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RELATIONSHIP BETWEEN THEOPHYLLINE UPTAKE AND INOTROPIC EFFECT IN THE GUINEA-PIG HEART

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1 The time course of the positive inotropic effect of theophylline was compared with the time course of the uptake and release of [^{3}H]-theophylline in guinea-pig isolated, electrically driven hearts perfused by the Langendorff method.

2 Formation of theophylline metabolites could not be detected under the experimental conditions used.

3 Theophylline entered myocardial tissue very rapidly in two different phases. The first process (half-time 21 s) amounted to 93% and the second (half-time 5 min 50 s) to 7% of the total uptake. The development of the positive inotropic effect of theophylline was about four times faster than even the rapid component of the uptake of the drug into the myocardium.

4 The amount of theophylline accumulated in myocardial tissue (after 10 min perfusion) increased proportionally with theophylline concentrations in the perfusion media and no signs of saturation were detected. The tissue-medium ratio did not exceed 1. The water content of the myocardial tissue amounted to about 80% at all theophylline concentrations examined.

5 The uptake of theophylline (3 mg/ml) was diminished by 9.2% after pretreatment of the hearts with caffeine (1 mg/ml). Theophylline uptake was also decreased by 13.5% when caffeine-pretreated hearts were perfused with a solution containing theophylline (300 μg/ml) plus caffeine (1 mg/ml).

6 Theophylline release from the hearts was also very rapid. The efflux curve was composed of three components (half-times: 24 s; 1 min 24 s; 6 min 18 seconds). The intercepts of the linear portions of the efflux curve occurred at 61%, 38% and 1%, respectively. Contractile force and theophylline content in myocardial tissue declined in a similar manner.

7 It is concluded that theophylline enters myocardial tissue very rapidly by passive diffusion. Theophylline distributes itself in the heart as freely as in the perfusion medium. A very small amount may be bound within the cell in a relatively specific way.

8 It seems possible that the positive inotropic effect of theophylline is partly due to an action of the drug on intracellular calcium binding or storage sites. However, the principal action of theophylline is assumed to be on the sarcolemma where it increases calcium influx from the extracellular space. This conclusion is based on the fact that the time courses of the increase in contractile force and of theophylline uptake into the cell were dissimilar.

Introduction

Previous studies on mammalian isolated heart muscle preparations have shown that the theophylline-induced augmentation of myocardial twitch tension is not modified by previous treatment with reserpine (Scholz & de Yazikof, 1971) and is associated with concomitant increases in the rate of ^{45}Ca exchange (Scholz, 1971a) and in the slow calcium inward current during the cardiac action potential (Scholz, 1971b). From these results it has been suggested (Scholz, 1971a, b) that the positive inotropic effect of theophylline in mammalian cardiac muscle is similar to that of, but is not mediated by, catecholamines and is mainly due to an enhancement in the calcium permeability of the sarcolemma, leading to an enhanced uptake of extracellular calcium during the excitation process. However, theophylline and other methylxanthines have also been found to cause a release of calcium from, and an inhibition of calcium sequestration by, sarcoplasmic reticulum preparations isolated from cardiac and skeletal muscle *in vitro* (Weber &

Herz, 1968; Weber, 1968; Fuchs, 1969; Pretorius, Pohl, Smithen & Inesi, 1969; Johnson & Inesi, 1969; Ogawa, 1970; Taniguchi & Nagai, 1970). These findings led several authors, e.g. Blinks, Olson, Jewell & Braveny (1972) and Nayler (1973) to assume that not only changes in surface membrane permeability for calcium but also interference with intracellular calcium binding and storage sites could be part of the events leading to the positive inotropic effect of theophylline in cardiac muscle. Such an intracellular action of theophylline would imply penetration of the drug into the myocardial cell and penetration should have a time course similar to the very rapid development of the positive inotropic effect. The present study was designed to obtain further information on these points. [^{3}H]-theophylline was used to investigate whether theophylline enters myocardial tissue and whether the kinetics of theophylline uptake and release are correlated to the time course of the positive inotropic effect of the drug.

Methods

The experiments were performed on electrically stimulated (frequency 3 Hz) guinea-pig hearts perfused by the Langendorff technique. The animals (either sex, weight 380-450 g) were kept on a standardized diet of Altromin and water *ad libitum*. They were injected intraperitoneally with heparin (20 mg/kg) 1 h before the experiments, killed by a sharp blow on the head and bled from the carotid arteries. The hearts were rapidly removed, trimmed and cannulated in oxygenated Tyrode solution at room temperature. They were mounted on a double-barrelled perfusion apparatus and perfused through the aorta at a constant perfusion pressure of 60 cm of water. A two-way tap system above the aortic cannula allowed rapid changes (within 0.75 s) to be made between a saline perfusion medium and the test medium containing [^{3}H]-theophylline. The Tyrode solution used (composition (mM): NaCl 136.9, KCl 5.4, $MgCl_2$ 1.05, NaH_2PO_4 0.42, $NaHCO_3$ 11.9, $CaCl_2$ 0.9, glucose 5.5; pH 7.4, temperature 35° C) was continuously gassed with 95% O_2 and 5% CO_2. All perfusion fluids were filtered before each experiment through a glass filter (Schott 25 D 3, pore size 15-40 μm).

After the dissection all preparations were equilibrated for 20-30 min in Tyrode solution. Constant electrical stimulation during the equilibration and the subsequent experimental periods was with rectangular pulses (Grass stimulator S6; frequency 3 Hz; duration 3 ms; intensity twice threshold value) by means of platinum stimulating electrodes implanted in each atrium. Since propranolol (10^{-6} M) did not decrease the contractile force, it can be assumed that liberation of endogenous catecholamines, if any, was minimal under these conditions of stimulation.

Mechanical, coronary flow and analytical measurements were performed on the same preparations. Development of tension in the right ventricle was recorded isometrically according to Beckett (1970) with a force-displacement transducer (Grass Ft 03) on a Hellige Helco Scriptor recorder. Ventricular diastolic tension was adjusted to, and maintained at, 5 grams. Coronary flow was observed at 1 min intervals by measuring the effluent from the inferior vena cava with a graduated cylinder.

[^{3}H]-theophylline uptake

After equilibration the hearts were perfused for the desired periods with Tyrode solution containing [^{3}H]-theophylline (0.05 μCi/ml) and various concentrations of non-radioactive theophylline. At the end of the uptake period four sections (40-60 mg each) were cut from each ventricle (left atria were also employed for the experiments with various concentrations of theophylline) and dipped three times for 2 s in theophylline-free Tyrode solution to remove superficially adherent radioactivity. The tissue samples were then blotted on ashless filter paper for 90 s under constant pressure (280 g), transferred into Packard scintillation counting glass vials, weighed (wet weight; wet wt.), dried for 1.5 h at 100° C, reweighed (dry weight; d.w.) and solubilized with 1 ml of Soluen TM 100 (Packard) at 60° C overnight. To the resulting clear yellow solution 1 ml of 1 N HCl and 10 ml of liquid scintillator (Unisolve 1, Koch-Light Laborat.) were added. The perfusion media (0.1 ml portions) were prepared in the same manner in each experiment. The radioactivity of the samples was measured in a Packard Tri-Carb liquid scintillation spectrometer (model 3380) after storage for 2 h in the dark at 4° C. All counts were corrected for quenching by external standardization. Adequacy of this correction was checked by the use of an internal standard in occasional samples. The measured radioactivity (d/min) was related to the tissue wet weight, the radioactivity of the loading solution and to the applied theophylline concentration. Thus, theophylline uptake could be expressed as μg/100 mg wet tissue weight. The tissue-medium ratio was calculated from

$$\frac{\mu g \text{ theophylline}/100 \text{ mg tissue (wet wt)}}{\mu g \text{ theophylline}/0.1 \text{ ml perfusion fluid}}$$

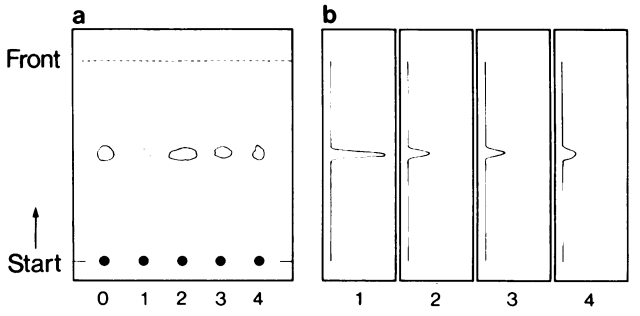

Fig. 1 (a) Thin layer chromatography on a silica gel plate (DC-Alufolie, Kieselgel F_{254}, Merck) to compare non-radioactive theophylline with various [^{3}H]-theophylline samples. 0 = non-radioactive theophylline; 1 = authentic [^{3}H]-theophylline; 2 = Tyrode solution containing radioactive theophylline before heart perfusion; 3 = Tyrode solution containing radioactive theophylline after heart perfusion; 4 = homogenate of a heart previously perfused with [^{3}H]-theophylline Tyrode solution. (b) Radiochromatographical analysis of thin layer chromatograms cut into strips. Same samples and symbols as in (a).

The water content of the tissue ($[H_2O]_T$) was calculated from the difference between wet weight and dry weight of each preparation.

[^{3}H]-theophylline release

The hearts were perfused for 15 min with Tyrode solution for equilibration. They were then exposed to radioactive medium (0.9 mM Ca^{++}; 100 μg/ml theophylline; 0.05 μCi/ml [^{3}H]-theophylline) for 10 minutes. The perfusion fluid was changed to theophylline-free Tyrode solution for 20 min and the perfusate was collected at 1 min intervals. The time course of the decline of tissue radioactivity was calculated as follows. [^{3}H]-theophylline activity of the perfusate samples and the residual radioactivity of the ventricles were determined as described above. From the remaining activity in muscle and from the amount of activity lost from the muscle into each sample of rinsing solution the tissue radioactivity during each collecting period could be calculated. To obtain the total radioactivity in muscle at the beginning of the washout period the tissue activity during the first minute was corrected for the volume of the cannula (2.3 ml) and of the coronary vessels (11.1% of the coronary flow during the first minute; Krebs, 1970). Then tissue radioactivity during the efflux period was expressed as % of the initial tissue radioactivity which was taken as 100%.

Materials

Theophylline (mol. wt., 180.17) and caffeine (mol. wt. 194.2) were purchased from Boehringer Sohn, Ingelheim. Both drugs were dissolved directly in the perfusion media by warming carefully (not over 60°C). Theophylline-[^{3}H] (G) (specific activity 1.28 Ci/mmol; radiochemical purity 98%) was obtained from The Radiochemical Centre, Amersham, England. It was generally labelled with tritium in the 8-position of the purine ring and was supplied in distilled water.

Several experiments were performed to demonstrate that the counted tritium represented [^{3}H]-theophylline and not possible radioactive metabolites of the drug or tritiated water. First, authentic [^{3}H]-theophylline solution, radioactive medium before and after the heart perfusion, a 0°C saline homogenate (Ultraturrax homogenizer, Janke & Kunkel) of hearts previously perfused with Tyrode solution containing [^{3}H]-theophylline, and non-radioactive theophylline were compared by thin layer chromatography on silica gel plates (DC-Alufolie, Kieselgel F_{254}, Merck) in chloroform, ethanol and formic acid (85 : 5 : 10). After development and drying at room temperature, the theophylline spots were visualized with Dische's diazoreagent (Figure 1a). The dried chromatograms were then cut into strips and were also examined on a Packard Radiochromatogram scanning system (Figure 1b). It was found that chromatography of all samples yielded single coloured spots with the same R_F-values and the radiochromatographical analysis also yielded identical radioactive peaks.

The content of tritiated water (T_2O) in muscles and perfusion solutions was determined by drying and distilling, respectively. The average radioactivity loss of dried muscles amounted to 2.3% ($n = 16$). T_2O in the perfusion medium distilled

under reduced pressure at 34°C amounted to 0.51% (n = 7).

Finally, the specific activity of the theophylline contained in the hearts was determined, i.e. the radiochemically measured theophylline content of the tissue was compared to the amount of theophylline determined by chemical analysis. The chemical determination was performed according to Strubelt, Steffen & Stutz (1970). It was found that 94.4% (n = 6) of the radioactive theophylline measured was determined by chemical analysis. Thus, at most 5.6% of the counted radioactivity was not [^{3}H]-theophylline and this was disregarded.

Evaluation of results and statistical analysis

The time courses of theophylline uptake and release and of the build-up of the theophylline-induced increase in systolic tension above the predrug level (positive inotropic effect) were calculated according to Rescigno & Segre (1966). Uptake and inotropic effect build-up curves were analyzed by plotting 100 minus the fraction of maximum values (= 100%) against time on a semilogarithmic graph. The time to peak inotropic effect was measured in each experiment; in the case of theophylline uptake, the mean 20 min value was regarded as maximal. The resulting curves were separated into components by the usual peeling technique. The half-time of each exponential component was derived graphically from the linear portions of the curves. Analysis of the theophylline release curve was performed in the same manner, the theophylline content of the hearts at the beginning of the washing period being regarded as 100%.

All experimental values are given as means with s.e. mean. In the graphs without s.e. mean, the latter was smaller than the size of the symbols. Statistical comparisons were performed with Student's t-test. A P value of less than 0.05 was considered significant.

Results

Time course experiments

The time course of the effect of theophylline on contractile force and on coronary flow and the time course of theophylline uptake were studied at a theophylline concentration of 100 μg/ml (5.55×10^{-4} M).

The positive inotropic effect of theophylline (100 μg/ml) began immediately after drug addition, reached a maximum after 49.7 ± 2.35 s (n = 35) and then declined gradually (Figure 2). At

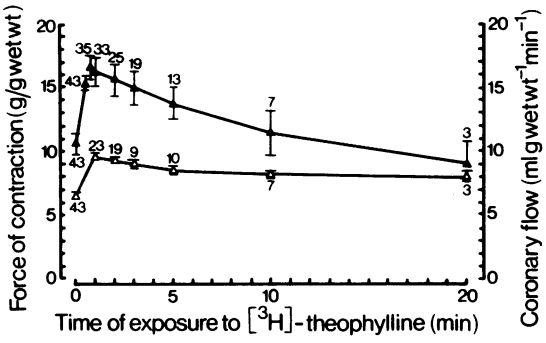

Fig. 2 Effect of theophylline (100 μg/ml) on contractile force (▲) and coronary flow (△) of guinea-pig isolated perfused hearts. Ordinates: contractile force (g/g wet wt) and coronary flow (ml g wet wt^{-1} min^{-1}). Drug addition at zero time. n = 3 to 43 (numbers beside the symbols).

the end of the 20 min theophylline perfusion the positive inotropic effect had disappeared. Coronary flow also increased to a maximum within the first minute of drug perfusion and then decreased up to the fifth minute; afterwards it remained at about 25% above the predrug level as long as theophylline was infused (Figure 2).

[^{3}H]-theophylline uptake was studied in the same experiments. The results are summarized in Figure 3. Theophylline uptake obviously proceeded very rapidly. After 3 min perfusion, the theophylline content of the hearts already amounted to more than 95% of the final 20 min level (Figure 3a). The analysis of the uptake curve (Fig. 3b) yielded two different components: 93% of the total theophylline uptake occurred during the first phase (half-time 21 s) whereas the half-time of the second process which amounted to 7% of the total uptake was 5 min 50 seconds.

The distribution of theophylline between perfusion medium and myocardial tissue can be seen from the tissue-medium ratios summarized in Table 1. The ratio increased in parallel with the uptake described above and reached a maximum of 1.0 towards the end of the 20 min period of theophylline infusion. Under steady-state conditions, the concentration of theophylline in the tissue thus corresponded to that in the perfusion medium. The effect of theophylline on the water content of the tissue is also shown in Table 1. The water content amounted to approximately 80% of the wet tissue weight and remained unchanged during the different loading periods.

In Fig. 4, the rate of theophylline uptake during the first, i.e. most rapid phase (curve b) was compared with the time course of the

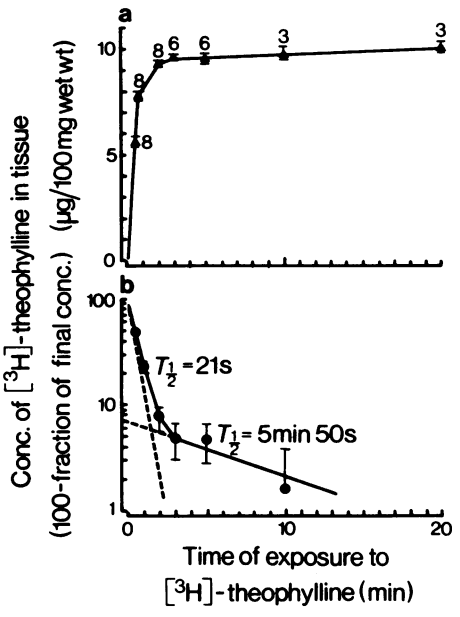

Fig. 3 (a) Time course of [^{3}H]-theophylline uptake in guinea-pig isolated perfused hearts. Ordinates: concentration of [^{3}H]-theophylline in myocardial tissue (µg/100 mg wet wt). Abscissae: perfusion time (min). n = 3 to 8 (numbers beside the symbols). (b) Analysis of the [^{3}H]-theophylline uptake curve. The data from Fig. 3a are replotted on semi-logarithmic coordinates. The mean 20 min value (final concentration) was taken as 100%. Each point was expressed as percentage of, and subsequently subtracted from, the final value. $T_{½}$ = half-time.

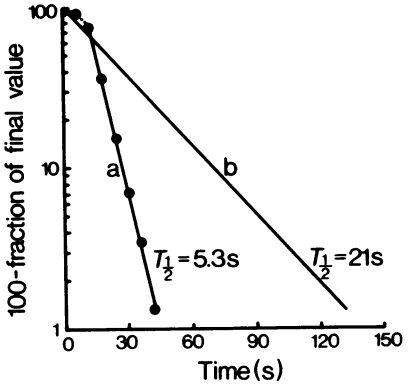

Fig. 4 Time course of [^{3}H]-theophylline uptake (curve b) as compared with the time course of the development of the theophylline-induced positive inotropic effect (curve a). Curve b represents the fast component of the theophylline uptake curve shown in Figure 3b. In curve a, the values were measured at 6 s intervals and subtracted from the peak inotropic effect (= 100%); the points represent the means of 12 experiments. $T_{½}$ = half-time.

development of the positive inotropic effect of the drug (curve a). Curve b of Fig. 4 was taken from Figure 3b. The development of the inotropic effect was measured at 6 s intervals and it was found that the half-time of the development of the positive inotropic effect was 5.3 (4.2-6.5) s whereas the half-time of the first component of the theophylline uptake was 21 s as already stated. Under these conditions, the positive inotropic effect of the drug thus developed more rapidly than the theophylline uptake by a factor of about 4.

Experiments with various concentrations of theophylline in the perfusion solution

The concentration-dependency of the effects of theophylline on twitch tension and coronary flow and of theophylline uptake was studied at concentrations of 10 µg-3 mg/ml (5.55×10^{-5}-1.67×10^{-2} M). The results are illustrated in Figure 5. The experiments were performed under the same conditions as those already described

Table 1 Concentration of [^{3}H]-theophylline in myocardial tissue related to concentration of [^{3}H]-theophylline in perfusion medium (tissue-medium ratio) and water content (% wet weight) of the preparations as a function of perfusion time (0.5-20 minutes)

Perfusion time (min)	*Tissue-medium ratio*	*Water content (% wet wt)*	n
0.5	0.56 ± 0.02	79.1 ± 0.76	8
1	0.77 ± 0.03	80.4 ± 0.71	8
2	0.93 ± 0.02	80.9 ± 0.42	8
3	0.96 ± 0.02	80.1 ± 0.34	6
5	0.97 ± 0.02	80.1 ± 0.41	6
10	0.99 ± 0.02	79.7 ± 0.83	3
20	1.00 ± 0.02	79.7 ± 0.41	3

n = number of preparations. Same preparations as in Figure 3.

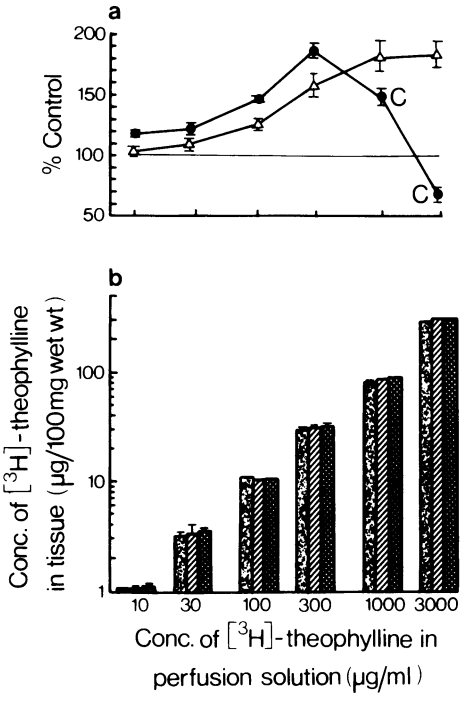

Fig. 5 (a) Effect of theophylline (10 µg-3 mg/ml) on contractile force (•) and coronary flow (△) in guinea-pig isolated perfused hearts. Ordinates: contractile force and coronary flow as percentage of control values measured before drug addition, control = 100%. Occurrence of contractures is marked with C. (b) [^{3}H]-theophylline uptake in left atria as well as in right and left ventricles of guinea-pig isolated perfused hearts at various concentrations of theophylline. Ordinates: concentration of [^{3}H]-theophylline in myocardial tissue (µg/100 mg wet wt). Base-line (log scale): concentration of [^{3}H]-theophylline in the perfusion solution (µg/ml). $n = 3$ for each value. Same preparations in a and b. Perfusion time 10 minutes. Stippled, left atrium; hatched, left ventricle; cross-hatched, right ventricle.

except that the loading period was held constant at 10 minutes. The positive inotropic effect of theophylline, i.e. the increase in systolic tension, began at a threshold concentration of 10 µg/ml, reached a maximum at 300 µg/ml (1.67 mM) and then decreased, i.e. the concentration-response curve was bell-shaped (Figure 5a). The highest concentration examined (3 mg/ml) led to a decrease in systolic tension and evoked contractures which occasionally occurred also at theophylline, 1 mg/ml. Coronary flow increased in parallel with the inotropic effect at concentrations up to 300 µg/ml. Higher concentrations which evoked only submaximal or no positive inotropic effects further enhanced coronary flow (Figure 5a).

The theophylline concentration in the tissue rose proportionally with the theophylline concentration in the perfusion medium (Figure 5b). No signs of saturation were detected as the theophylline concentration was increased up to 3 mg/ml, both in ventricular and in atrial preparations which were also examined in these experiments. The tissue-medium ratio was about 1 at all concentrations examined (Table 2) and the water content (about 80%) also remained unchanged in all cases (Table 2).

Pretreatment with caffeine

Theophylline uptake was studied in guinea-pig isolated hearts pretreated with non-labelled caffeine. Caffeine causes a positive inotropic effect similar to that produced by theophylline (e.g. Blinks *et al.*, 1972) and we assumed that the uptake of theophylline would be diminished by caffeine if the action of theophylline were due to a binding to some specific cellular receptor sites. After equilibration the hearts were perfused with caffeine (1 mg/ml) for 10 min; thereafter they were loaded with [^{3}H]-theophylline (300 µg-3 mg/ml) in the presence or absence of caffeine for an additional 10 minutes.

After a previous caffeine perfusion, isometric twitch tension was not further increased but rather decreased by theophylline, regardless of the presence (Fig. 6d) or absence (Fig. 6a-c) of caffeine during the [^{3}H]-theophylline infusion.

Figure 7a illustrates the results of the uptake experiments in which caffeine-pretreated hearts were subsequently treated with [^{3}H]-theophylline alone. It was found that pretreatment with caffeine did not alter the theophylline uptake when the hearts were perfused with theophylline in concentrations of 300 µg and 1 mg/ml (Fig. 7a, left and middle columns). However, caffeine significantly diminished the uptake of theophylline by 9.2% when a high theophylline concentration (3 mg/ml) was used (Fig. 7a, right columns). In this case, the tissue concentrations of theophylline were 291.3 and 263.5 µg theophylline/100 mg wet weight without and with caffeine pretreatment, respectively ($n = 5$-6; $P < 0.001$). When caffeine (1 mg/ml) was also present during [^{3}H]-theophylline perfusion of caffeine-pretreated hearts the theophylline uptake was decreased even at a theophylline concentration of 300 µg/ml. This is shown in Figure 7b. In these experiments, the caffeine-induced depression of theophylline uptake was 13.5% (decrease from

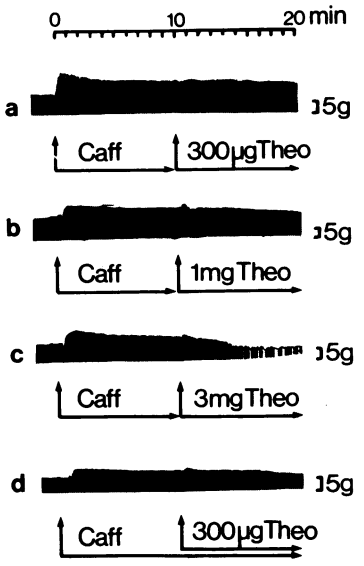

Fig. 6 (a-c) Effect of theophylline (Theo; 300 μg-3 mg/ml; perfusion time 10 min) on contractile force of guinea-pig isolated perfused hearts after a 10 min pretreatment with caffeine (Caff; 1 mg/ml). (d) Contractile force of a guinea-pig isolated perfused heart in the presence of caffeine (1 mg/ml) and caffeine (1 mg/ml) plus theophylline (300 μg/ml).

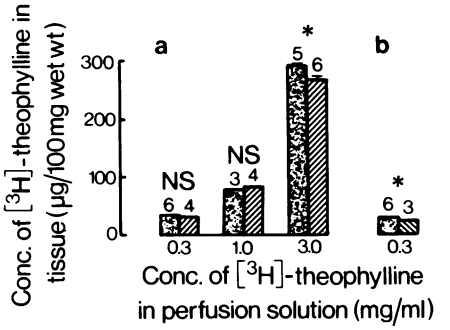

Fig. 7 Influence of caffeine (1 mg/ml) on the uptake of [^{3}H]-theophylline in guinea-pig isolated perfused ventricles. Stippled columns: No caffeine pretreatment. Hatched columns: 10 min pretreatment with caffeine (1 mg/ml) and uptake of [^{3}H]-theophylline in the absence (a) or in the presence of caffeine 1 mg/ml (b). Ordinates: concentration of [^{3}H]-theophylline in ventricular tissue (μg/100 mg wet wt). Base-lines: concentration of [^{3}H]-theophylline in the perfusion solution (mg/ml).
* $P < 0.001$; NS = not significant. The number of preparations is given above the columns.

29.6 to 25.6 μg theophylline/100 mg wet wt.; $n = 4$-6; $P < 0.001$).

Theophylline release

Hearts were perfused with [^{3}H]-theophylline (100 μg/ml) for 10 min and were then washed with drug-free medium for 20 minutes. The theophylline content of the hearts obtained by extrapolation to zero time (end of [^{3}H]-theophylline infusion) was 8.53 ± 0.49 μg/100 mg wet weight ($n = 8$). This value correlates well with the theophylline content in myocardial tissue obtained in the uptake experiments. The theophylline desaturation curve is shown in Figure 8. Theophylline release was about 84% complete after 2 min and more than 99% complete after 9 min of washing; that is theophylline release was also very rapid. The efflux curve was found to be

Table 2 Concentration of [^{3}H]-theophylline in myocardial tissue related to concentration of [^{3}H]-theophylline in perfusion medium (tissue-medium ratio) and water content (% wet wt) of the preparations as a function of the theophylline concentration in the perfusion medium

Concentration of theophylline in the perfusion solution (per ml)	*Tissue-medium ratio*	*Water content (% wet wt)*	n
10 μg	1.09 ± 0.03	78.2 ± 0.64	3
30 μg	1.08 ± 0.03	77.3 ± 1.24	3
100 μg	0.99 ± 0.02	79.9 ± 1.18	3
300 μg	1.03 ± 0.03	79.2 ± 0.74	3
1 mg	0.95 ± 0.01	79.6 ± 0.70	3
3 mg	0.98 ± 0.01	79.8 ± 0.77	3

Same preparations as in Figure 5. n = number of hearts. In each heart, the atrial and ventricular data were the same and therefore have been combined.

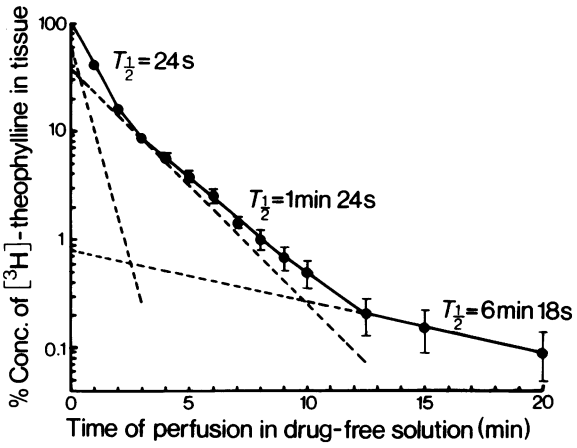

Fig. 8 Theophylline release from guinea-pig isolated perfused hearts. Ordinates: percentage [^{3}H]-theophylline concentration in myocardial tissue, log scale. Abscissae: Perfusion time in drug-free solution (min). n = 8; $T_{½}$ = half-time.

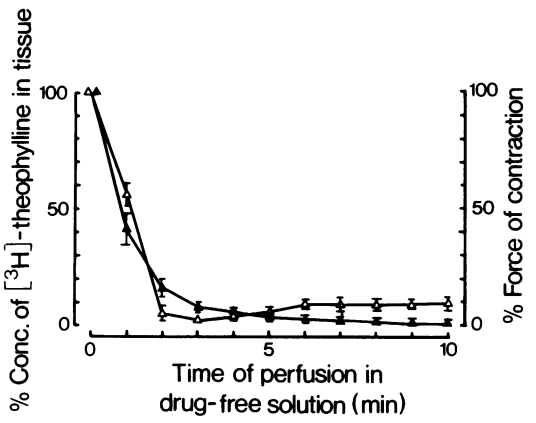

Fig. 9 Theophylline release (▲) and decrease of contractile force (△) during perfusion of guinea-pig isolated hearts in drug-free solution. Ordinates: percentage [^{3}H]-theophylline concentration in the tissue and percentage contractile force. 100% values are the [^{3}H]-theophylline concentration at zero time and the difference between systolic tension at zero time and the minimum level to which tension fell, respectively. Abscissae: perfusion time in drug-free solution (min). n = 8. Same hearts for both curves.

composed of three different components. The intercepts of the linear portions of the curve occurred at 61%, 38% and about 1%, the half-times of the components being 24 s, 1 min 24 s and 6 min 18 s, respectively.

The time courses of theophylline release and of the decline of contractile force upon washing are compared in Figure 9. It is evident that both parameters decreased in the same manner.

Discussion

The present experiments have shown that the uptake of theophylline into the myocardium proceeds very rapidly. The uptake process was more than 95% complete within 3 minutes. The steady-state tissue-medium ratio was 1. Concentrations of theophylline in myocardial tissue increased proportionally with the theophylline concentrations in the perfusion media and theophylline uptake did not approach saturation when the theophylline concentration of the perfusion fluid was raised. These results, which are similar to those obtained with caffeine in frog sartorius muscle (Bianchi, 1962) and in rabbit auricles (Holland & Wassermann, 1972), indicate that the myocardial uptake of theophylline is mainly a rapid diffusion process and that theophylline distributes itself in heart muscle as freely as in the perfusion medium. Thus, the cell membrane does not appear to be a permeability barrier for the penetration of the drug and an intracellular accumulation of theophylline against a concentration gradient is also unlikely.

However, the theophylline uptake did not follow the pattern of a single exponential process. A second component was found the half-time of which was more than 15 times slower than that of the first process (5 min 50 s as against 21 seconds). The slow process amounted only to about 7% of the total uptake but it probably suggests a compartment within the cell where theophylline may be bound in a more specific manner. The latter view is indicated by the finding that the uptake of theophylline was slightly decreased by caffeine under certain experimental conditions. Using the high concentration of 3 mg/ml theophylline, the uptake of the drug was decreased by 9.2% when the hearts were first perfused with caffeine (1 mg/ml). In the presence of caffeine, theophylline uptake was also reduced by 13.5% when caffeine-pretreated hearts were perfused with a solution containing only 300 μg/ml theophylline.

Similar conclusions can be drawn from the washout experiments. The release of theophylline from the hearts was completed by more than 95% within 5 min, indicating again that theophylline moves very readily across the cardiac cell membrane. The efflux of theophylline occurred in three different phases. The first two processes

(99% of the total efflux) probably reflect efflux of theophylline from coronary vessels, extracellular space and rapidly releasable cellular sites but a convincing relation to one of these sites cannot be ascertained. The half-time of the third component (about 1% of the total 'theophylline space') was 6 min 18 seconds. This figure corresponds to the half-time of the slow uptake process. It suggests again that a small amount of theophylline may be bound within the cell. The site of this binding may be the sarcoplasmic reticulum but in this case, too, an exact location cannot be derived from these experiments with intact muscle.

The second object of this study was to investigate whether the myocardial uptake of theophylline, if any, occurs so rapidly that it could be causally related to the rapidly developing positive inotropic effect of the drug. We have already mentioned that the increase in twitch tension induced by theophylline has been suggested to be due, at least in part, to an intracellular action leading to a release of calcium from intracellular calcium storage or binding sites, e.g. from the sarcoplasmic reticulum. By the use of sarcoplasmic reticulum preparations isolated from cardiac and skeletal muscle, such calcium releasing effects have indeed been shown with caffeine and theophylline *in vitro* at threshold concentrations of 1 mM or more (Weber & Herz, 1968; Weber, 1968; Fuchs, 1969; Pretorius *et al.*, 1969; Johnson & Inesi, 1969; Ogawa, 1970; Taniguchi & Nagai, 1970). These findings are in keeping with the hypothesis of an intracellular site of theophylline action. At first sight, this view is also supported by the present result that theophylline readily enters the myocardium and by the assumption that a small amount of theophylline may be bound within the cell in a relatively specific manner. However, in the present study it has also been found that the development of the positive inotropic effect of theophylline is about four times faster than the myocardial uptake of the drug. From this particular result it is concluded that the theophylline-induced increase in myocardial twitch tension is not, in the main, initiated by an intracellular action. It seems more reasonable to assume that theophylline, at least in concentrations of 1 mM or less, acts mainly at a level prior to the intracellular space, e.g. on the surface membrane to increase the calcium influx from the extracellular space during the cardiac action potential as has been suggested previously (Scholz, 1971a, b). Nevertheless it should be kept in mind that the positive inotropic effect of theophylline is accompanied by an increase in the total duration of the isometric contraction (Blinks *et al.*, 1972). This effect can best be explained by a methylxanthine-induced inhibition of calcium sequestration by the sarcoplasmic reticulum which has also been shown in the above mentioned *in vitro* studies. The possibility that an additional intracellular action may contribute to maintain the positive inotropic effect of theophylline, at least at large concentrations, should therefore not be rejected.

Another question at present widely discussed is whether the inotropic effects of theophylline are mediated by cyclic AMP. It is beyond the scope of the present paper to attempt a detailed interpretation of the results in terms of phosphodiesterase inhibition and subsequent cyclic AMP accumulation as a possible mechanism of the inotropic theophylline action. However, it should be briefly mentioned that our findings are not incompatible with the cyclic AMP concept because part of the theophylline-inhibited phosphodiesterase activity seems to be bound to the sarcolemma (Kukovetz & Pöch, 1970). It should also be noted that the theophylline (and adrenaline)-induced changes in the calcium permeability of the cardiac cell membrane can be mimicked by dibutyryl cyclic AMP (Meinertz, Nawrath & Scholz, 1973).

In summary, we suggest that the positive inotropic effect of theophylline in mammalian cardiac muscle may be due to an intracellular action because the drug readily enters the myocardium. However, the theophylline-induced increase in twitch tension has been found to be appreciably faster than even the rapid component of the uptake process. The main effect of the drug, regardless of whether cyclic AMP is involved or not, is therefore thought to be on the sarcolemma.

This paper is dedicated to Prof. Dr G. Kuschinsky on the occasion of his 70th birthday.

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