Tl^+-Ions: Influence on Cardiac Contractility

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Attention has recently focussed on the heavy metal thallium as an environmental contaminant of increasing importance. From accidental or suicidal ingestions of thallium it has been known for many years that cardiovascular disorders regularly emerge, and for this reason, a variety of investigations of cardiological interest have been conducted. Amongst these, the effects of thallium on the contractile force of isolated myocardial tissues have been studied. Previous experiments were all carried out at concentrations far beyond those encountered during intoxication and yielded controversal data. We therefore reinvestigated the effects of thallium on myocardial tissue at levels between 10^{-8} and 10^{-3} M, thus covering the range of thallium concentrations encountered after uptake from a polluted environment through those seen after unintentional or intentional ingestion to levels at which previous studies were performed.

Sheep interventricular cordis muscles were used at a stimulation frequency of 0.4 Hz showing three types of responses to thallium exposure. From a total of 32 experiments in 15% of all cases thallium caused a persistant increase in contractility which tended to decrease with time and thallium concentration but always remained greater than the control value.

50% of the experiments showed a progressive loss of contractile force with time and thallium concentration, despite transient increases in contractility which lasted for only 2–5 min after the application of each new thallium concentration. A combination of these types of reaction was observed in the remaining experiments in that at low thallium concentrations myocardial contractility increased considerably but then decreased progressively with time and thallium concentration. Guinea pig papillary muscles were used to test one thallium concentration only for up to 75 min. At 10^{-8} M there was no effect, at 10^{-7}, 10^{-6}, 10^{-5} M Tl^+ there were positive inotropic transients followed by an inotropic decay; at 10^{-4} M Tl^+ only a progressive decrease of contractility was observed.

The relationships between time and thallium concentration at different rates of stimulation were examined in two series of experiments at 0.1, 0.2, 0.4, 1.0, and 2.0 Hz. The effects of thallium were accelerated with increased beating rate and the decay of contraction also proceeded to markedly lower levels. In the rested state, thallium was also very effectual; this was illustrated in two series of experiments in which after 10 min intervals of quiescency 15 or more test stimuli were applied at different beating rates (0.1 to 2.0 Hz). The configurations of the resulting staircase phenomena were analyzed with respect to control behaviour for each frequency of the test stimuli and for each thallium concentration. These results suggested an involvement of the slow inward current. The steady state values after quiescency showed a pronounced thallium-induced decay similar to that obtained at high constant stimulation rates. The effects are discussed in terms of distortion of cellular energy production by thallium.

Introduction

Thallium (I)-compounds have long been known as classical poisons and their toxicology has been described in detail [1–14]. In recent years it has become evident that Thallium (I)-ions are possibly a considerable environmental burden. One of the main industrial sources of thallium contamination are coal – burning power plants which produce an emission of solid fume constituents containing 50–90 ppm thallium [15].

A typical order of magnitude of thallium traces in many plants was determined to be around 0.5 ppm [16]. Uptake of thallium from food, water, and air of the normal human was estimated to be approx. 1.8 µg per day [6]. Weinig and Zink [17] measured the overall human body level of thallium to be approx. 2 ppb and, for example, reported a value of 0.3 ppb for the urine. 10 to 20 ppb have been

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defined as the upper tolerable limit for thallium in human urine [18].

In addition to the encumbrance by thallium from natural and industrial sources, thallium is also used in clinical cardiology in its isotope 201 for myocardial imaging (for further information see [19]).

Tl^+ and K^+ ions have similar ionic radii (Tl^+: 1.44 Å, K^+: 1.33 Å), and thus thallium-ions may be expected to interfere with potassium in biological systems. According to preliminary experiments of Mullins and Moore [20] the rates of influx and efflux of thallium-ions in skeletal muscle in low concentrations are comparable to those of potassium. In particular, "Tl^+ reaches a steady state distribution between fibre water and Ringer solution that is very close to the corresponding ratio for K^+" [20]. Gehring and Hammond [21] showed that in erythrocytes Tl^+ is carried to the interior of the cell by means of the active K^+-transport system. As early as 1967 [22] these authors also presented a detailed study demonstrating further interrelationships between Tl^+ and K^+ as to their metabolism in the whole organism and their effects on membrane transport systems and intracellular enzyme systems. This study [22] was also a first approach to the time dependence of thallium distribution in a variety of organs as was later confirmed by Achenbach *et al.* [23]. Gehring and Hammond [22] showed that Tl^+-ions activate the Na/K-ATPase in a similar manner as do K^+-ions. This observation was confirmed by Skulskii *et al.* [24–26] and Lishko *et al.* [27] for thallium concentrations up to 10^{-3} M. Higher thallium concentrations supposedly block the ouabain sensitive sodium efflux. Since the reports of Landowne [28, 29], Hagiwara *et al.* [30], and Hille [31] it is known that active thallium fluxes are larger than potassium fluxes. From these studies, experimental information became available for the additional passive Tl^+-flux which cannot be blocked by ouabain [26]. The passive Tl^+-flux has also been seen in the smooth muscle [32] and ascites cells [33]. On the basis of the latter studies three pathways for thallium transport across the cell membrane can be distinguished:

1) a ouabain sensitive pump flux
2) a passive transport
3) a furosemide or nitrate sensitive exchange flux.

In all cases the transport of thallium is favored compared to that of potassium, for instance in activating the Na/K-ATPase in rabbit kidney [34], in K^+ activated phosphatase in brain [28, 29], in nerve [30, 31, 35], in skeletal muscle [20, 36, 37], cardiac muscle [9, 10, 36, 38–42], erythrocytes [21, 24–27, 43], ascites cells [33], bacteria [44–48], and mitochondria [49, 50].

In this study we wish to demonstrate the influence of thallium on the contractility of mammalian cardiac tissue. From experiments with frog sartorius muscles Mullins and Moore [20] concluded that "it was considered likely that muscles in 1 mM Tl^+ had undergone some irreversible damage". On the other hand, Rusznyak *et al.* [36] found that Tl^+ reinstated heart activity when administered via a Straub cannula to frog hearts which were arrested by a K^+-free medium. 2 mM Tl^+ were seen to be of equivalent activity as 4 mM K^+, whereas 4 mM Tl^+ damaged the heart pacemaker irreversibly. Hughes *et al.* [9] replaced the potassium content of the perfusion solution by thallium in isolated Langendorff hearts and observed a marked and rapid decrease in heart frequency under these conditions leading to cardiac arrest after about 7 min. Much lower concentrations of Tl^+ ($1-4\times10^{-6}$ M) produced a transient acceleration of heart frequency. Higher doses of Tl^+ depressed the magnitude of contractions, whereas lower concentrations – especially in perfused diaphragm preparations – caused a transient increase of contraction force.

In contrast to these observations, Ku and coworkers [39, 40] reported a marked positive inotropic effect of Tl^+ in guinea pig atrial muscle preparations ranging from a 30% increase in contraction force at 0.25 mM Tl^+ to a rise of 120% at 2 mM Tl^+.

These experiments had been carried out under the assumption that high Tl^+ concentrations block the Na/K ATPase thus leading to an intracellular accumulation of sodium and therefore cause a positive inotropic effect in cardiac muscle fibres via a pathway similar to that assumed for cardiac glycosides.

Methods and Materials

Sheep hearts were obtained from the slaughter house (Fa. Kind, Grevenbroich, FRG) immediately after sacrifice. After the ventricles were opened and rinsed, the hearts were placed in a cooled and

oxygenated solution (about 8 °C). Purkinje fibres (used for voltage clamp measurements) and interventricularis cordis muscles (used for contraction measurements) were cut out of both ventricles not later than 60 min after slaughter and kept in oxygenated bathing solution at about 22 °C. The interventricularis cordis muscle accessible in the right ventricle only was ligated with silk thread at four points approx. 4 mm apart and excised to yield two separate preparations. These preparations, then, consisted of approx. 4 mm contractile tissue and of 2 mm of tissue beyond the points of ligation at each end which did not contribute to contractile force. Muscles of more than approx. 1.2 mm in diameter were usually discarded owing to poor performance and durability. Thinner muscles kept well and after a recovery period during which regular stimuli were applied, stabilized and maintained constant contractile forces for three or more hours. The preparation of guinea pig papillary muscles was essentially identical to the procedure outlined above. Since it was conducted in conjunction with the preparation of sinoatrial strips please refer to the second communication in this series for details.

The experiments were carried out in thermostated (36 °C ± 0.2 °C) perspex chambers described previously, which were modified to allow for contraction measurements [7]. The loose ends of one of the ligation threads were firmly hitched into a notch of a rigid stainless steel wire at one end of the chamber. The other thread was likewise clasped by a similar notch attached to the arm of an induction force transducer (HBM, Q 11/5 P). Both nooses ensured that the preparation was completely immersed in the bathing solution. The distances between the notches and the ligations were around 1 – 2 mm. Two silver stimulation electrodes were gently mounted across the preparation near the immobile end of the active portion of the muscle. The resting length of the muscle could be adjusted by positioning the transducer under microscopic control to allow measurements of isometric twitches at given resting length. Resting tension and twitch tension were registered as a current fed into the transducer to maintain zero position (Hellige 19). Resting tension, twitch tension, and elongation of the muscle were recorded continuously.

Stimuli of 1 ms were applied by the two silver electrodes mentioned above and threshold voltages were determined and checked during experimental work. Stimulation voltages were 15–20% above threshold (4–8 V). Driving rates were maintained at 0.4 Hz throughout except in the experiments at different beat frequencies in which the rates were given in the figures.

The composition of the bath solution was the following: Na^+: 149.16; K^+: 4.0; Ca^{2+}: 1.8; Mg^{2+}: 0.5; Cl^-: 145.5; HCO_3^-: 12.0; $H_2PO_4^-$: 0.36; glucose 15 (mM).

Thallium-containing Tyrode solution was prepared from a stock solution of thalliumsulfate (Tl_2SO_4, Merck, p.A.). The stock solution was prepared by dissolving 0.252 g thalliumsulfate in 5 ml destilled water. This was added to 995 ml Tyrode solution and yielded 10^{-3} M Tl^+ final concentration. Addition of this small amount of destilled water did not seriously affect the ionic composition of the Tyrode solution but greatly facilitated the procedure of dissolving the thallium salt. If given directly to Tyrode deposits of comparatively insoluble TlCl formed on the surface of the Tl_2SO_4 crystals since the solubility product of TlCl was exceeded in the immediate vicinity of the undissolved crystals.

Solution flow through the chamber was controlled by an electrical flowmeter.

Results

After ensuring constant control twitch contractility at optimal elongation by driving the sheep preparation at a constant rate of 0.4 Hz (see Methods) for 30 to 40 min various perfusates containing Tl^+ were applied to the fibres. Doses were increased in intervals of 30 min during which contractility was continuously registered. Beginning at moderately low thallium levels of 10^{-6} M and subsequently increasing thallium doses to 10^{-5} M and 10^{-4} M yielded three distinct types of time-dependent responses in over 30 single experiments.

These are exemplified in Fig. 1 for three individual fibres. In the upper panel of Fig. 1 thallium caused a persistent increase in contractility which tended to decreased with time and thallium concentration. Fibres responding to thallium according to type 1 behaviour (approx. 15% of all fibres) always showed large initial transient contraction surges after exposure to each new thallium level.

In the middle panel of Fig. 1 an experiment is displayed which was typical for the reverse kind of

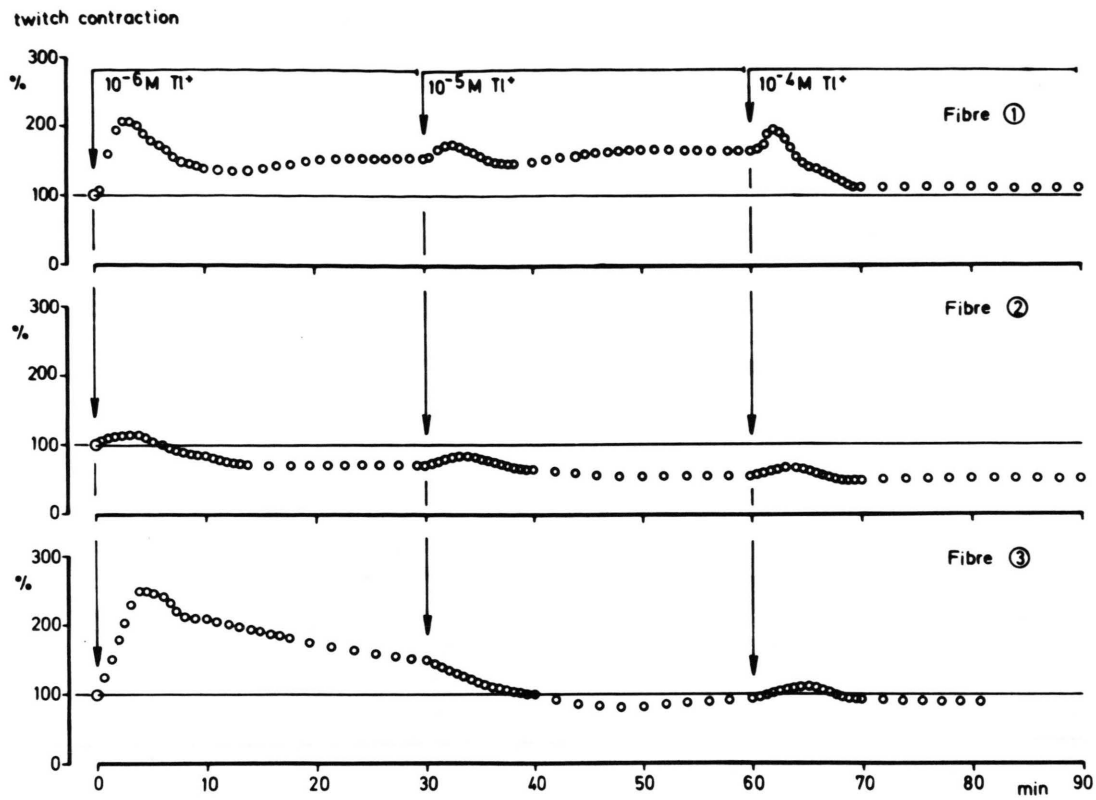

Fig. 1. Twitch contraction measurements of sheep interventricularis cordis muscles exposed to various increasing concentractons of Tl^+. The figure illustrates three typical types of responses to thallium seen in a total of 32 single experiments. Top panel: Transient positive inotropic responses are followed by generally elevated levels of twich tension, which deteriorates only at comparatively high thallium doses. Middle panel: Transient positive inotropic responses are not very pronounced due to an early decay of inotropic force. Lower panel: An apparent combination of the above two types of responses, early inotropism at low thallium levels is large but contractile force decreases with time and thallium concentration. For further information see text.

fibre response to thallium exposure (approx. 50% of the experiments). Here, no sustained positive inotropic effect was noted; after first contact with thallium, small initial increases of contractility were observed but the overall reaction was that of a progressive loss of contractile force with time and thallium concentration.

A combination of these two types of reaction was observed in the remaining experiments: At moderately low thallium concentrations there was a considerable transient increase of myocardial contractility which then rapidly decreased with time and increasing thallium levels (lower panel).

The decline of contractile force following the positive inotropic action of thallium was not uniformly attributable to certain thallium concentrations. There were large individual differences between particular fibres. On the whole, however, one constantly observed transient positive inotropic reactions following the administration of thallium in concentrations up to 10^{-4} M Tl^+. In some cases, such reactions occurred even at 10^{-3} M Tl^+ provided that the fibres were not pretreated with lower thallium levels.

Positive inotropism was in no case a stable event, nor was its transient form reproducable with any reasonable degree of accuracy. In some cases it was practically completely concealed by the rapid onset of inotropic decay. These findings determined from a relatively large number of experiments are in contrast to the results reported by Ku *et al.* [39] who had seen consistent positive inotropic responses in 5

experiments. The reasons for this dissimilarity of data will be treated in detail in the Discussion.

The experiments were repeated on guinea pig papillary muscles using only single doses of thallium applied for 60 min. The number of experiments performed on individual fibres at different thallium levels is specified in the caption of Fig. 2.

At the lowest dose of 10^{-8} M thallium there is no significant effect on the contractile force. Thallium in levels of 10^{-7} and 10^{-6} M caused a slight transient positive inotropic reaction during the first 10 to 20 min followed by a steady decline in contractility. Application of 10^{-5} and 10^{-4} M Tl^+ resulted in an immediately loss of contractile force.

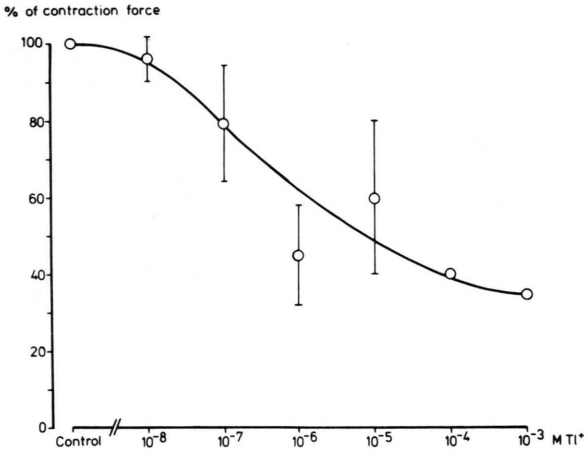

Fig. 2. Dose response relationship of loss of contractility of guinea pig papillary muscles exposed to thallium for 60 min. The numbers of experiments at each Tl^+ concentration were: 10^{-8} M: 3; 10^{-7} M: 9; 10^{-6} M: 6; 10^{-5} M: 6; 10^{-4} M: 2; 10^{-3} M: 1.

As far as the general negative inotropism of thallium is concerned, the effects described for sheep heart preparations were confirmed in guinea pig papillary muscles. However, positive transients appeared only at low concentrations in the latter preparation. The dose-response data are shown in Fig. 2 for the percentage loss of contractile force after 60 min; these data are more homogenous than those of the less sensitive sheep muscles.

As a further point of interest we investigated the extent to which the effects of thallium depend on either resting or active state of the fibres.

Two different kinds of experiments were considered necessary to do so. Firstly, fibres were subjected to continuous constant rates of stimulation during which thallium doses were increased at certain intervals. This procedure was similar to the experiments shown in Fig. 1 the difference being that intervals of 15 min were chosen. Repeat experiments on new fibres at different constant driving rates (between 0.1 and 2.0 Hz) then yielded the frequency-dependence of thallium intoxication (Fig. 3). The effect of the driving rate was confirmed in two experimental series, *i.e.* each driving rate was examined in two individual fibres.

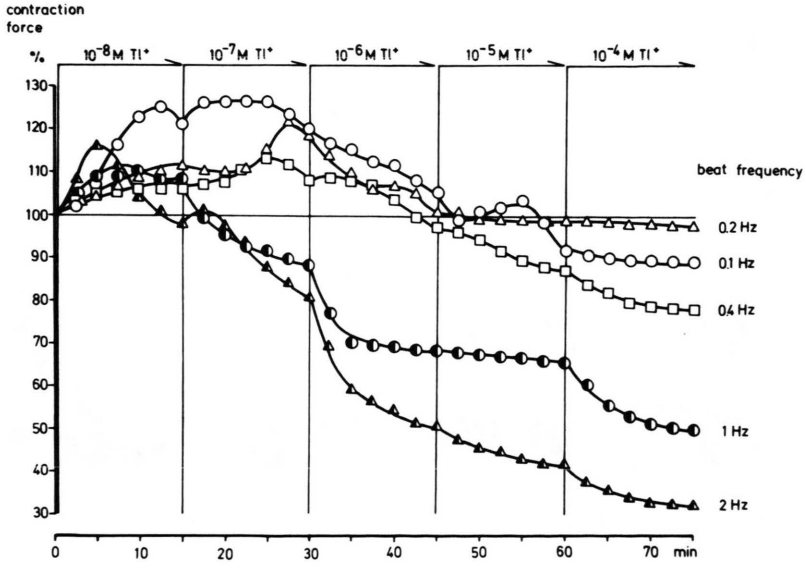

Fig. 3. Effects of time and increasing doses of Tl^+ on the contraction force of sheep interventricularis muscles at different driving rates. The results of each driving rate were confirmed on two to three individual fibres. At constant driving rates the negative inotropic effects of Tl^+ are apparently more pronounced the faster the preparations are stimulated.

One of these series is presented in Fig. 3. Referring to this figure we can clearly see that the decay of contractile force with increasing thallium doses was strongly affected by the heart rate. The time course of toxic events occurring at 0.4 Hz during 15 min intervals of exposure confirmed the results of the experients with 30 min intervals (Fig. 1). In general terms, the results indicated that negative inotropism is favored by increased heart rate, and conversely that the positive transient inotropic effects are considerably more stable at low stimulation frequencies.

Fast but short-lived positive inotropic responses were observed at low thallium concentrations at 2 Hz.

The strong beat-dependence of loss of contractility at steady stimulation rates can be interpreted on the basis of two different assumptions. On the one hand, there might be a work-dependent cellular thallium uptake as the determining factor. On the other hand increased net energy consumption accompanied by a progressive loss of energy production would be work-dependent. Which mechanism predominates can be estimated by comparison with the effect of thallium on the resting muscle ascertained by the following experiments.

The second kind of experiments designed to shed light on the dependence of thallium action on the state of fibre activity focussed on the resting state. Here, fibres exposed to increasing doses of thallium for 20 min remained quiescent during intervals of 10 min each. At the end of these resting periods the fibres were stimulated 15 times. The resulting contraction staircases were frequency dependent, and so the experiments were performed at different test stimulation frequencies (0.1; 0.2; 0.41 1.0; 2.0 Hz), using two fresh preparations for each frequency.

The results of one of these series are shown in Fig. 4. Please note that the controls (first vertical row) refer to experiments on different fibres and therefore correspond only to the experiments in the respective horizontal panel. Before determining the

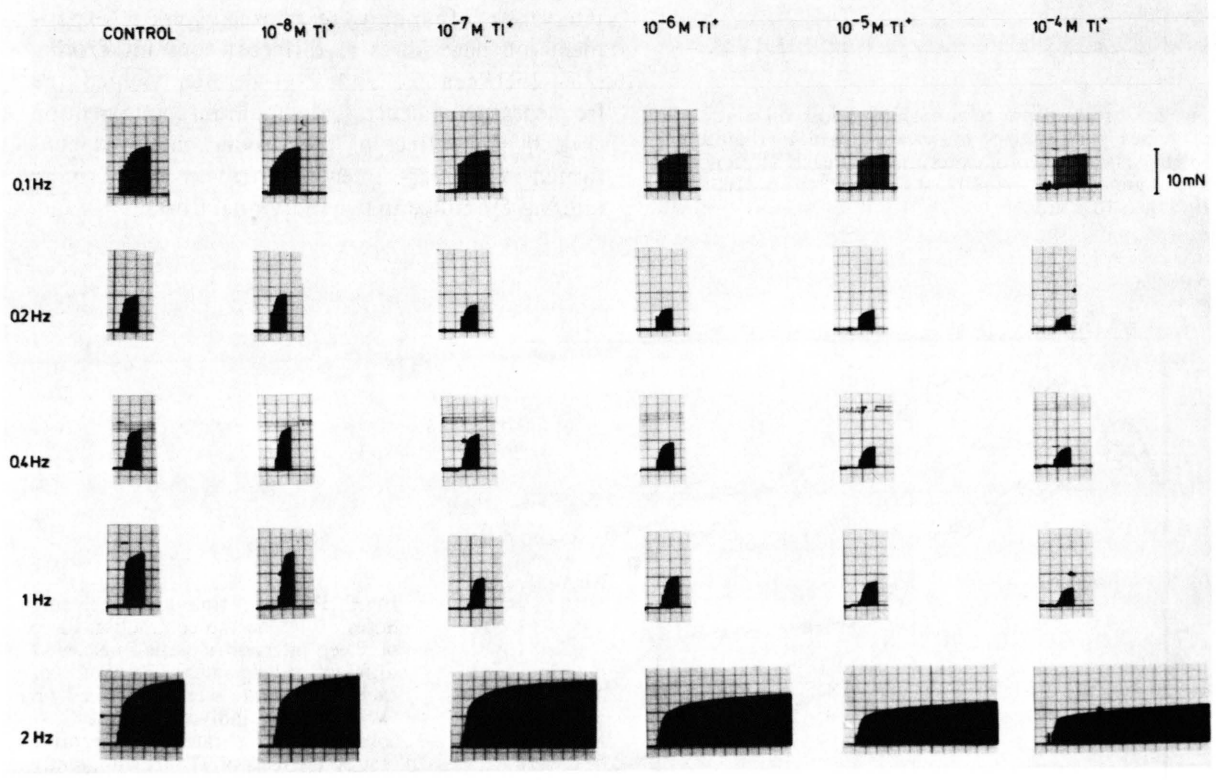

Fig. 4. Staircase phenomena elicited after periods of quiescency (10 min) at the end of 20 min exposure to various increasing concentrations of Tl^+. The frequency indicated at the left refers to stimulation during the staircase. For further details see text.

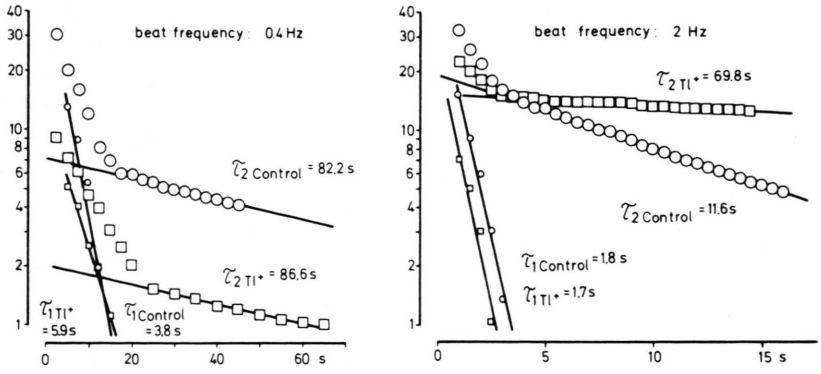

Fig. 5. Evaluation of the staircases shown in Fig. 6 illustrated for 0.4 Hz and 2 Hz under control conditions and after exposure to 10^{-4} M Tl^+. We assume the staircase phenomena to be composed of two distinct time dependent processes, a fast and a slow phase. To determine the corresponding time constants of both processes the semilogarithmic plots of steady state contraction force minus time dependent contraction force versus time were first determined for the slow phase. Subtraction of the slow component during the initial fast phase and plotting the differences semilogarithmically versus time yielded the fast time constant.

thallium and frequency dependence of the staircases, the frequency dependence of staircases under control conditions were established on several individual fibres (not shown).

At low thallium, the maximal tension obtained at the end of the staircase was slightly increased in all stimulatory conditions. Above 10^{-7} M Tl^+, there was a progressive loss of maximal contractility to values of between 60 and 30% of the control. At a test stimulation frequency of 0.1 Hz contractile force was reduced by only 25%. As an overall result, however, these experiments showed that prolonged periods of quiescency did not guard against thallium-induced deterioration of maximal twitch tension.

The loss of rested state contractility at the end of quiescent periods did not depend on the frequency of the test stimuli and was more pronounced than the loss produced during continuous fibre stimulation at low frequencies (see Fig. 3). This finding suggested that, at rest, there is an appreciable thallium uptake, and that beat-induced uptake can only account for a fraction of the total thallium intrusion. This finding is in accord with the results of Skulskii *et al.* [26], Johns [32] and Bakker-Grunwald [33].

Since the decline of tension observed during the experiments on "quiescent" and on rapidly stimulated fibres were comparable, one must assume a certain similarity of conditions in both cases. Given that the beat-dependence of thallium uptake is negligible, this implies that fibres either recovering from quiescency (staircase phenomenon) or subjected to rapid stimulation (tachycardia) are equally susceptible to thallium as far as their energy balance is concerned. The reasons for this assumption will be discussed later in this paper and corroborated with the results of the electrophysiological experiments described in the subsequent communications.

After exposure to thallium, the configurations of the recovery staircases were modified in a frequency-dependent manner. When beating was resumed after periods of rest, two phases could be distinguished during which active tension recovers. At first, there was a comparatively rapid increase which levelled off after approx. 5 beats. Contractile force then inclined gradually to reach steady state values. If the time course of these events was evaluated by the standard procedure of plotting the logarithm of steady-state tension minus time-dependent tension versus time, the time constants of the fast phase (τ_1) and the slow phase (τ_2) could be determined as shown for 0.4 and 2 Hz in 10^{-4} M Tl^+ in Fig. 5.

The relative changes of these (rate-dependent) time constants during thallium intoxication are illustrated by the ratios of τ_1 (control)/τ_1 (thallium) and τ_2 (control)/τ_2 (thallium) respectively. These ratios are presented in Fig. 6 in their relationship to the test stimulation frequency.

At low (10^{-8} M) thallium levels, *i.e.* in range in which thallium action is predominantly positive

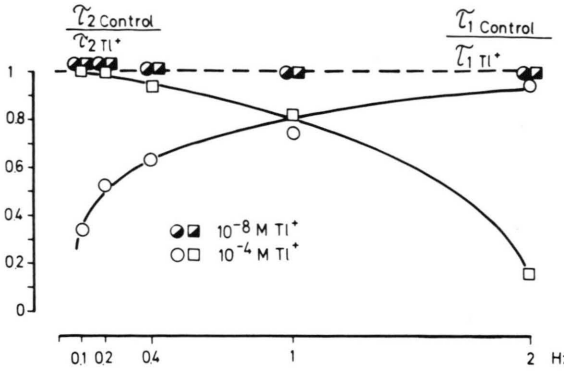

Fig. 6. The ratios of the time constants of control and Tl^+ experiments evaluated according to the procedure outlined in the caption of Fig. 5 are plotted with respect to the frequency of the test stimuli after quiescency. At 10^{-8} M Tl^+ the time constants of both the slow and the fast phases do not differ from those of the control and are not frequency dependent. At high levels of Tl^+ (10^{-4} M) the time constants of the fast phase are increased with respect to the control at low frequencies; at high frequencies the ratio of the time constants is practically 1. Contrary to this behaviour, the time constants of the slow phase are not affected by Tl^+ at low frequencies but rather at high frequencies at which they are considerably prolonged with respect to the control.

inotropic, no effects of thallium on the time constants and their frequency dependence were observed. At high (10^{-4} M) thallium levels, *i.e.* in the range of distinct negative inotropism, the recovery of contractility is delayed ($\tau_{1/2}$ (control)/$\tau_{1/2}$ (thallium) < 1).

The interesting point in Fig. 6 is that the effect of thallium on the recovery time constants during intoxication is largely determined by the rate of stimulation during the test periods. At low stimulation frequencies, the fast recovery phase is more strongly affected than the slow phase. Conversely, at high stimulation frequencies the time constant of the fast recovery phase is practically identical to the control, whereas the slow recovery period is prolonged markedly.

Discussion

In the present paper, the influence of thallous ions on myocardial contractility was studied. In experiments during which sheep interventricularis cordis muscles were exposed to increasing doses of thallium at the constant stimulation frequency of 0.4 Hz, positive inotropic effects were often observed at moderately low doses (10^{-6} M), whereas negative inotropism occured at high doses (10^{-4} M). After first contact with any level of thallium, short lived transient increases in contractility usually resulted. Longer exposure periods commonly favored the loss of contractile force at any thallium level.

Since the time courses and the resulting final levels of the inotropic events produced by thallium could not be foreseen and, in particular, could not be analyzed with respect to their dose response behaviour, the study was extended to other cardiac preparations and to investigations on the influence of the stimulation frequency on contractility after thallium poisoning.

It should be remarked that there are large differences in sensitivity toward thallium intoxication not only for different species but also for different tissues. Similar experiments to ours on sheep and guinea pig preparations are currently in progress on rat preparations (Schüller [51]), showing that positive inotropic effects occur even up to relatively high thallium concentrations (10^{-4} M).

Our findings differ from those of Ku *et al.* (39, 40) obtained at a somewhat lower temperature (30 °C) and for shorter periods of exposure (30 min) yielding consistent and dose-dependent increases of contractility after administration of thallium. Ku *et al.* [39, 40] therefore interpret their results in terms of the inhibition of the Na/K ATPase and base the positive inotropism on a specific membrane effect of thallium which involves the sodium pump. In this context it is the opinion of other investigators (Gehring and Hammond [22], Skulskii *et al.* [24–26], and Lishko *et al.* [27]) that thallous ions are 3 [27] to 10 [24] times more effective in activating the Na/K ATPase than potassium in concentrations below 1 mM. Only at higher concentrations does one find an inactivating effect of thallium. A stimulatory effect of thallium on the Na/K pump has also been demonstrated in the squid axon [29], crab nerve [52], and rabbit vagus nerve [53].

Skulskii *et al.* [25] proposed an "interaction of Tl^+ with the K^+-selective site on the external surface of the membrane" for concentrations of 0.1 to 1 mM, and Hammond and Gehring [22] concluded that the "activation of Na/K-activated adenosine triphosphatase by the substitution of thallium for potassium supports the belief the mechanism involved in the active transport of potassium cannot differentiate between thallium and potassium". For

higher concentrations of thallium Skulskii *et al.* [25] stated: "Competition between the internal Na^+ and rapidly penetrating thallous ions at the inner Na^+-specific binding sites of the erythrocyte membrane could account for the inhibitory effect of Tl^+". Cavieres *et al.* [43] reported an inhibition of the ouabain sensitive K^+-influx in erythrocytes by Tl^+ in high Na^+-media, whereas Ku *et al.* [40] observed a reduced inhibition by Tl^+ in high Na^+.

Insofar as the effect of thallium on the Na/K exchange pump were estimated from the electrical activity of excitable tissues, there is agreement that thallium activates the pump after it has been inactivated. Appropriate measures to do so are for instance the reduction of external potassium (K_0). Early experiments of Rang and Ritchie [53] on the post-tetanic hyperpolarizations in mammalian non-myelinated nerve fibres showed that Tl^+ is three times as effective as K^+ in stimulating the pump. This was confirmed by Eisner and Lederer [54, 55] using the effects of depleted K_0 on contractile behaviour and membrane potentials and currents of sheep cardiac Purkinje fibres. A somewhat smaller reactivating effect of thallium in depleted K_0 (1.5 times that of potassium) had previously been reported by the same group [56]. Similarly, Den Hertog [57] found thallium to be only about 1.5 times as effective as potassium as assayed from hyperpolarizing responses following bathing of desheathed vagus nerves of rabbits in K_0-free Locke solution. Hyperpolarizing (K-like) responses of membrane potential were also seen in rat diaphragm muscles treated with thallium after K^+-depletion (Den Hertog and Mooij [58]) and were between 14% and 55% larger than in equimolar potassium solutions. Using the same experimental protocol as Den Hertog [57], Smith [35] reported larger effects of thallium on electrogenic responses of rat desheathed vagus nerve (three times that of potassium). The inhibitory effect of thallium (and potassium readmission) on the spontaneous activity of rabbit myometrium bathed in potassium and chloride-free Krebs-Henseleit-type solution was interpreted by Johns [32] in terms of Tl^+-being "about twice as potent as potassium in turning on the electrogenic pump". In experiments on human trabeculae of young non-pretreated patients submitted to open heart surgery, Christé observed that the "effects of low (0–0.5 mM) K_0 were readily reversed by addition of 2 mM TlCl". Similarly, Rusznyak *et al.* [36] found that isolated frog hearts arrested by washing with zero potassium could be restarted by a solution containing 2 mM $TlNO_3$ or 4 mM K^+ and subsequently re-arrested reversibly by 4 mM of thallium and 12 mM potassium.

Hence, the view held by Ku *et al.* [39, 40] that the biochemically assayed inhibition of the Na/K activated ATPase be explanatory for the positive inotropism observed by them is not in contrast to the findings of other investigators provided one assumes that thallium inactivates the already fully activated pump (in normal K_0) whilst the fully or partially inactivated pump (*e.g.* in low K_0) is stimulated by thallium. Eisner and Lederer [54], for instance have attributed the reduction of twitch tension of Purkinje fibres bathed in 1 mM thallium compared to the tension in zero potassium to an activation of the pump by thallium.

In the investigation cited above, the experimental conditions of high (millimolar) thallium concentrations and short to very short exposure periods were used throughout. Under these circumstances the concept of relating the inotropic effects observed to what is confidently assumed to be the corresponding effect of thallium on the Na/K mediated pump is certainly tenable. This model, which was originally proposed for the inotropism of digitalis, however, fails to explain the effects of long-term exposure to low and moderate levels of thallium as are encountered during intoxication. Moreover, the complex time course of inotropic events following thallium administration (as described here and in pharmacological and toxicological reports) eludes description by this model completely. In particular, a consistent dose dependent inotropic action of thallium is at best negative (cf. Fig. 2), preceeded by transient positive responses in many cases. In the framework of the model, positive transients and positive inotropy at low levels followed by negative inotropy at high levels imply that thallium first inhibits the pump and then stimulates it. This is clearly incompatible with the provision of the model that stimulation of the pump take place at external sites and inhibition at internal sites. From the model one would expect thallium first to reduce contractile force and, after thallium has entered the cell, eventually to increase it. This type of behaviour was not observed, nor has it been reported.

We therefore feel that low level effects of thallium are not associated specifically to changes of

membrane activity but rather secondary events following intracellular modifications. Thallium ions are able to cross the cell membrane rapidly and then interfere with many enzymatic processes in which potassium is involved. These include the Na/K ATPase (see *e.g.* Grisham *et al.* [60] for location of cation binding site) and adsorption to sites in the A-band normally occupied by K^+ (Ling [37]). Other enzymes affected by thallium are: pyruvate kinase, homoserine dehydrogenase, AMP deaminase, vitamin B_{12}-dependent dioledehydrogenase, and l-threonine dehydratase (cited from Sabbioni and Manzo [6]). On the whole the available biochemical data confirm the interpretation of the effects of thallium presented here in many respects.

In the experiments on the influence of fibre activity (rate dependence) on the action of thallium, we found that increased activity accelerated the deterioration of contractile force. By comparison with the decay of tension during periods of rest we could exclude that beat dependent intrusion of thallium is responsible for this effect.

Inspection of the time constants of the staircase phenomena elicited after periods of rest revealed that the fast and the slow phases of contractile restoration were modified by thallium in a frequency-dependent manner. The two phases apparent in staircases are consistent with literature data ([61, Fig. 1, 3, and 5). Schulze [62] evaluated the time constants quantitatively after changes in stimulation frequency and obtained values very similar to ours.

At low concentrations of thallium (10^{-8} M), *i.e.* in the region of positive inotropism, the time constants are identical to the control. This implies a functional integrity of the cell and the positive inotropic effect of thallium is possibly due to a slight activating effect of intracellular enzymes by thallium. The fact that thallium did not alter the resting tension and that no contractures were observed nor had they been reported supports the idea that the Na/K pump is not involved.

At high levels of thallium which induce progressive negative inotropy, the rate-dependent changes of the time constants are typical for the phases of the staircases. During the initial fast phase the recovery of contractility ensues largely from readmission of calcium to labile calcium stores (called "SR_l" by Allen *et al.* [63]) via the slow inward current. At high beating rates to restitute contractility after rest (2 Hz), the intermittant intervals of calcium reextrusion are short so that the slight reduction of i_{si} encountered at high thallium levels (see last paper in this series) is of no account for the calcium accumulation. Therefore, τ_1(Tl) is equal to τ_1 (control). At low rates of stimulation (0.1 to 0.4 Hz) during the test period the dual effect of reduced calcium influx and of larger intervals of calcium extrusion results in a slower recovery of contractile force and hence τ_1 (Tl) is larger than τ_1 (control). Moreover, the Na/Ca exchange pump is driven by the sodium gradient and not by metabolic processes [64], and particularly metabolic inhibitions (such as dinitrophenol) promote calcium extrusion, so this may be the reason that larger beat intervals delay recovery during the fast phase.

In contrast to the time constants of the fast phase, the slow phase of the stairchases is not affected by thallium at slow rates and greatly delayed at high rates of test stimuli. During restitution of contractility to final steady state values, the limiting factor for the height of twitch tension is not calcium intrusion, but rather the functional equilibrium of the contractile proteins with regard to their energy supply. On the assumption that thallium affects the energy supply, it is not surprising that, at low rates and low energy demand, this equilibrium is attained with comparatively little delay and τ_2 (Tl) is equal τ_2 (control). Conversely at high rates, the energy demand is increased, steady state levels are reached later and hence $\tau_2(\text{Tl}) > \tau_2(\text{control})$.

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