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The Effect of Lithium on Strophanthidin Toxicity in Cardiac Purkinje Fibers[1] (40850)

CHENG I LIN AND MARIO VASSALLE

Department of Physiology, State University of New York Downstate Medical Center, 450 Clarkson Avenue, Brooklyn, New York 11203

Cardiac glycosides inhibit Na^+, K^+-ATPase activity (1) and this could be related to the onset of arrhythmias. Lithium has been reported to stimulate the Na^+, K^+-ATPase (2, 3), although it may inhibit the sodium pump (see (2, 4)). Thus, it is not clear how lithium might be expected to affect the arrhythmias induced by cardiac glycosides. When lithium was tested *in vivo* as a possible antiarrhythmic agent for digitalis-induced arrhythmias, contrasting results were obtained. In one report (5), lithium was found to potentiate digitalis toxicity (onset of ventricular fibrillation), while in another report (6) lithium significantly shortened the duration of ouabain-induced arrhythmias. In the latter study, it was postulated that the antiarrhythmic action of lithium might involve either a direct effect on the heart or a depression of catecholamine metabolism (centrally or at the heart level).

In the *in vivo* studies of Osman *et al.* (5) and of Polumbo *et al.* (6), complex reactions may occur in response to the administration of cardiac glycosides and lithium. For this reason, the action of lithium on strophanthidin toxicity was studied on cardiac Purkinje fibers perfused *in vitro*. In this manner, changes in the central nervous system function in response to lithium, and changes in heart rate, in blood pressure, in the level of anesthesia are avoided and the action of lithium on administered catecholamine can be evaluated. The results show that lithium (5 m*M*) has little action of its own on the electrical and mechanical activity of canine Purkinje fibers, accelerates the onset of strophanthidin-induced spontaneous activity and modifies little the effect of norepinephrine either in the absence or in the presence of strophanthidin.

Materials and methods. Mongrel dogs of either sex were anesthetized with sodium pentobarbital (30 mg/kg, iv) and the heart was excised through a thoracotomy. Purkinje fibers strands were removed from the ventricles and perfused in tissue bath with oxygenated (97% O_2 and 3% CO_2), warm (37°C) Tyrode solution. The composition of the Tyrode solution was as follows (m*M*): NaCl, 137; KCl, 2.7; $NaHCO_3$, 11.9; NaH_2PO_4, 0.45; $MgCl_2$, 0.5; $CaCl_2$, 2.7; and dextrose, 5.5. Lithium chloride, strophanthidin, and tyramine chloride were obtained from Sigma Chemical Company, propranolol–HCl from Ayerst Laboratories and norepinephrine (Levophed) from Winthrop Laboratories. The preparations were fixed at one end by means of a stainless pin insulated except for the tip and was tied at the other end to a rigid rod by means of a short silk thread. The rod was attached to a force displacement transducer (Grass Model FT03C). The preparations were driven at 60/min by means of electrical stimuli of suprathreshold strength and of a 2-msec duration. The stimulator (Grass S4) was connected by means of a stimulus isolation unit (Grass SIU 4678) to steel pins in contact with the preparation.

The resting force applied to the preparations was 60% of that necessary to allow maximal force development. The membrane potentials were recorded by means of glass microelectrodes filled with 3 *M* KCl connected to a cathode follower stage. The tip of one microelectrode was immersed in the perfusion fluid and the other was inserted intracellularly. The electrical and mechanical events were displayed on a dual beam oscilloscope (Tektronix Model 502A) and were photographed on film with a Grass Kymograph Camera (Model C4C).

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The contractile force was also recorded continuously with a Grass Model 7 polygraph at a speed of 0.25 mm/sec.

The preparations were equilibrated for 1 hr in Tyrode solution. Strophanthidin was administered at a concentration of 10^{-6} *M* until spontaneous activity overcame the driving rate of 60/mm: this was taken as evidence of toxicity. After full recovery, the preparation was exposed first to lithium alone and then to lithium and strophanthidin. The time to toxicity and mode of intoxication in the presence and absence of lithium were compared.

Values are expressed as the mean ± standard error. Statistical analysis was performed with Student's *t* test and a *P* value smaller than 0.05 was regarded as significant.

Results and discussion. The effect of lithium on the action potential and contractile force. It has been repeatedly suggested that the positive inotropic effect of cardiac glycosides is due to an inhibition of cardiac Na^+, K^+-ATPase (see (7) for references) and lithium has been shown to have positive inotropic effect at certain concentrations (3). In view of these findings, it was of interest to find out whether lithium increases the force of contraction at a concentration (5 m*M*) which shortens the duration of digitalis arrhythmias (6).

In Fig. 1, the top strip shows the action potential and contractile force in Tyrode solution (first panel) and after 5, 13, and 19 min of perfusion with 5 m*M* lithium (second, third, and fourth panel, respectively). The bottom strip shows a low-speed record of the contractile force. It is apparent that lithium caused insignificant changes in the magnitude and configuration of the action potential and in the force curve. In six experiments, there was no apparent effect of 5 m*M* lithium on contractile force. In 10 other experiments, two concentrations of lithium were used: neither the lower concentration (5 m*M*) nor the higher concentration (10 m*M*) changed the force of contraction significantly (0.8 ± 6.7 and 3.3 ± 3.7%, respectively).

These results suggest that, at the concentrations used, the inhibition of the pump by lithium should be small, if any. In fact, a

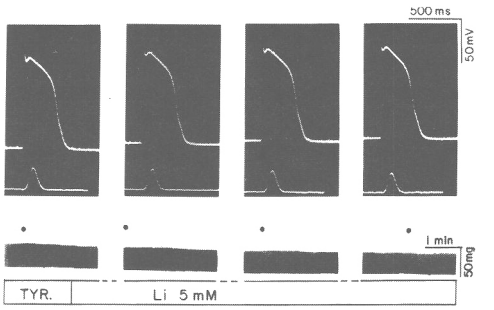

FIG. 1. Effect of lithium on the mechanical and electrical activity of Purkinje fibers. The top strip shows the control recording (first panel) and the recordings during 5 m*M* Li (lithium) exposure (subsequent panels). The bottom strips show the slow-speed recording of the mechanical activity before and during lithium exposure. The dots on top of the bottom traces indicate the points at which the top panels had been recorded. The action potential trace shifted in an upward direction with time but the action potential revealed little or no changes. Time, voltage, and force calibration are indicated at the right-hand side of the figure.

significant inhibition of the sodium pump has been found with much higher concentrations of lithium (58 m*M* or higher) but not with a concentration of 29 m*M* (see table 4 in Ref. (7)). Furthermore, at a concentration of 5 m*M*, lithium has been found to stimulate the Na^+, K^+-ATPase (2, 3) and not to increase the force of contraction either in Purkinje fibers (present experiments) or in atrial fibers (3). Since the present experiments were intended to evaluate the action of lithium on digitalis toxicity in reference to *in vivo* studies, there was little point in using higher concentrations of lithium, since plasma concentrations of lithium in excess of 10 mg/liter are toxic (8).

The effect of strophanthidin in the absence and in the presence of lithium. The effect of lithium on strophanthidin-induced spontaneous activity was studied by first exposing the fibers to strophanthidin and then (after full recovery) to lithium and strophanthidin. In Fig. 2, strip A shows the action potential in Tyrode solution (first panel) and in the presence of strophanthidin (following three panels). Strip B shows a slow-speed recording of the mechanical activity. The dots above strip B show the time at which the panels in A were recorded. As

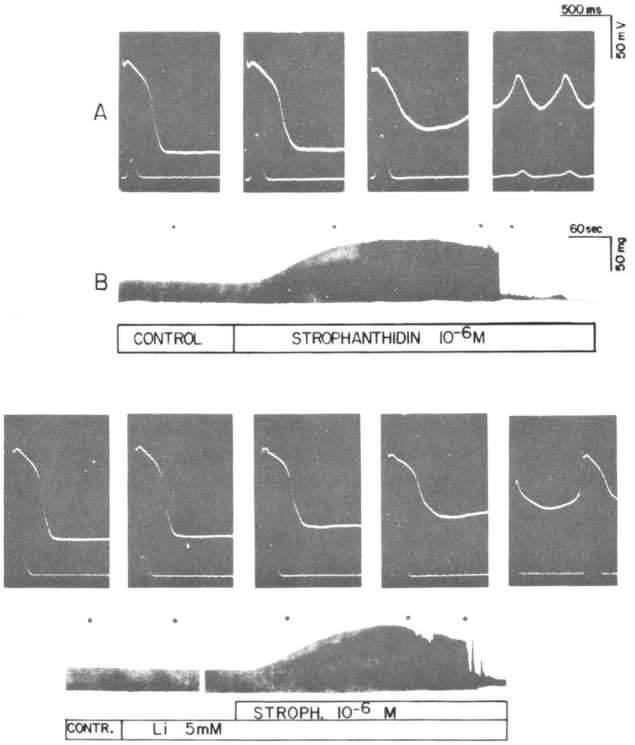

FIG. 2. Effect of strophanthidin in the absence and in the presence of lithium. Strip A shows the control records (first panel) and the records in the presence of strophanthidin (subsequent panels). Strip B shows a slow-speed record of mechanical activity before and during strophanthidin. The third strip shows the control records (first panel), the records in the presence of lithium (second panel) and those in the presence of lithium plus strophanthidin (subsequent panels). The fourth strip shows the slow-speed recording in the absence and presence of lithium and strophanthidin.

usual (9–11), strophanthidin caused a lengthening and then a shortening of the action potential, a gradual fall in maximum diastolic potential (E_{max}), a steepening of diastolic depolarization, and eventually spontaneous activity. The force typically (10, 11) at first increased and then decreased. When the spontaneous activity developed (last upper panel) not only the twitch was much smaller but force increased progressively during diastole.

The third strip shows the action potentials and the force in Tyrode solution (first panel), in the 5 m*M* lithium solution (second panel) and in the presence of lithium plus strophanthidin (subsequent panels) in the same preparation. The fourth strip shows a continuous record of the mechanical activity. The tracings show that, in the presence of lithium, strophanthidin caused the usual changes in the magnitude of E_{max} and the slope of diastolic depolarization; and induced spontaneous discharge. In fact, the onset of the spontaneous activity occurred sooner in the presence than in the absence of lithium.

Similar results were obtained in six experiments in which the time to the onset of spontaneous activity was 10 ± 3% ($P < 0.05$) shorter in the presence than in the absence of lithium. The time to the peak inotropic effect was similar (strophanthidin 652 ± 100 sec and lithium plus strophanthidin 643 ± 102 sec, $n = 5$). The peak inotropic effect was somewhat higher (+16%, $n = 6$) but not significantly ($P > 0.05$) when strophanthidin was given in the presence of lithium.

These results make clear that lithium does not counteract strophanthidin induced

spontaneous activity but instead favors its onset, in agreement with the results of Osman *et al.* (5). It is possible then the shortening of the duration of the arrhythmias (6) might have been due to a depressant effect on catecholamines metabolism in CNS during late digitalis toxicity.

The role of catecholamines in the action of lithium. The above results exclude the possibility that lithium has a direct action in shortening the duration of the arrhythmias, since *in vitro* the onset of spontaneous activity was not retarded. The possibility has been put forward that lithium may affect catecholamine metabolism in cardiac tissues (6). That there are indeed catecholamine stores in the preparation studied is shown in Fig. 3. In the top trace, tyramine (6×10^{-5} *M*), an agent known to liberate catecholamines, increased the force of contraction. That the increase in force was due to a liberation of catecholamines is shown in the bottom trace: tyramine failed to alter the force after the administration of 3.4×10^{-6} *M* propranolol. Similar results were obtained in two experiments. As the experiments show that there are local stores of catecholamines, possible effects of lithium on catecholamine action were tested by studying the effects of exogenous norepinephrine on force of contraction in the presence and in the absence of lithium. In Fig. 4, top strip, norepinephrine (10^{-6} *M*) increased force of contraction. In the middle strip, norepinephrine was administered in the presence of 5 m*M* lithium and its inotropic effect was little affected. In the bottom strip, norepinephrine was administered first: when lithium was added, again the force of contraction was little modified. In 10 experiments, 5 and 10 m*M* lithium caused a small decline in force in the presence of norepinephrine ($-12.3 \pm 4.5\%$, $P < 0.05$, and $-8.6 \pm 4.5\%$ $P > 0.2$, respectively).

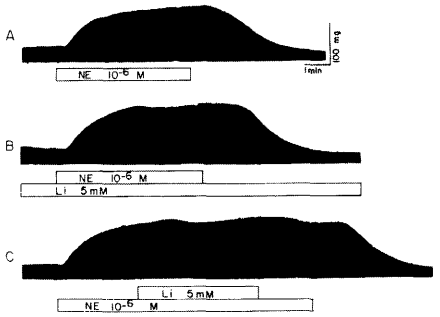

FIG. 4. The action of norepinephrine in the absence and in the presence of lithium. Norepinephrine and lithium were administered during the period indicated beneath each of the three strips.

Norepinephrine in a concentration which does not cause arrhythmias in Tyrode solution may do so in the presence of strophanthidin (12). As illustrated in Fig. 5, lithium does not appear to alter the induction of spontaneous activity by norepinephrine in the presence of strophanthidin. In the top strip, norepinephrine caused spontaneous activity shortly after its application in the presence of strophanthidin as shown

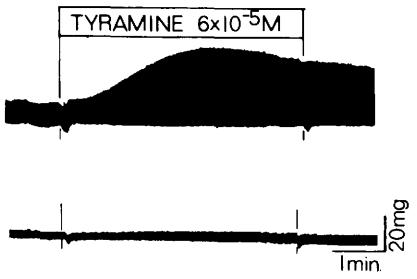

FIG. 3. The effect of tyramine on the force of contraction in the absence and in the presence of propranolol. The strips show the slow-speed recording of contractile force. Tyramine was perfused between the two vertical lines in the absence (top strip) and in the presence of propranolol (3.4×10^{-6} *M*, bottom strip).

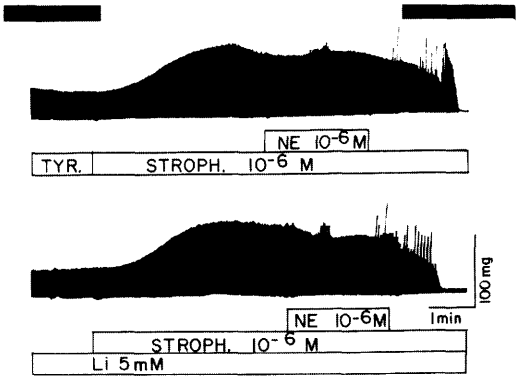

FIG. 5. The arrhythmogenic effect of norepinephrine and strophanthidin in the absence and in the presence of lithium. Norepinephrine, strophanthidin, and lithium were present in the solution during the periods indicated beneath the two strips.

by the irregularities in the mechanical record. In the bottom strip, the onset of spontaneous activity by norepinephrine in the presence of strophanthidin was not much different during the perfusion of lithium chloride.

Thus, it appears that lithium has little effect either on the endogenous stores of catecholamines (a depression of metabolism would have retarded the onset of spontaneous activity) or on the action of exogenous catecholamines in cardiac tissues. The possibility remains that the results of Polumbo *et al.* (6) were due to a diminished release of catecholamines due to central effects of lithium. While lithium potentiated the arrhythmogenic action of digitalis in the *in vivo* experiments of Osman *et al.* (5), this could be due to a different role of the central nervous system in different species. Whatever the case may be, the present results show that the direct effect of lithium is to facilitate the onset of strophanthidin-induced spontaneous activities. Since lithium enters Purkinje fibers as sodium does, it would add an inward current to facilitate the onset of spontaneous activity. It has been proposed that a transient increase in sodium concentration in a small intracellular compartment near the cell membrane occurs with each action potential and that digitalis enhances such a transient increase (see (7)). Therefore, an alternative possibility is that lithium (while having little effect on its own at low concentrations) may magnify the effect of strophanthidin on the transient increase in sodium and this could lead to an earlier onset of spontaneous discharge. This would agree with the slightly greater inotropic effect of strophanthidin in the presence of lithium and with the increased calcium content in the myocardium of animals treated with lithium and cardiac glycosides (5).

Summary. The effect of lithium (5 m*M*) on strophanthidin-induced spontaneous activity was studied in canine Purkinje fibers perfused *in vitro*. Lithium did not alter the configuration of the action potential and had little effect on contractile force. Strophanthidin induced the usual increase and then a decrease in contractile force and eventually spontaneous activity. Lithium modified little the effect of strophanthidin on force but spontaneous activity occurred sooner. Catecholamines are stored in Purkinje fibers but these stores are unimportant in the facilitatory action of lithium on digitalis toxicity, since lithium did not increase the effects of exogenous norepinephrine, either in the absence or in the presence of strophanthidin. It is concluded that lithium favors (and does not antagonize) the onset of digitalis-induced spontaneous activity and this action does not involve a change in cardiac catecholamine metabolism.

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