Tl^+-Ions: Comparison of the Effects on the Slow Inward Current and Contractility of Ventricular Tissues

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Slow Inward Current, Thallium Intoxication, Voltage Clamp, Available Intracellular Energy

To conclude our investigation of thallium effects on cardiac tissues, we studied the slow inward current of sheep cardiac Purkinje fibres exposed to 10^{-7} to 10^{-5} M Tl^+ for extended periods of up to 80 min. Our previous results had suggested a possible involvement of the slow inward current during thallium intoxication: a) the modification of contractility staircases observed during thallium exposure, b) action potential recordings of ventricular muscle, c) changes in spontaneous beating in sino-atrial preparations. The thallium levels chosen were between those yielding strong positive inotropic transients and those producing a marked long-term decay of contraction force.

The slow inward current was measured using a conventional two-microelectrode-technique and the standard voltage clamp protocol for this current system. The experimental work was restricted to the determination of $\bar{d}_\infty$, the kinetics of activation of the slow inward current and of $\overline{i_{si}}$, the current voltage relation of the current system. This was necessary since the effects of thallium were known to be short-lived and therefore frequent repeat runs of the voltage clamp program had to be performed in order to obtain the time courses of possible transient changes.

The results showed that the slow inward current was first increased and then declined at the low concentration of 10^{-7} M Tl^+. At 10^{-5} M Tl^+ the initial increase was smaller, whereas the decay of the slow inward current proceeded to lower values. Comparison with contractility measurements at the same concentrations of thallium showed a distinct parallelism between changes of the slow inward current and myocardial contractility. Despite this apparent relationship, we do not conclude that the contractile events are primarily a result of changes of the slow inward current, since thallium does not seem to specifically alter the parameters of the slow inward current at the membrane level.

Introduction

In this, the final communication of a series of three on the cardiac effects of thallium ions on isolated tissues we have investigated the changes observed in the slow inward current following exposure to thallium. The slow inward current was included in the study for a number of reasons that had become apparent during the previous work [1, 2]. Involvement of the slow inward current was suggested from results of contractility measurements and from recordings of action potentials. Contractile force was affected quite severely by thallous ions and during transient positive inotropic responses a slight elevation of action potential plateaus of myocardial tissue was observed. Hence, there was some indication of a possible relationship between changes of electrical and mechanical properties. The first question was: Does the slow inward current follow the time course of the inotropic events observed after administration of thallium? More specifically, do transient positive inotropic responses and subsequent inotropic decay proceed in parallel to changes in the slow inward current (i_{si})?

Secondly, are possible changes of i_{si} primarily membrane effects in the sense that slow channel properties are altered (as for instance Ca-antagonists do) or are they rather to be considered secondary occurrences based on intracellular events? In this context, the findings of the previous paper relating to the parameters of excitability and pacemaker current had shown that membrane effects of thallium on ventricular current systems, i_{Na} and i_{K2} do not seem likely. On the other hand, however, in the case of disrhythmic effects of thallium on the sinus node the pacing activity of which is brought about by the "slow response", *i.e.* calcium influx, a direct membrane effect could not be excluded.

Thirdly, would the complicated force-frequency behaviour of thallium-treated ventricular muscle fibres reveal a certain regularity in view of measurable changes in the slow inward current. This

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possibility has been suggested and discussed in the first communication [1].

Lastly, our aim is to suggest a possible mechanism for the course of arrhythmias, positive inotropic transients, and contractile deterioration after thallium administration from the data compiled in these studies. We have pointed out before that some of the classical mechanisms of inotropic changes as have been suggested for other agents do not seem to apply for thallium. The classical mechanism of digitalis-induced inotropism has been suggested for thallium [3, 4] but so far as our results are concerned the effects of thallium delude unambiguous description in terms of this mechanism.

Methods and Materials

Purkinje fibres of sheep and occasionally calf hearts obtained by a nearby slaughterhouse were used in this study. The details of the removal of hearts and their transport to the laboratory, and the subsequent excision and storage of Purkinje strands have been presented previously [2].

For reasons of homogenous voltage control the preparations were restricted to 2 mm in length. Viability of these short preparations was tested with impaled microelectrodes to ensure normal resting and action potentials.

The composition of the standard Tyrode solution was as follows: Na^+: 149.16; K^+: 4; Mg^{2+}: 0.5; Ca^{2+}: 1.8; Cl^-: 145.5; HCO_3^-: 12; $H_2PO_4^-$: 0.36 and glucose 15 (all in mM).

Thallium was added to this solution as its sulfate salt (Tl_2SO_4, Merck, p.A. grade). For preparation of thallium Tyrode solution see the first paper [1].

Voltage clamp experiments

The properties of the microelectrodes and the auxiliary electronic equipment have been documented previously on several occasions [2, 5, 6]. Essentially, the technique was a modification of the method introduced by Deck *et al.* [7]. The measurement of the properties of the slow inward current followed the procedure described by Vitek and Trautwein [8] and Reuter and Scholz [9].

In this study d_∞ and $\overline{i_{si}}$ were determined. For d_∞, the fast sodium current was inactivated by maintaining a holding potential of -50 mV. Increasing 20 ms depolarizing pulses activated the slow inward current to various levels, the relative magnitude of which could be determined from inward current tails after clamping back to the holding potential. The current-voltage relationship of $\overline{i_{si}}$ was measured by changing the potential of the return clamps at given constant degrees of activation. Thereby the driving force of the current activated during the 20 ms depolarizing pulse was changed. In the complete experiments described later the voltage clamp protocol was repeated every 5 min after administering thallium in order to obtain the time course of the effects of thallium on the slow inward current.

Interference by other time dependent currents overlapping in the potential range of i_{si} was not observed. A transient outward current could not be obtained at any frequency of clamp pulses from a holding potential of -50 mV (compare Boyett [10, 11]). Interference with the transient outward current was at worst 10 to 15% as calculated previously [16] on the basis of the literature [12, 13]. The time constants of the repolarizing potassium current i_{x2} are too large to have a marked effect on the comparatively fast current tails of the slow inward current, the time constants of i_{x1} are comparable with those of i_{si} but the magnitude of i_{x1} too small to interfere [14].

Due to a number of limiting factors only a few of the experiments could be completed and hence be used as a comparison of the time courses of i_{si} and cardiac contractility after administrating thallium in this study. There were three main reasons for the low success within the projected scope of the study: Short fibres such as were used to study the slow inward current were highly susceptible to damage which could result from comparatively large voltage clamp currents. Maintenance of a holding potential of -50 mV often caused oscillatory responses in the plateau range, particularly during further depolarization to $+10 - +20$ mV. Experiments with such fibres were then discontinued. A number of healthy fibres that would have responded well to current strain during voltage clamping had to be discarded due to obvious voltage and current inhomogenity as a result of large series resistances and large total membrane capacities. This has been a topic of discussion particularly for cardiac Purkinje fibres. Despite its relative puniness compared to the fast sodium current voltage control of the slow inward current can be poor if the cleft resistance is high. Furthermore, the time constants of the slow inward current are of the order of the time constant of the

passive membrane capacity, so that a large series resistance can distort the time course of the slow inward current considerably. For practical purposes it was necessary to judge whether the possible error was still permissible and in many instances we were forced to abandon otherwise promissing experiments.

A final draw back for the experiments in this study aiming to correlate the time course of possible changes of i_{si} to changes of contractile force was that the fibres were exposed to a highly toxic agent. The necessary frequent repeat cycles of the voltage clamp protocol to determine the slow inward current were sometimes impossible and in a number of cases the experiments were terminated because the fibres had obviously passed away under the combined effects of loss of structural integrity, continuous voltage clamp current strain, and thallium intoxication. However, we eventually succeeded in bringing to a satisfactory conclusion three complete experiments at 10^{-7}, 10^{-6}, and 10^{-5} M Tl^+ during which i_{si} was recorded at 5–10 min intervals for up to 80 min after admission of thallium. It is these experiments with which this communication is mainly concerned.

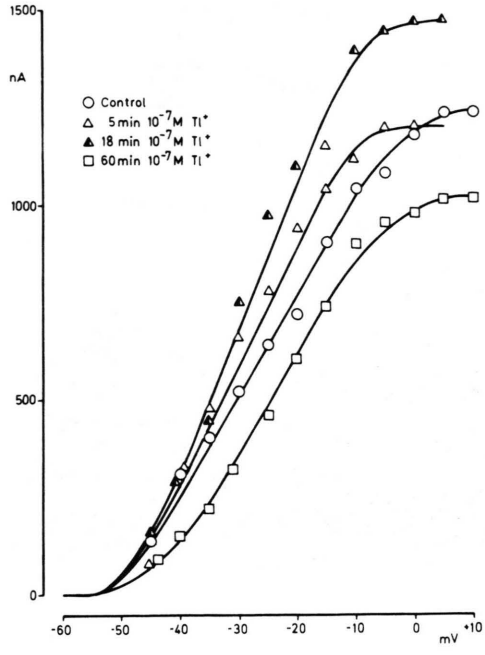

Fig. 1a. Amplitudes of inward current tails obtained at holding potentials of − 50 mV after 20 ms activating voltage clamp pulses to different potentials. Abscissa: Potential of activating pulse. Ordinate: Current amplitude. Selected voltage clamp runs during exposure of fibre 1 to 10^{-7} M Tl^+.

Results

Activation of the slow inward current

The activation kinetics of the slow inward current were determined by clamping the membrane to a holding potential of − 50 mV and applying activating voltage clamp pulses of various heights for 20 ms. After return to the holding potential a decaying inward current tail was obtained. The amplitude of the current tail was a function of the potential of the activating pulse and was plotted versus this potential. Fig. 1a, 2a, and 3a show the results of the three experiments at different thallium concentrations recorded after various intervals of exposure. In Fig. 1a (10^{-7} M Tl^+) the maximal amplitude of the tail current was increased after a period of 18 min and dropped to below control values after 60 min. In Fig. 2a (10^{-6} M Tl^+) the maximal amplitude increased more rapidly and declined to below control values after 20 min. In Fig. 3a (10^{-5} M Tl^+) only the decreases of the maximal amplitudes with respect to time are shown. As will be seen in Fig. 7 the

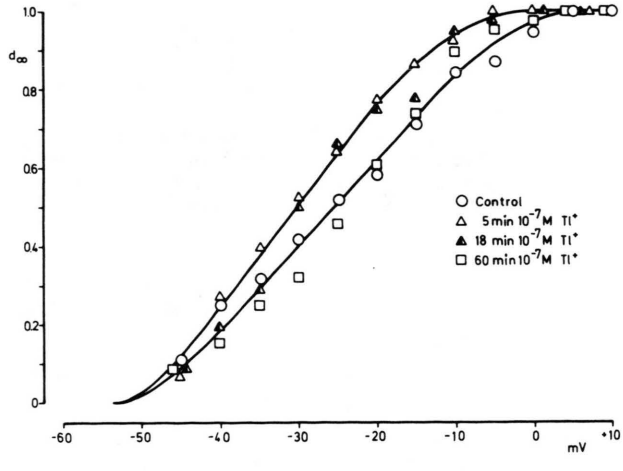

Fig. 1b. Activation curve of the slow inward currend d_∞. Abscissa: Potential of the activating 20 ms pulse. Ordinate: Current tails normalized with respect to the maximal current amplitude. Experimental data from Fig. 1a.

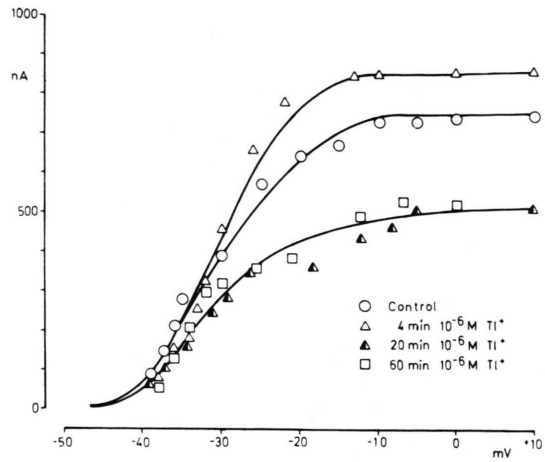

Fig. 2a. Amplitudes of inward current tails obtained at holding potentials of − 50 mV after 20 ms activating voltage clamp pulses to different potentials. Abscissa: Potential of activating pulse. Ordinate: Current amplitude. Selected voltage clamp runs during exposure fibre 2 to 10^{-6} M Tl^+.

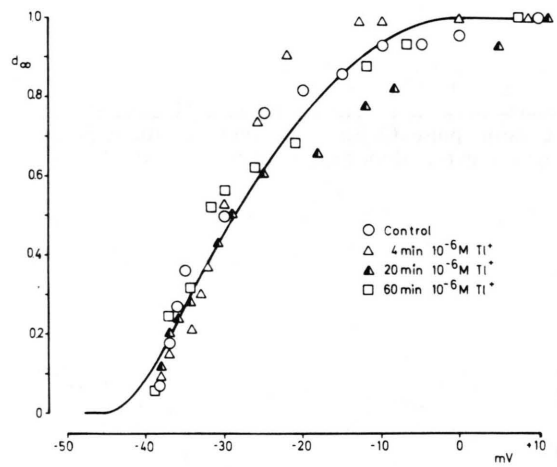

Fig. 2b. Activation curve of the slow inward current d_∞. Abscissa: Potential of the activating 20 ms pulse. Ordinate: Current tails normalized with respect to the maximal current amplitude. Experimental data from Fig. 2a.

recording of the slow inward current after 5 min showed a slight increase.

In Figs. 1b, 2b, and 3b the current tail amplitudes were normalized with respect to maximal amplitudes at full activation of the slow inward current. The curves corresponded to the same experiments as in the unnormalized curves. Normalization showed that the parameter of activation of the slow inward current d_∞ was slightly increased with respect to potential at 10^{-7} M Tl^+ (Fig. 1b) but resumed its original position after 60 min. At 10^{-6} M Tl^+ (Fig. 2b) we did not observe a significant change of d_∞ after exposure to thallium. Under the influence of 10^{-5} M Tl^+ d_∞ was apparently more activated during the course of the experiment. It was not possible to determine whether this effect

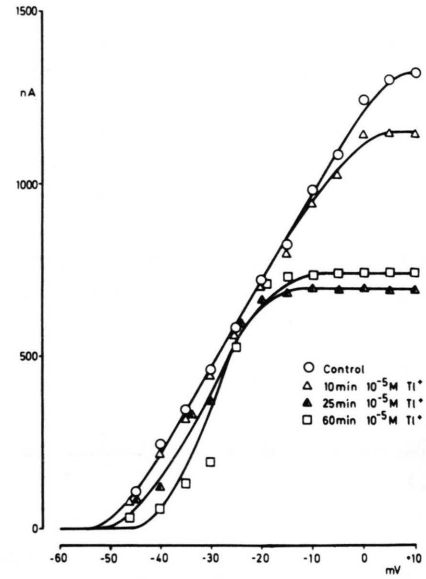

Fig. 3a. Amplitudes of inward current tails obtained at holding potentials of − 50 mV after 20 ms activating voltage clamp puses to different potential. Abscissa: Potentials of activating pulse. Ordinate: Current amplitude. Selected voltage clamp runs during exposure of fibre 3 to 10^{-5} M Tl^+.

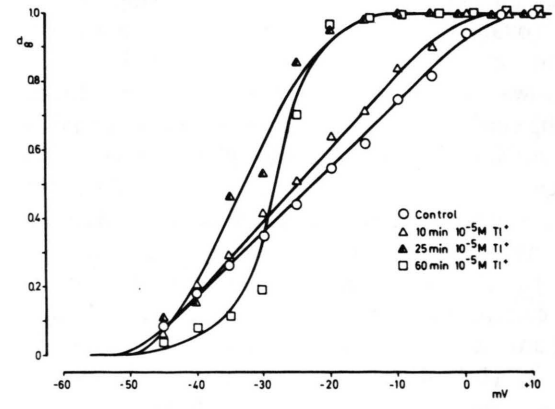

Fig. 3b. Activation curve of the slow inward current d_∞. Abscissa: Potential of the activating 20 ms pulse. Ordinate: Current tails normalized with respect to the maximal current amplitude. Experimental data from Fig. 3a.

was coincidential for reasons given in the Methods section. However, the important point is that the maximal amplitude was decreased at this concentration as will also be seen in Fig. 6.

Current voltage relationship of the slow inward current

The current voltage relationship of the slow inward current was determined by setting the voltage clamp protocol to a constant holding potential of − 50 mV and activating for 20 ms at a potential of + 10 mV. The slow inward current followed as decaying current tails after clamping to various test potentials. The amplitudes of these current tails were plotted against the potential of the test clamp as is seen in Figs. 4, 5, and 6. These plots yielded the current voltage relationship of the slow inward current at constant maximal activation. Within reasonable limits the plots were linear with intercepts on the voltage axis corresponding to the "reversal potential" of the slow inward current. The nature of the current and its experimental determination is such that only a relative measurement can be achieved with reasonable accuracy. It is for this reason that the reversal potentials of the slow inward current scatter considerably in the literature and that in a study concerned with the toxic action of an agent during a long period of exposure it must suffice to establish only the relative effects of the agent on the slow inward current.

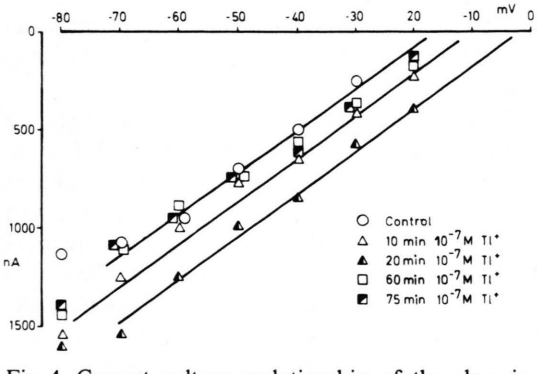

Fig. 4. Current voltage reslationship of the slow inward current determined from the amplitudes of current tails versus driving force. Holding potential before stimulation: − 50 mV; potential of 20 ms activating pulse: + 10 mV. Abscissa: Potential of test clamp after activating. Ordinate: Amplitude of current tails. Preparation: Fibre 1, 10^{-7} M Tl^+.

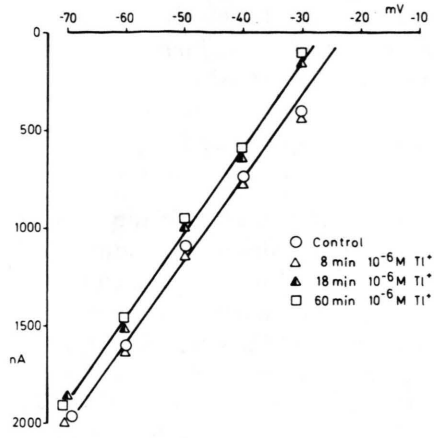

Fig. 5. Current voltage relationship of the slow inward current determined from the amplitudes of current tails versus driving force. Holding potential before stimulation: − 50 mV; potential of 20 ms activating pulse: + 10 mV. Abscissa: Potential of test clamp after activating. Ordinate: Amplitude of current tails. Preparation: Fibre 2, 10^{-6} M Tl^+.

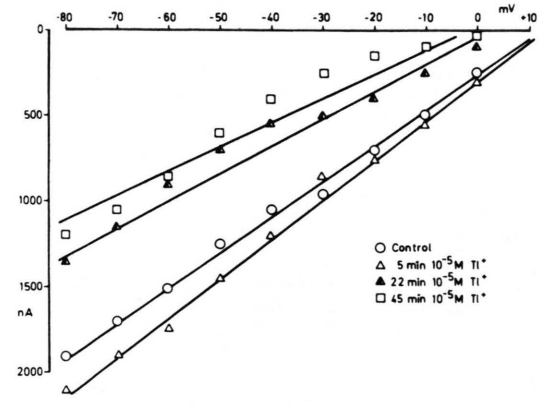

Fig. 6. Current voltage relationship of the slow inward current determined from the amplitudes of current tails versus driving force. Holding potential before stimulation: − 50 mV; potential of 20 ms activating pulse: + 10 mV. Abscissa: Potential of test clamp after activating. Ordinate: Amplitude of current tails. Preparation: Fibre 3, 10^{-5} M Tl^+.

In Fig. 4 (10^{-7} M Tl^+) there was a transient increase in current during the first 20 min and a subsequent return to approx. control values. A more rapid but considerably smaller increase was observed at 10^{-6} and 10^{-5} M Tl^+ (Figs. 5 and 6). The subsequent decrease at these concentrations extended to well below control levels after longer exposure. The time courses of the transient increase and subsequent decrease as well as the absolute

magnitudes of the current voltage relationships are in good agreement with the maximal amplitudes determined during measurement of d_∞.

Comparison of slow inward current and contractile force

Fig. 7 illustrates the time courses of the relative changes of the slow inward current at various levels of thallium. The triangles indicate the percentage change of maximal current tail amplitudes obtained from d_∞ determinations and the percentage change of current tails at test potentials of − 50 mV from measurements of current voltage relationships. In most cases these values were identical but owing to the time lag between one measurement and the other different values were sometimes obtained. It can be seen from Fig. 7 that the initial increase of slow inward current was more pronounced at low thallium concentrations and that the final decrease of the slow inward current was dose dependent. At higher thallium concentrations the maximal transient increase occurred somewhat earlier.

For comparison with the time courses of changes of contractility we chose the data from guinea pig papillary muscles (see the first communication in this series). The effects of thallium on the guinea pig papillary muscles were more homogenous than those on sheep cardiac muslces. Referring to Fig. 7 there is considerable agreement in the time courses of slow inward current and myocardial contractility at all levels of thallium. In particular, the positive transient responses and the final levels are quite similar.

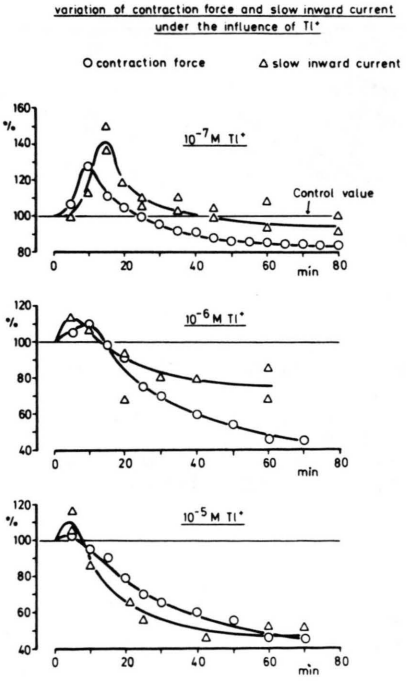

Fig. 7. Comparison of the changes of slow inward current and myocardial contractility under the influence of different thallium concentrations. The slow inward current was determined in sheep cardiac Purkinje fibres at holding and test potential of − 50 mV. Myocardial contractility was obtained in guinea pig papillary muscles and the means of at least five experiments are shown.

Discussion

Fig. 7 illustrates that there is a qualitative agreement between the time course of the slow inward current and the contraction force of ventricular tissues after exposure to thallium. In the case of the inotropic responses this finding confirms the results of action potential recordings reported in the previous paper [2]. Action potentials had shown a slight elevation of plateau accompanied by a rise in active tension. The action potentials during the decline of contractile force did not reveal an opposite effect. Possibly this can be explained because at 10^{-7} and 10^{-6} M Tl^+ the reduction of i_{si} is less than the average loss of contractile force, whereas at 10^{-5} M the time course of i_{si} is practically superimposible on the decline of contractility. This could either mean that the experiment performed at 10^{-5} M Tl^+ revealed too large a loss of i_{si} or that there is a difference in the response of the slow inward current of Purkinje fibres compared to that of ventricular muscle.

In the first communication in this series [1] we reported that the recovery of contractility following periods of rest proceeded in two distinct phases which depended on the beat frequency of the test stimulation and on thallium concentration. In particular, the fast (first) recovery phase supposed to reflect resumed calcium influx, was delayed by thallium at high concentrations and low test stimulus frequency. At high frequency no difference was observed compared to the control. This can be interpreted by assuming that a high beat rates calcium influx is saturated and no longer determines the rate of recovery from quiescency. On the other

hand, at low frequencies small reduction of i_{si} can cause a significant prolongation of the recovery of fibre tension.

The slow (second) recovery phase which follows the initial surge of contractility after quiescency would not depend on i_{si} alone; this can be seen from the converse behaviour of its time constants with regard to stimulation frequency thallium intoxication and also from the loss of contractility at different rates of continuous stimulation. If i_{si} were the only factor determining the loss of contractility, one would expect fibres to be more sensitive to thallium at low rates of continuous beating than at high rates.

If therefore seems most likely that despite the apparent similarity between i_{si} and contractility after intoxication by thallium as illustrated in Fig. 7, there must be another more decisive factor governing the toxicity of thallium to cardiac muscle.

Before attempting to present a formal mechanism which could possibly explain this additional factor, we should like to discuss the question of whether the effects of thallium on the slow inward current are genuine "membrane" effects. As was pointed out in the previous paper [2] the membrane properties determining the fast sodium current and the pacemaker current were not affected by thallium. A specific membrane effect of thallium at concentrations of 10^{-3} M on the electrogenic sodium pump has been discussed [1] but this is not associated with the events at normal toxic thallium levels.

It can be argued for the slow inward current that the fully activated current voltage relationship was not greatly affected in its slope conductance which would reflect a reduction of membrane channels permeable to calcium but rather in the position of its reversal potential. The reversal potential, however, is essentially a feature related to intracellular conditions and not to membrane properties. A negative shift of the reversal potential can be attributed to an increase in intracellular calcium and hence to a reduction of the driving force of the slow inward current. Agents that have been observed to shift the slow inward current reversal potential in the negative direction (dihydroouabain [15] and AR-L 115 BS [16]) show positive inotropism which is compatible to the concept of increased intracellular calcium.

The critical point is that under the conditions of a more negative slow inward current reversal potential indicating increased intracellular calcium, thallium is definitely negative inotropic. This strongly suggests that despite higher intracellular calcium levels the fibre cannot maintain even normal contractile tension. A possible conclusion from this behaviour is that a factor other than intracellular calcium now limits the development of active tension, one that normally is available in a comparative surplus in myocardial tissue.

We have previously suggested that the limiting factor of myocardial contractility shifts from the availability of intracellular calcium to the availability of intracellular energy during the intoxication of thallium. Evidence for this behaviour was provided by the finding that the slow phase of the recovery of myocardial tension was not prolonged at low frequencies of test stimuli but rather at high frequencies. At high constant beating rates the energy balance of the fibres is unfavorable and the fibres are more sensitive to thallium than at low rates. This was confirmed by experiments at constant stimulation at different frequencies. On the other hand a quiescent fibre is also highly susceptible to thallium intoxication. There is no contradiction between the findings within the context of disorted energy balance after exposure to thallium, since the energy supply in a non-stimulated fibre is restricted whereas the energy consumption in a rapidly stimulated fibre is comparatively high.

To interpret the effects of thallium it is feasible to assume a low concentration mechanism in which thallium substitutes for potassium as is known for a number of potassium enzymes [17–20]. This results in an activation of these enzymes and an overall increased output of the cells. In this way the transient positive inotropic responses of myocardial cells can be explained.

On the other hand a high concentration mechanism may be assumed to account for the toxic manifestations of thallium. It is known that mitochondria of cells of a variety of organs (kidney, liver, intestine, brain, seminal vesicles, and pancreas) show swelling after exposure to thallium [21]. This also applies to the axon of peripheral nerve fibres as was shown by Spencer *et al.* [22]. The penetration of mitochondria by thallium seems to proceed via different mechanisms: Melnik *et al.* [23] provided evidence of an active transport different from that of potassium which is followed by the uncoupling of the oxydative phosphorylation. Skuls-

kii *et al.* [24, 25] observed a collapse of diffusion potential due either to increased proton permeability or release of endogenous calcium followed by calcium reuptake (Ca^{2+}-cycling) after penetration of the mitochondria by thallium. A high proton gradient and a potential across the mitochondrial membrane are considered essential factors for the coupling of electrons during oxydative phosphorylation and production of ATP [26, 27]. The findings of Melnik *et al.* [23] and of Skulskii *et al.* [24, 25] strongly support the view that the ATP-production is reduced by thallium although thallium shows no chemical resemblance to classical uncouplers of chemiosmosis such as dicumarol, dinitrophenol, and CCCP which are lipophilic acids that permeate the mitochondrial membrane in both protonated and unprotonated states and thus reduce the proton gradient. Similarly, thallium does not resemble an ionophore which would reduce the membrane potential due to enhanced permeability of sodium, potassium, and protons as do gramicidine, valinomycine, and nigericine.

The cited biochemical observations possibly also apply to cardiac tissue as far as our results are concerned with the restriction that the actual biochemical mechanism by which mitochondrial damage is caused remains uncertain. However, we feel that cellular thallium intoxication and its clinical manifestations cannot be interpreted in terms of any specific membrane effect of thallium.

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