related to inhibition of passive fluxes of Na^+ and K^+ with ensuing alterations in the steady-state distribution of these ions across the plasma membrane. The present data indicate that the concentration gradients for Na^+ and K^+ across the plasma membrane can to a significant extent be increased by inhibition of passive leaks without any direct stimulation of the active Na^+–K^+ pump.

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