LITHIUM ATTENUATES THE ACTIVE TRANSPORT OF CALCIUM IN THE LARVA OF *AËDES AEGYPTI*

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Larvae of the freshwater mosquito *Aëdes aegypti* (L.) are able to maintain their principal electrolytes against a steep concentration gradient between the haemolymph and the external medium. The concentration of monovalent ions such as Na^+, K^+ and Cl^- is kept far above that in the external medium by processes of active transport which are located in the anal papillae, the rectum and the Malpighian tubules (Koch, 1938; Ramsay, 1953; Treherne, 1954; Stobbart, 1959, 1960, 1965, 1967). It has been demonstrated that over 90 % of the exchange of monovalent ions occurs through the anal papillae (Treherne, 1954), but these organs are not permeable to bivalent ions (Wigglesworth, 1933). The processes which regulate the exchange of bivalent ions between the larva and the external medium have not yet been elucidated. Recently, we have demonstrated that larvae of *Aëdes aegypti* maintain a saturable transport system for the uptake of calcium ions from dilute calcium solutions (Barkai & Williams, 1983). This system obeyed Michaelis-Menten kinetics and its activity was susceptible to ruthenium-red which selectively inhibits Ca^{2+}-activated ATPase (Watson, Vincenzi & Davis, 1971).

We now present evidence that lithium ions (Li^+) act in a similar manner to that of ruthenium-red in attenuating the accumulation of Ca^{2+} from dilute solutions. The net Ca^{2+} uptake and the Ca^{2+} fluxes were estimated with ^{45}Ca in early fourth instar larvae. The net Ca^{2+} uptake ($nmol\,h^{-1}\,larva^{-1}$) was determined at Ca^{2+} concentrations ranging between 0·01 and 50 mM in the presence and absence of 2 mM-LiCl or 2 mM-NaCl. Calcium fluxes were determined at a concentration of 0·1 mM-$CaCl_2$ from the curve representing the accumulation of ^{45}Ca in the larva with time (Barkai & Williams, 1983).

When values for the quantity of ^{45}Ca in the larva ($^{\bullet}Q_L$) were divided by the concentration of ^{45}Ca in the medium ($^{\bullet}Q_M$) and plotted against time, the resulting data could be fitted quite closely to the following exponential function of time:

$$^{\bullet}Q_L/^{\bullet}Q_M = [K_{in}/(K_{in} + K_{out})]\cdot[1 - e^{-(K_{in} + K_{out})t}]\,, \tag{1}$$

where K_{in} and K_{out} are fractional rate constants for the influx and efflux of calci██ respectively. Non-linear regression analysis of the data from control larvae resulted in a mean K_{in} of $0.335\ \mu l\ h^{-1}\ larva^{-1}$, representing the entry of $33.5\ pmol\ h^{-1}$ into an average larva at a Ca^{2+} concentration of 0.1 mM. The mean value for K_{out} under these
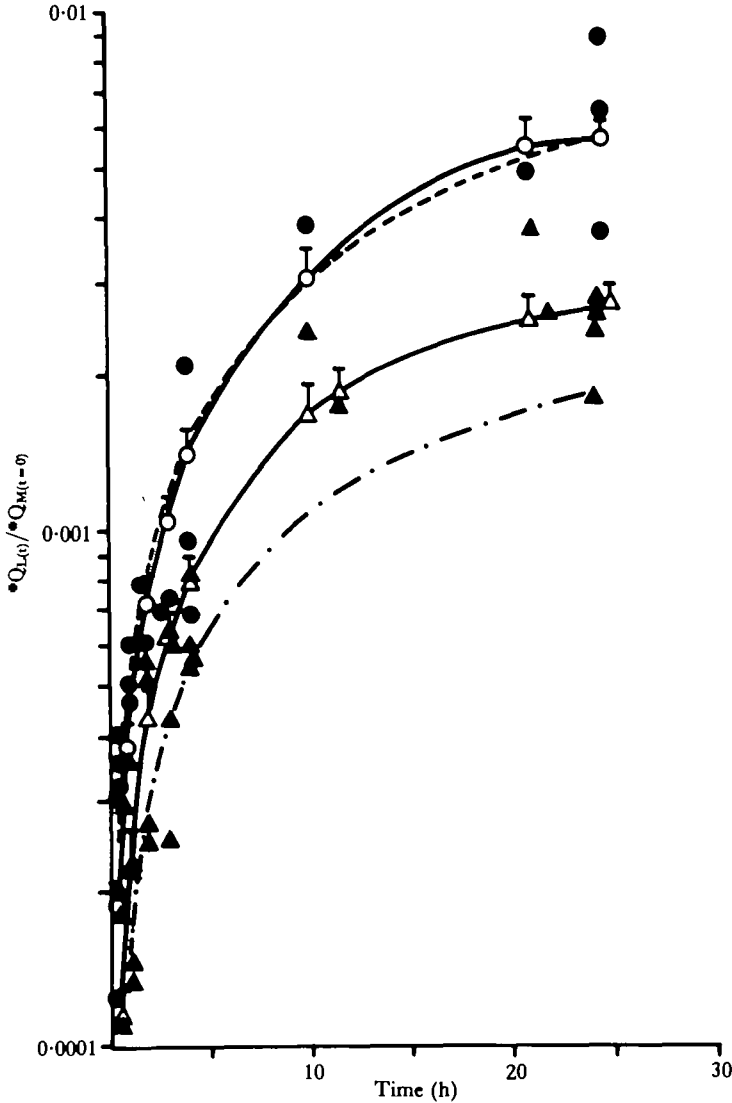

Fig. 1. Change of [^{45}Ca] in the larva with time [$^*Q_{L(t)}$]. Larvae were placed at time zero in a beaker containing 0.1 mM-CaCl$_2$, a tracer amount of ^{45}Ca, and either NaCl (circles) or LiCl (triangles) at a final concentration of 2 mM. Data points are depicted as a fraction of ^{45}Ca in the medium [$^*Q_{m(t=0)}$]. Each curve was obtained after fitting of the data to the function presented in equation (1) in the text using a computer programme for non-linear regression analysis. Empty circles or triangles with attached vertical bars represent predicted values and their corresponding standard deviations as produced by the BMDP3R computer programme. Also shown are a control curve (- - - - -) and a curve representing 0.1 mM ruthenium-red in the external medium (— · — · —) obtained previously (Barkai & Williams, 1983). The fractional rate constants K_{in} and K_{out} which were found from these curves according to the theoretical relationship for a closed two compartmental system are presented in Table 1.

nditions was $0\cdot027\,h^{-1}\,larva^{-1}$, indicating that $0\cdot027$ of the readily exchangeable calcium pool in the larva is released into the medium each hour (Barkai & Williams, 1983). When NaCl (2 mM) was present in the external medium there were no significant changes in either K_{in} or K_{out}, but when the external medium contained LiCl (2 mM), the accumulation of ^{45}Ca was much slower (Fig. 1). This slower accumulation resulted from both a decrease in K_{in} and an increase in K_{out} compared to the corresponding control values (Table 1).

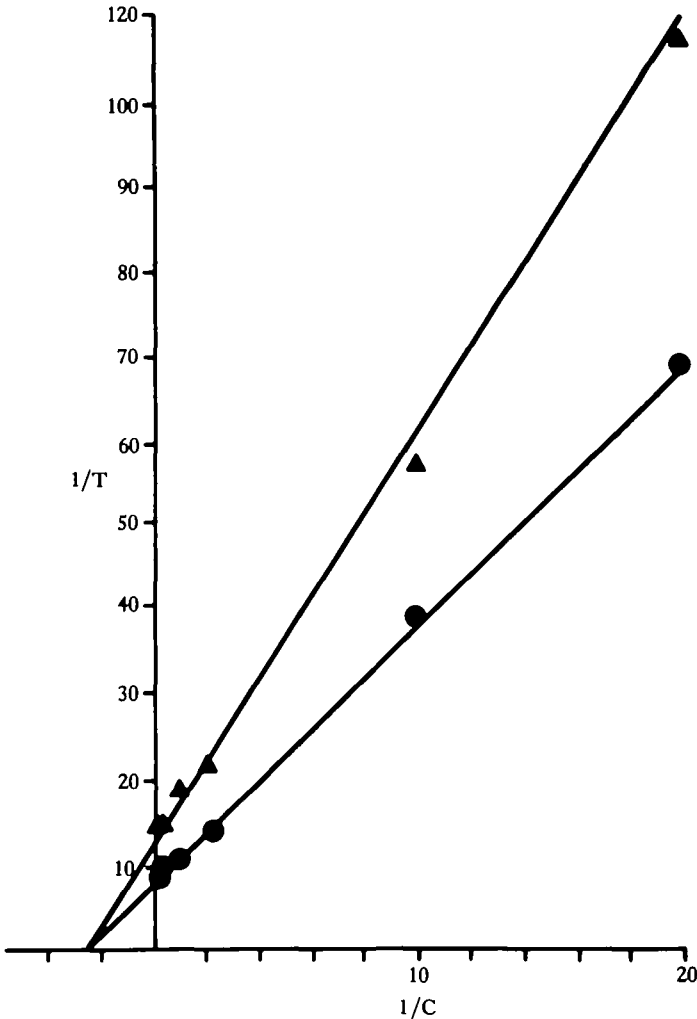The effects of Li^+ on the saturable transport of calcium were studied in separate

Fig. 2. Kinetic analysis of the saturable transport, T, as a function of calcium concentration, C, in the external medium in the presence of 2 mM-NaCl (circles) or 2 mM-LiCl (triangles). Each point is the mean of five or six separate determinations. Intercepts were determined by linear regression analysis using the method of least squares. The affinity constant (K_m) of $0\cdot45$ mM obtained for both experimental conditions was similar to the K_m value obtained for controls (Barkai & Williams, 1983). The maximum transport velocity (V_{max}) in larvae exposed to LiCl was $0\cdot07$ nmol h^{-1} larva^{-1}, much slower than the value of $0\cdot12$ nmol h^{-1} larva^{-1} in larvae exposed to 2 mM-NaCl or in control larvae (Barkai & Williams, 1983).

Table 1. *Transfer constants for calcium exchange in* Aëdes aegypti *larvae under various conditions*

Experimental condition	K_{in}* (μl h^{-1} larva^{-1})	K_{out}* (h^{-1} larva^{-1})	Total calcium pool (nmol larva^{-1})
Control†	0·335 ± 0·077	0·0266 ± 0·0143	7·6 ± 0·8
NaCl (2 mM)	0·037 ± 0·128	0·0320 ± 0·0171	8·1 ± 0·6
LiCl (2 mM)	0·232 ± 0·037‡	0·0708 ± 0·0233‡	6·8 ± 0·7
Ruthenium-red (0·1 mM)†	0·153 ± 0·033‡	0·0668 ± 0·0281‡	5·9 ± 0·6‡

Values are given as means ± 1 s.d.
* Values for K_{in} and K_{out} were obtained after data representing the accumulation of ^{45}Ca with time were fitted to equation (1) in the text using a computer programme for non-linear regression (BMDP3R, Health Sciences Computing Facility of UCLA in Los Angeles, 1979 revision).
† Data from Barkai & Williams (1983).
‡ Significantly different from corresponding control value ($P < 0.01$).

experiments. The saturable transport was analysed, according to the method of Lineweaver & Burk (1934), in the presence and absence of Li$^+$ (2 mM). This analysis revealed that the maximum transport velocity (V_{max}) decreased markedly in the presence of LiCl (Fig. 2), a phenomenon which was not observed with NaCl. The apparent affinity constant (K_m) was not appreciably different. These results indicate that a marked decrease had occurred in the number of 'calcium carriers' when the larvae were exposed to LiCl and show that Li$^+$ may influence carrier sites which are linked to the Ca^{2+} pump. The finding that Li$^+$ acts to increase K_{out} and decrease K_{in} in a manner similar to that of ruthenium-red (Table 1) suggests that Li$^+$ influences two independent processes; one is the absorption of Ca^{2+} from dilute solutions into the larva and the other is the prevention of Ca^{2+} loss to the medium. The entry of Ca^{2+} is most likely to occur through the gut, whereas Ca^{2+} loss occurs in the urine. Lithium may therefore interfere with the action of calcium pumps which are located in both the gut and the rectum. The calcium pumps in the gut act to enhance the entry of Ca^{2+} from the swallowed medium, and therefore inhibition of their activity is expected to result in a lower K_{in}. In contrast, the calcium pumps in the rectum act to reabsorb Ca^{2+} that has been excreted in the urine by the Malpighian tubules, and therefore inhibition of their activity might be expected to result in a higher K_{out}.

Effects of Li$^+$ on Ca^{2+} transport are of some importance in biological psychiatry in view of the therapeutic role of Li$^+$ in the treatment of affective disorders (Shopsin & Gershon, 1978). Although Li$^+$ is widely used in psychiatry, its mode of action has not yet been elucidated. It has been suggested that Li$^+$ acts at the presynaptic membrane, preventing release of biogenic amines and facilitating their uptake (Bunney, Gershon, Murphy & Goodwin, 1972), probably by inhibiting the action of Ca^{2+} on the presynaptic membrane (Katz & Kopin, 1968), but direct evidence for the antagonism between Li$^+$ and Ca^{2+} is still obscure. The uptake of Li$^+$ into rat cerebral cortex slices has been shown to be inversely related to the concentrations of Ca^{2+} in the medium (Wraae, Hillman & Round, 1976). Recent studies with red blood cells indicated that Li$^+$ and Ca^{2+} may share the same transport system (Meltzer, 1979), thus implying that the therapeutic effects of Li$^+$ in certain affective disorders may be associated with changes in calcium transport.

■he present findings that Li^+ but not Na^+ inhibits the active transport of Ca^{2+} in the mosquito larva is consistent with the observation that Li^+ efflux is linked to the operation of Ca^{2+} pumps in the red blood cell (Meltzer, 1979) and suggests that larvae of *Aëdes aegypti* provide a useful and convenient model to study the effects of Li^+, or other agents, on the saturable system for the active transport of calcium.

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