Tl^+-Ions: Effects on the Automaticity of Sinoatrial Tissue and on dV/dt_{max} and i_{K2} of Cardiac Purkinje Fibres

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The disrhythmic effects of thallium were investigated in various cardiac tissues to determine the primary site of intoxication with respect to ensuing arrhythmias. In isolated cardiac tissue Lameijer and van Zwieten [1] had contended that arrhythmias arise from the sinus node after thallium poisoning. To test this hypothesis we administered concentrations of Tl^+ between 10^{-7} and 10^{-4} M to guinea pig sino-atrial preparations, to guinea pig papillary muscles and to sheep cardiac Purkinje fibres. In sino-artial preparations thallium provoked increases and decreases of spontaneous beat frequency which were not linked to corresponding changes in contractile force. In conductive tissue, Purkinje fibres, the inactivation kinetics of the fast sodium current and the pacemaker current i_{K2} were investigated by voltage clamp experiments. Here, thallium was seen to be essentially without toxic effects which could account for arrhythmias. In ventricular muscle actions potentials and contractile force were recorded simultaneously. Here again, ventricular arrhythmias are not to be expected from thallium intoxication in rather high concentrations. The findings support the view that arrhythmogenic effects of thallium are restricted to the sinus node.

Introduction

Numerous clinical reports have shown that after ingestion of Tl^+ a variety of acute disorders evolve in a sequential pattern which is typical of this poison. At first there are severe gastro-intestinal symptoms. Within two to five days neurological disturbances become predominant during the course of which cardiovascular manifestations commence. These are seen to be quite diverse and range from sinus-tachycardia and hypertension to disrhythmias, hypotension and cardiac failure. The victim experiences angina-like pain and shows ECG-abnormalities. It is upon the background of the diversity of the cardiovascular symptoms of thallium intoxication that this study was performed. (For references see the first paper in this series.)

Animal experiments with Tl^+ have revealed a similar multiplicity of cardiovascular events as in humans but they do not fully clarify the mechanisms of Tl^+ intoxication. The information resulting from work on isolated cardiac tissue is mainly restricted to inotropic effects of thallium, and to cellular Tl exchange and has been cited in the previous communication. The data of interest to us in the present study are those concerning the disrhythmic effects of Tl^+-ions in spontaneously beating isolated tissue. Lameijer and van Zwieten [1] showed that in guinea pig atrial preparations thallium decreased heart rate. This effect could be reversed by atropine, cocaine, or considerable changes in external potassium. The authors presume a direct influence on the sinus node. Similarly, Hughes *et al.* [2] observed a steady decay of frequency after administering high doses of thallium and transient accelerations depending on whether the preparations were completely immersed in thallium containing solutions or exposed to thallium during pulse injection into the perfusion fluid.

As yet, these findings on isolated tissues have not been followed up by the corresponding electrophysiological experiments. Thallous ions, however, have been of considerable interest to cardiac electrophysiologists in studying the electrogenic sodium pump. This aspect of electrophysiological work with thallium has also been dealt with in detail in the previous communication in relationship to its possible significance for thallium induced changes of myocardial contractility. However, specific current

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systems known in cardiac tissue have as yet not been investigated at concentrations of thallium encountered during manifestation of the cardiac disorders mentioned above. Our aim in this study is therefore to provide data on the effects of thallium on currents relevant to the function of the heart.

Specifically, we aim to test the hypothesis of Lameijer and van Zwieten [1] that the location of thallium induced disrhythmias may be the sinus node.

To do so, we compared the effects of thallium on sinoatrial tissue to those on ventricular tissue. In sinoatrial tissue of guinea pigs automaticity and contractility were recorded as a function of time and thallium levels. In ventricular tissue guinea pig papillary muscles, sheep Purkinje fibres, and interventricularis cordis muscles were used to record action potentials and effective refractory periods intracellularly in conjunction with changes in contraction force. Finally, dV/dt_{max} and i_{K2} current were assessed using a conventional two-microelectrode voltage-clamp technique. This was done to identify small changes of phases 0 and 4 of action potentials. These can be estimated in action potentials only if rather large, whereas a voltage-clamp technique can reveal small effects which may be of significance for an overall disrhythmic behaviour of hearts exposed to thallium.

From our previous experience with thallium it seemed important to us to employ a relatively delicate technique and to repeat experiments frequently during the course of exposure to low levels of thallium since the effects of thallium have been observed to be rather inconstant. We have not yet successfully applied the two-microelectrode voltage-clamp technique to sinoatrial tissue in order to study the influence of thallium.

Methods

Preparations

In the present study we employed various cardiac tissues suitable for the investigation of cardiac disorders related to chronotropism. For the detection of thallium induced intoxication of the automatic pacemaker, guinea pig sino-atrial preparations were used. Sheep cardiac Pukinje fibres were chosen since they can be considered to represent the properties of conductive tissue and can be imployed for voltage clamp experiments. In conclusion guinea pig papillary muscles were feasable for the recording of ventricular action potentials and refractory periods in conjunction with thallium effects on contractile force.

Guinea pig preparations

Male and female guinea pigs weighing 200 to 500 g were rapidly killed by a blow to the neck and cardiectomized. The beating hearts were bathed in warm (36.5 °C) oxygenated Tyrode solution and rinsed until no further discoloration of the rinsing fluid could be discerned. To obtain papillary muscles the atria were carefully removed and the ventricles opened. Before dissecting the papillary muscles these were ligated with two silk threads which were later used to mount the preparations by insertion into appropriate nooses in the measuring chamber. Sino-atrial preparations were obtained by first removing the left atrium from the dissected combined atria described above. This was followed by a careful inspection of the remaining right atrium to determine the spread of excitation. When one felt confident to have located the approximate origin of excitation a strip of tissue one mm in width and five to six mm long was excised such that the assumed location of the primary pacemaker was contained within the strip. There were two criteria to determine whether this was indeed the case: 1) Activity ceased in the expended tissue and did not recommence. 2) The strip of tissue presumed to contain the sinus node resumed its activity at a rate approximately equal to the former rate of the right atrium. This took up to 10 min after excision.

The procedure of removing a sino-atrial preparation from the right atrium was fraught with the danger of destroying the sinus node completely in which event automaticity ceased immediately. The number of failures could, however, be limited to about 10–15%.

After about 10 min after automaticity had returned the preparations were ligated with silk threads for mounting in the chamber and left at slack for at least another 20 min for complete recovery. The procedure of mounting has been described in detail in the previous communication [3]. Experiments were performed by adjusting the resting length and the resting tension of the prepara-

tion to approx. 110 to 120% of slack length such that constant activity ensued. The control frequency and the resulting control contractile force was kept under close observation for at least 1 h before Tl^+ was added.

Sheep Purkinje fibres

Sheep hearts were obtained from a nearby slaughterhouse (Fa. Kind, Grevenbroich). Immediately after slaughtering, hearts were removed, opened, and rinsed three times in cooled, oxygenated Tyrode solution. They were then transported to the laboratory in fresh Tyrode solution at approx. 6 °C and continuously aerated with 95% O_2 and 5% CO_2. Purkinje fibres were excised from both ventricles within 60 min from time of dead and cut to appropriate lengths between 2 min (i_{K2}) and 6 mm (dV/dt). They were then stored in oxygenated Tyrode at room temperature and were left for at least 1 h for healing over and recovery from dissection before use in the first experiment. To experiment, single fibres were carefully transferred via a pipette to a perspex chamber maintained at 36.5 °C by a thermostated fluid. The preparations in the chamber were continuously perfused with oxygenated Tyrode at a constant flow rate of 2.5 ml/min controlled automatically. After switching to different (test) solutions from other reservoirs the exchange of solution surrounding the preparations was complete after about 3 min.

Solutions

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

Thallium was added to this solution as its sulfate salt (Tl_2SO_4, Merck, pa grade). For preparation of thallium Tyrode solution see the previous paper [3].

Experimental design

Sino-atrial preparations

To determine the automaticity and the contractile force of the sino-atrial preparations the experimental set-up was used as described in Ziskoven *et al.* [3] for sheep interventricularis cordis muscles and guinea pig papillary muscles.

Guinea pig papillary muscles

These were mounted in the manner identical to the sino-atrial preparations and the sheep interventricularis cordis muscles. In addition, they were impaled by a microelectrode at sites of least movement near the end of the muscles in order to record intracellular action potentials.

Sheep cardiac Purkinje fibres

Sheep cardiac Purkinje fibres were used for voltage clamp experiments. The relationship between dV/dt_{max} and prestimulation membrane potential was ascertained by the method of Weidmann [4]. The pacemaker current i_{K2} was studied using the technique of Noble and Tsien [5]. The experimental procedure and the apparatus has been described extensively in former publications from our laboratory [6, 7]. In particular, the intracellular microelectrodes and the electronic devices used were identical to those described previously [6, 7].

Results

Guinea pig sino-atrial preparations

The results of a typical experiment on a guinea pig sinoatrial preparation is demonstrated in Fig. 1. The preparation was kept under control conditions for about 1 h at the end of which the control frequency and contractile force were recorded. Exposure to low concentrations of Tl^+ (10^{-7} M) showed slight changes in frequency and rather more pronounced changes in contractile force. The time course of these changes was recorded at various intervals and is shown in the second panel at 3, 9, and 25 min. After 30 min a higher dose (10^{-6} M) was added. This resulted in a surge in frequency and contractile force after 2 min followed by a subsequent decrease in both parameters. Again, after 30 min 10^{-5} M Tl^+ were added and a further loss of frequency and contractile force was observed.

The experiment shown in Fig. 1 was typical for other experiments in so far as there was no relationship between changes in frequency and contractility. As was confirmed in three other similar experiments no prediction could be made on how the preparations would react. In the case shown in Fig. 1 transient increases of frequency were observed

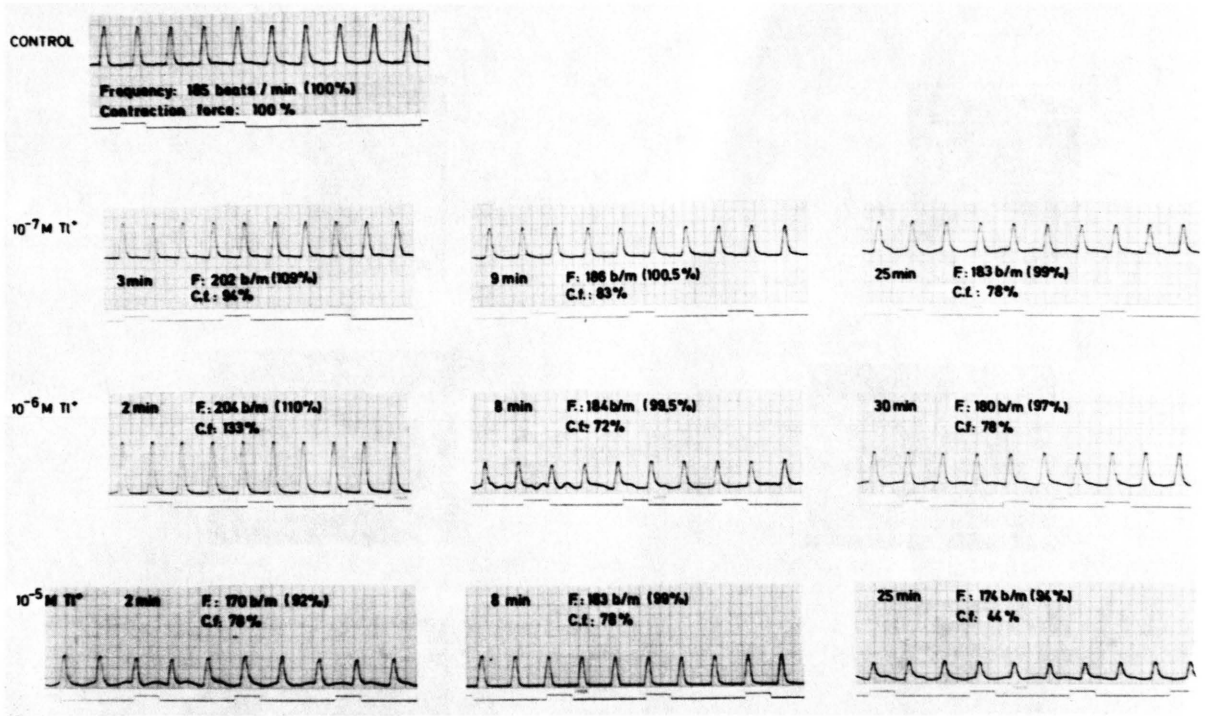

Fig. 1. Original recordings of contractile force of one of four spontaneously beating guinea pig sino-atrial preparations before and after the exposure to various Tl^+ concentrations. The time marker on the recordings indicated seconds. For further explanation see text.

at 10^{-7} and 10^{-6} M. At 10^{-5} M the spontaneous rate decreased but showed a tendency to recover after about 8 min.

Coinciding with previous experiments on guinea pig papillary muscles [3] contractility decayed with time and thallium levels. Only at 10^{-6} M thallium was there a short-lived increase which occurred in approx. 50% of experiments on sheep interventricularis cordis muscles during early exposure periods.

Guinea pig papillary muscles

In guinea pig papillary muscles 10^{-7} M Tl^+ produced a slight increase in contractile force during short term exposure at a stimulation frequency of 0.4 Hz (Fig. 2, upper panel). For long term behaviour see the previous communication [3]. Action potentials were recorded simultaneously in the preparations showing that during the increase in contractile force the conformation of the action potentials was altered in a manner associated with a positive inotropic intervention. Referring to Fig. 2, upper panel, inspection reveals that the plateau of the action potentials was slightly elevated and the action potential duration was shortened. Further details of the relationship between the enhanced levels of the plateau as related to the slow inward current and changes in inotropism after exposure to thallium will be presented in the next communication [8].

At concentrations of 10^{-5} and 10^{-4} M Tl^+ (middle and bottom panels) the contractile force is reduced markedly but the action potentials were not visibly affected. We consider these results to be of interest for the interpretation of the effects of thallium on myocardial tissue. Since the conformation of the action potential was changed during a positive inotropic effect of thallium this may be related to a direct or indirect effect on the cell membrane. Negative inotropism, however, was not related to marked changes of the action potentials; the resting potentials, overshoot, plateau, and repolarization showed no interference by thallium. This clearly

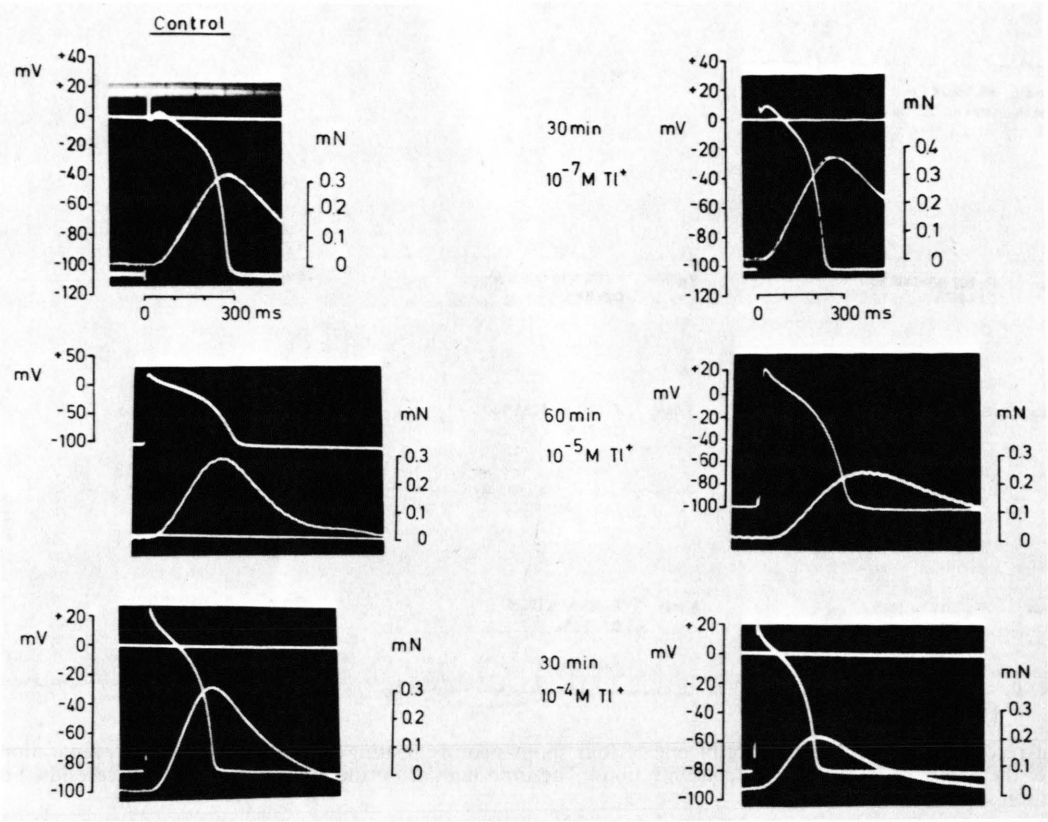

Fig. 2. Original recordings of action potentials and contractile force of guinea pig papillary muscles before and after exposure to various concentrations of Tl^+. The stimulation frequency was 0.4 Hz. The experiments were each performed on individual fibres *i.e.* the control (left column) refer to the corresponding thallium experiment (right column) only.

indicates that the loss of contractility is not accompanied by a deterioration of electrical activity and thus does not seem to be a consequence of membrane effects.

Effective refractory periods were also recorded in guinea pig papillary muscles at comparatively high levels of Tl^+ (10^{-5} M). As shown in Fig. 3 the excitability of ventricular tissue was not affected by thallium. This supports the conclusions drawn above.

Sheep cardiac Purkinje fibres

The influence of Tl^+ on dV/dt_{max}

Measurements of action potentials of various ventricular tissues did not reveal a pronounced effect of thallium. Since it is uncertain that recordings of action potentials would uncover thallium effects of small magnitude which might be significant in the overall behaviour of the intact heart voltage clamp experiments were performed. In the case of the parameters of excitation the upstroke velocity was determined as a function of the membrane potential. Using the classical procedure of Weidmann [4] Purkinje fibres were clamped to a given potential ranging from -110 to -50 mV for 300 ms. Action potentials were elicited 1 ms after termination of the clamp and the upstroke velocity was measured by electronic differentiation. The maximal upstroke velocity dV/dt_{max} was plotted against the prestimulation membrane potential. The resulting sigmoid curves are a measure of the inactivation kinetics and the availability of the fast sodium influx across the cell membrane. Fig. 4 shows the results of a typical voltage clamp experiment performed at a concentration of 10^{-7} M Tl^+. From the time course of the effects of thallium on myocardial contractility we were aware of the fact that the

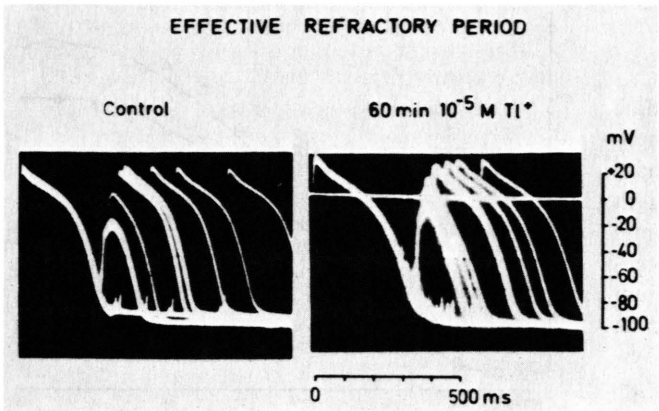

Fig. 3. Recordings of the effective refractory period of control after 60 min exposure to 10^{-5} M Tl^+. There was a slight prolongation of the effective refractory period concommittant with the action potential duration. Like the inactivation of the sodium system, reactivation also did not seem to be affected by thallium.

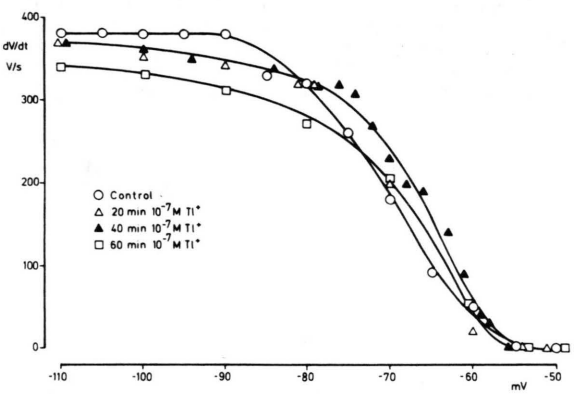

Fig. 4. Plots of maximal rates of rise (dV/dt_{max}) of action potentials of cardiac Purkinje fibres elicited after 300 ms conditioning voltage clamp pulses. Abscissa: membrane potential preceding fast depolarization. Ordinate: maximal rate of rise. Exposure of 10^{-7} M Tl^+ over the periods of time indicated produced some instability of sigmoid curve related to the inactivation kinetic of the fast sodium influx but no consistent or continuous effect. Recordings of complete inactivation curves were repeated in 3 to 4 min intervals for reasons given in the text. Fig. 4 shows those obtained after 20, 40, and 60 min only.

action of thallium could be short-lived and fleeting so that a steady state value of a parameter investigated would not necessarily be attained. We therefore repeated our experiments in 3 to 4 min intervals at all potentials in order to be able to identify transient effects of Tl^+ should they occur. Figs. 4 and 5 show only a fraction of the data obtained during the experiments. However, the curves obtained in the experiments shown in Figs. 4 and 5 (10^{-7} and 10^{-5} M Tl^+) are representative for at least 80 experiments on 6 fibres in that small variations in the behaviour of the sodium system could be discerned. There was, however, no consistent change that could be reproduced on a quantitative scale. In the experiments shown in Fig. 4 the peak dV/dt_{max} values obtained after conditioning pulses negative to -85 mV were reduced by approx. 15%. At more positive potentials there was a slight increase of dV/dt_{max} after 40 min which returned to control values after 60 min. At the higher concentration of 10^{-5} M peak dV/dt_{max} values were not affected although the inactivation curve was shifted to more negative values after 35 min. All in all, fibres tended to respond to Tl^+ by a slight reduction of their sodium system either by loss of peak influx or by a hyperpolarizing shift of its inactivation kinetics.

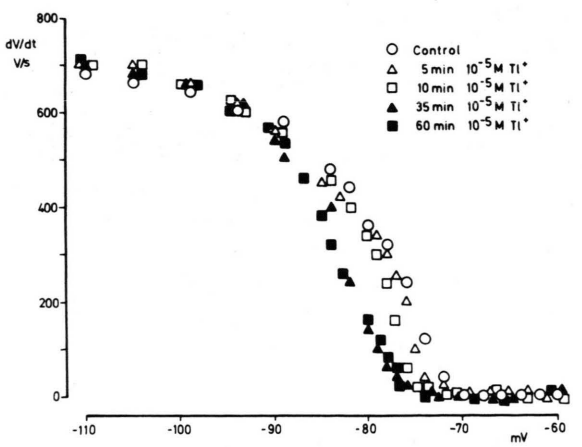

Fig. 5. Sigmoid inactivation curves obtained from sheep cardiac Purkinje fibres after exposure to 10^{-5} M Tl^+ under the conditions specified in the caption of Fig. 4. Here, the effect of thallium is rather that of a hyperpolarizing shift of the inactivation kinetics normally associated with local anaesthetics. Again, the figure illustrates a selection of recordings performed in intervals of 3 to 4 min.

The influence of Tl^+ on the current underlying pacemaker activity, i_{K2}

The so-called pacemaker current, i_{K2}, of cardiac Purkinje fibres is activated during the action potential. Its deactivation after the repolarization phase of the action potential is responsible for the slight depolarization of the membrane from the maximal diastolic potential to either a stabile resting potential or to the threshold potential of the fast sodium current. This current system is partly involved in determining the parameters of the conduction system and also important in various forms of arrhythmias. Due to the known arrhythmogenic potency of thallium is seemed necessary for us to investigate the i_{K2} system carefully in spite of the fact that action potential recordings had not revealed any significant effect after exposure to thallium. However, the same reasoning as for the fast sodium current also applied here, since even small effects may be consequential for cardiac disorders the origin of which are located elsewhere.

According to the theory of Hodgkin and Huxley [9] as adapted by Noble [10] the current underlying pacemaker activity, i_{K2}, is described by two factors:

$$i_{k2} = \overline{i_{K2}} \cdot s$$

where $\overline{i_{K2}}$ is the instantaneous current voltage relationship depending on voltage only and showing inward going rectification with a marked negative slope in the region positive to -70 mV. s is a time dependent dimensionless variable which controls the degree of activation of this current at given potentials.

This current system can be investigated by a voltage clamp program of long (5 to 20 s) test pulses from a holding potential which is chosen in the vicinity of the membrane resting potential. The voltage clamp step to the test potential is accompanied by an instantaneous current jump and a time dependent current tail determined by the time dependent activation or deactivation of i_{K2}. Similarly, after return to the holding potential the current response is composed of an instantaneous current jump and a time dependent current tail. The time dependent currents on the return to the holding potential can be plotted against membrane potential of the test pulse yielding sigmoid curves. For comparison foot points of these curves are normally set to zero so that the amplitudes of the full curves reflect the total available currents. Thus, single curves from different test solutions in one experiment can be compared. The effects of 10^{-7} M Tl^+ after 10 and 100 min of exposure are demonstrated in Fig. 6a. The total current amplitude was reduced by about 18%. This effect was reversed after wash-out.

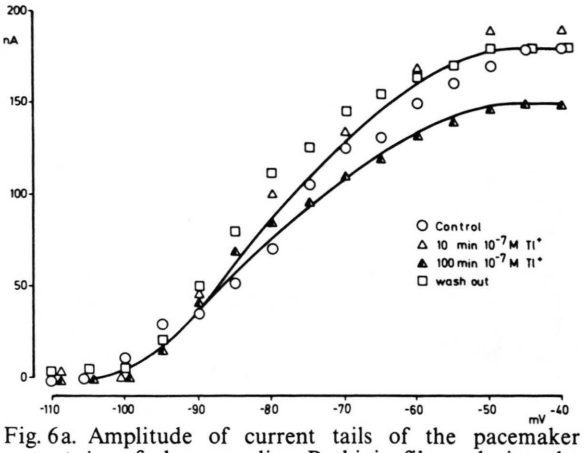

Fig. 6a. Amplitude of current tails of the pacemaker current i_{K2} of sheep cardiac Purkinje fibres during the return clamp to the holding potential of -80 mV. Maximal inward decaying tails were arbitrarily set to zero and outward decaying current tails were augmented by that amount. Exposure to 10^{-7} M Tl^+ produced a slight and reversible diminution of the maximal time dependent current.

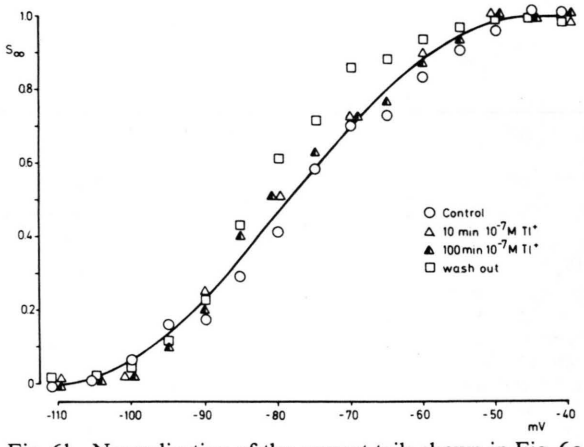

Fig. 6b. Normalization of the current tails shown in Fig. 6a amounting to s_∞. The potential dependence of the activating kinetics of i_{K2} was not affected by exposure to 10^{-7} M Tl^+. The experiments performed after 10 and after 100 min exposure as shown in Fig. 6a and b are representative for the experiments performed at intermediate periods. During wash out a slight negative shift was discerned.

Normalizing these current curves yielded the voltage dependence of the steady state activation

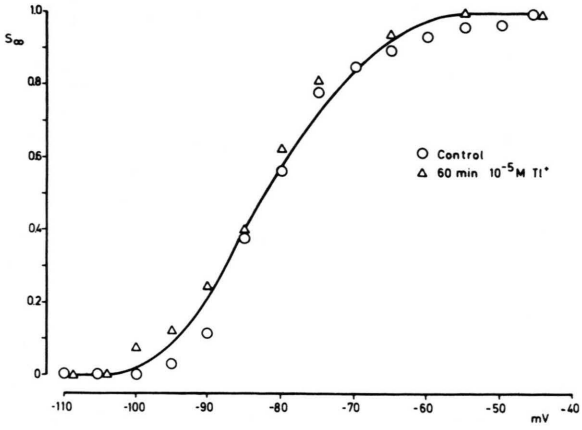

Fig. 7. The effects of thallium at a concentration of 10^{-5} M on the s_∞ kinetics of i_{K2} after 60 min exposure are shown in this figure. At this higher level of thallium the pacemaker current in conductive tissue was essentually not affected by thallium.

parameter s_∞ as is shown in Fig. 6b. As can be seen in this figure Tl^+ did not alter the voltage dependence of the kinetics of the pacemaker current. This could also be illustrated for 10^{-5} M Tl^+ (Fig. 7), at which concentration the total current amplitude was again reduced by about 20%.

The amplitude of the total available current at all potentials of interest, the current voltage relationship of the fully activated current $\overline{i_{K2}}$ was obtained by calculating the rectifier ratio (quotient of the current tails during the test pulses over the current tails after return to the holding potential) and multiplying this ratio with the maximal current at

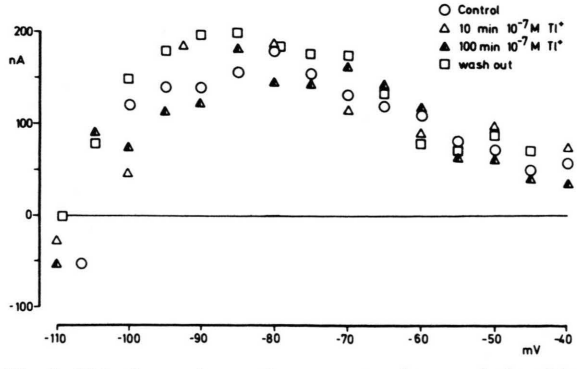

Fig. 8. This figure shows the current voltage relationship of the fully activated pacemaker current i_{K2} before and after administration of 10^{-7} M Tl^+. The reversible depression of maximal i_{K2} reflected the decrease of the time dependent current tails shown in Fig. 6a.

full activation (obtained from the values in Fig. 6a). As an example the experiment at 10^{-7} M Tl^+ is illustrated in Fig. 8. Neither the fully activated current nor the inward rectification nor the i_{K2} reversal potential (intercept of the curves with the voltage axis) were markedly influenced by thallium.

This also applied to the higher concentration of Tl^+ (10^{-5} M). In a total of five voltage clamp experiments it could be seen that Tl^+ did not affect the current underlying pacemaker activity in cardiac Purkinje fibres in a manner which could account for the disrhythmic effects of thallium.

Discussion

From the results of this study the conclusion may be drawn that the major disrhythmic effects of thallium probably occur in the sinus node. In the conductive tissue (Purkinje fibres) as well as in ventricular muscle currents and action potentials did not change significantly. Whilst the parameters of conductance and ventricular excitability should not be expected to be responsible for rhythmic irregularities of their own accord, they may contribute to ECG abnormalities.

With regard to dV/dt_{max} and i_{K2} in Purkinje fibres a comparison with another potassium-like ion, cesium, is interesting. We have shown that cesium also does not affect dV/dt_{max} at high concentrations [11]. However, it is well known that time dependent potassium currents react very sensitively to cesium at high concentrations [12–14]. Similar high doses of thallium cannot be applied to tissue without the additional removal of chloride due to the low solubility of TlCl. Moreover, the high toxicity of thallium to cardiac tissue in general restricts the use of high doses of thallium in order to study specific current systems in lengthy voltage clamp experiments due to the rapid deterioration of the fibres. High thallium is only feasible to study short-term events such as the inactivation of activation of the sodium pump.

We conclude that thallium at levels that are highly toxic to man does not exert an action on the membrane with respect to excitability or ventricular tissue and to the pacemaker current of Purkinje fibres. In sino-atrial tissue there is a pronounced disrhythmic effect of thallium but there is no connection between changes in contraction force and frequency. As yet we have no way of deciding

in which manner possible membrane effects of thallium in sino-atrial tissue may influence contractility and/or frequency. The evidenc however, suggests that frequency changes in the automatic pacemaker are not a result of a direct and specific attack at membrane sites but rather that they are complicated secondary events following the disruption of intracellular processes. This conclusions is drawn mainly from the finding that there is no concentration of thallium at which a discrete and reproducible alteration in sinus nodal activity can be produced.

In the following study data on the slow inward current of cardiac Purkinje fibres will be presented. In some respects this has confirmed the effects reported in sino-atrial tissue here.

We should like to stress that we consider the dissociation of rhythm and contractility observed in sino-atrial tissue, compared to the lack of membrane effects in ventricular tissue, to indicate that disrhythmic events originate solely in the sinus node as proposed by Lameijer and van Zwieten [1]. This conclusion applies to isolated tissues such as have been used by us and by Lameijer and van Zwieten [1]. In "intact" animals so far as this term is applicable after thallium poisoning, severe neurological disorders most likely enhance the thallium induced cardiovascular disturbances.

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