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DISSOCIATION OF THEOPHYLLINE UPTAKE AND INOTROPIC EFFECT IN MYOCARDIAL TISSUE: INFLUENCE OF TEMPERATURE, pH AND CALCIUM

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1 The myocardial uptake and the positive inotropic effect of theophylline (100 μg/ml; 0.56 mM) were studied in isolated electrically driven guinea-pig hearts perfused by the Langendorff technique under various extracellular conditions. [^{3}H]-theophylline was used.

2 Variations in temperature, hydrogen ion and calcium ion concentrations of the perfusion media changed the time course and magnitude of the effect of theophylline on myocardial twitch tension but did not affect the time course and amount of theophylline uptake.

3 Under all conditions, the build-up of the positive inotropic effect of theophylline was about three times faster than the uptake of the drug into the heart.

4 Since no relationship could be found between theophylline uptake and inotropic effect, the cardiac positive inotropic response to theophylline is thought unlikely to be due mainly to an interaction of the drug with intracellular receptor sites but is assumed to occur via an effect of the drug on the sarcolemma, that is at a site which the drug reaches before it enters the intracellular space.

Introduction

Previous work of the present authors (Bellemann & Scholz, 1974) has provided evidence that theophylline enters myocardial tissue very readily. However, development of the theophylline-induced increase in myocardial twitch tension was found to proceed appreciably faster than the uptake of the drug into the heart. From these findings it was concluded that the cardiac positive inotropic effect of theophylline is unlikely to be due mainly to an action of the drug within the cell, e.g. to the release of calcium from intracellular calcium binding or storage sites.

In order to obtain further information on the relationship between theophylline uptake and the positive inotropic effect, the present work was designed to investigate whether the time course and amount of the myocardial uptake of theophylline are affected by hypothermia or by varying the extracellular hydrogen and calcium ion concentrations. Such factors are known to exert a considerable influence on cardiac mechanical performance and on drug-induced positive inotropic effects (e.g. Reiter, 1963; Blinks & Koch-Weser, 1963; Yeatman, Parmley & Sonnenblick, 1969; Scholz & de Yazikof, 1971; Reiter, 1972; Schaer, 1974).

Methods

The preparation used was the isolated guinea-pig heart perfused by the Langendorff technique. The perfusion apparatus, the methods of preparation, analytical procedure, and the experimental schedule were described by Bellemann & Scholz (1974) and only a brief account of them will be given here.

The animals (either sex, weight 380-450 g), which were kept on a standardized diet of 'Altromin' and water *ad libitum*, were injected intraperitoneally with heparin (20 mg/kg) 1 h before the experiment, killed by a sharp blow on the head and bled from the carotids. Their hearts were rapidly removed, mounted on a double-barrelled Langendorff perfusion apparatus and immediately perfused through the aorta at a constant pressure of 60 cm of water with Tyrode solution of the following 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, 35°C); the perfusion fluid was continuously gassed with 95% O_2 and 5% CO_2. When desired, the extracellular Ca^{++}-concentration ($[Ca]_o$) was varied between 0.45 and 1.8 mM by the addition of $CaCl_2$ without allowing for the alteration of osmotic pressure. The investigations with hypo-

thermia were performed at 25° C, $[Ca]_0$ and pH being 0.9 mM and 7.4, respectively. The perfusion medium used in the studies with different pH (temperature 35° C) was of the following composition (mM): NaCl 136.9, KCl 5.4, $MgCl_2$ 1.05, $CaCl_2$ 0.9, Tris-(hydroxymethyl)-aminomethane-HCl 5, glucose 5.5 and was bubbled constantly with 100% O_2. All perfusion fluids were filtered (glass filter, Schott 25 D 3, pore size 15-40 μm) before each experiment. The Ca^{++}-content of the perfusion medium was monitored by atomic absorption spectrometry (Perkin-Elmer, Model 403). The pH of the solutions was adjusted with a digital precision pH-meter (WTW-DIGI 610).

After dissection, all preparations were first perfused for 15-20 min with Tyrode solution under 'normal conditions' (0.9 mM Ca^{++}; pH 7.4; 35° C). The hearts were then equilibrated for 15 min with a medium of the desired experimental composition (variation of $[Ca]_0$, pH or temperature) without drug. Afterwards they were incubated for various periods (1-20 min) with the same solution containing 0.05 μCi/ml [^{3}H]-theophylline and 100 μg/ml (0.56 mM) of non-radioactive theophylline.

Constant electrical stimulation (rectangular pulses; Grass stimulator S6; frequency 3 Hz; duration 3 ms; intensity twice threshold value) during the equilibration and the experimental periods was by platinum electrodes implanted in each auricle. Mechanical and analytical measurements were performed on the same preparations. Isometric tension of the right ventricle was monitored according to Beckett (1970) with a force-displacement transducer (Grass Ft. 03) and was recorded 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 with a graduated cylinder.

The [^{3}H]-theophylline content of the ventricular tissue and of the incubation medium was determined as described in the previous work. Drugs used were obtained from Boehringer Sohn, Ingelheim (non-radioactive theophylline; molecular weight 180.17) and from The Radiochemical Centre, Amersham (theophylline-[^{3}H] (G)). Formation of radioactive theophylline metabolites or tritiated water under the experimental conditions used here was not demonstrable; thus, the radioactivity measured was directly related to [^{3}H]-theophylline.

The analysis of the theophylline uptake and inotropic effect curves against time was performed according to Rescigno & Segre (1966). All data are given as means ± s.e. mean. Statistical comparisons were performed by Student's *t* test with unpaired values. A *P* value equal to or less than 0.05 was considered significant.

Results

Theophylline uptake

It is evident from Figure 1a that time course and magnitude of the theophylline uptake into myocardial tissue were neither altered by hypothermia (25° C) nor by variations of the calcium concentration and the pH of the perfusion fluid. In all cases, the uptake process proceeded very rapidly and followed the same pattern: after 3 min perfusion, the theophylline content of the hearts was already about 90% of the final 20 min level

Table 1 Amount of theophylline taken up by isolated electrically driven Langendorff-perfused hearts of guinea-pigs under different conditions

Series	*Condition* $[Ca]_0$ *mM*	*Temperature °C*	*pH*	*Tissue: medium ratio*
A	0.9	25	7.4	0.96 ± 0.06 (3)
	0.9	35	7.4	1.01 ± 0.02 (3)
B	0.9	35	6.6	1.01 ± 0.02 (3)
	0.9	35	7.4	1.01 (1)
	0.9	35	8.2	0.96 ± 0.04 (3)
C	0.45	35	7.4	1.03 ± 0.12 (3)
	0.9	35	7.4	1.01 ± 0.02 (3)
	1.8	35	7.4	1.02 ± 0.01 (3)

Data represent concentration of theophylline in the tissue related to concentration of theophylline in the perfusion medium (tissue: medium ratio) after 20 min perfusion with [^{3}H]-theophylline. The number of preparations is given in parentheses.

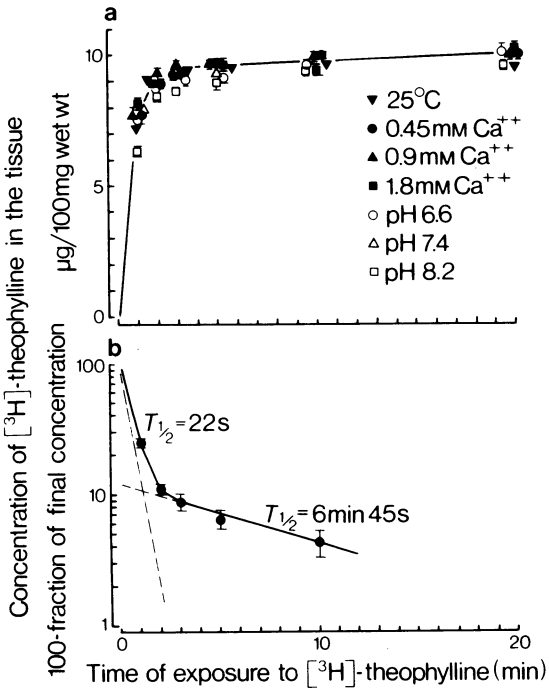

Figure 1 Isolated perfused hearts of guinea-pigs (a) Effect of hypothermia (25° C), extracellular Ca^{++} concentration (0.45-1.8 mM) and extracellular pH (6.6-8.2) on [^{3}H]-theophylline uptake. n = 3-8 for each value. Ordinates: concentration of [^{3}H]-theophylline in myocardial tissue (µg/100 mg wet weight). Abscissae: perfusion time (min). (b) Time course of [^{3}H]-theophylline uptake. The data from Figure 1a were averaged and were replotted on semi-logarithmic coordinates according to Rescigno & Segre (1966). Each experimental point was normalized as % of, and subsequently subtracted from, the respective final value which was set at 100%. The term final value refers to the mean 20 min concentration of [^{3}H]-theophylline in the tissue as measured in each individual series of experiments. $T_{½}$ = half-time.

which, in turn, corresponded to the theophylline concentration of the perfusion medium (10 µg/0.1 ml). The latter finding can also be seen from the 20 min tissue: medium ratios listed in Table 1: they were approximately 1 under all conditions. The water content of the tissue (about 80% wet weight) also remained unaltered in all series.

More detailed information on the time course of the theophylline uptake process is given in Figure 1b. The uptake curve shown was obtained by averaging the data of all series. It is evident that theophylline entered the tissue in two phases. On the average, the first process amounted to 88% and

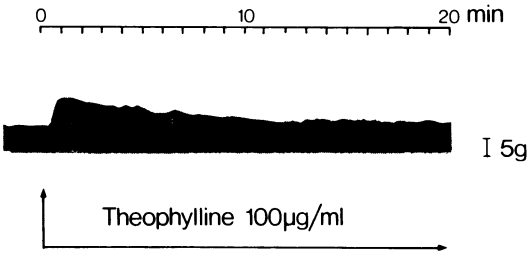

Figure 2 Isometric twitch tension in an isolated electrically stimulated (frequency 3 Hz) heart of guinea-pig perfused by the Langendorff technique before and during application of theophylline 100 µg/ml. The experiment was performed under normal conditions (temperature 35° C; pH 7.4; $[Ca]_o$ 0.9 mM).

the second one to 12% of the total uptake; the half-times of the components were 22 s and 6 min 45 s, respectively.

Twitch tension and inotropic effect of theophylline

The original tracing shown in Figure 2 illustrates an experiment under 'normal conditions' (pH 7.4, 0.9 mM Ca^{++}, 35° C). The positive inotropic effect of 100 µg/ml theophylline began almost immediately after perfusing with the drug-containing medium, reached its peak within less than 1 min and then declined gradually. Under normal conditions, the time to peak effect was, on the average, 49.7 ± 2.4 s and the half-time of the build-up of the drug's positive inotropic effect was 7.6 ± 0.29 s (Table 2, series A, 2nd line). The latter value is about 3 times smaller than the half-time of even the rapid component of the theophylline uptake which was 22 s, as stated above. Thus, the former process developed appreciably more rapidly than the latter.

However, theophylline uptake and positive inotropic effect were not only dissimilar with respect to their time course. In contrast to the theophylline uptake the inotropic effect of the drug was also considerably influenced by the experimental conditions used. The build-up of the theophylline-induced increase in twitch tension was significantly slowed by acidosis (pH 6.6) and low calcium (0.45 mM) and was found to be accelerated at 1.8 mM $[Ca]_o$. This is illustrated in Table 2 whereas the respective alterations in the magnitude of the inotropic effect and in its persistence are demonstrated in Figures 3-5 (upper panels). As compared to normal conditions, the peak effect of theophylline remained unchanged

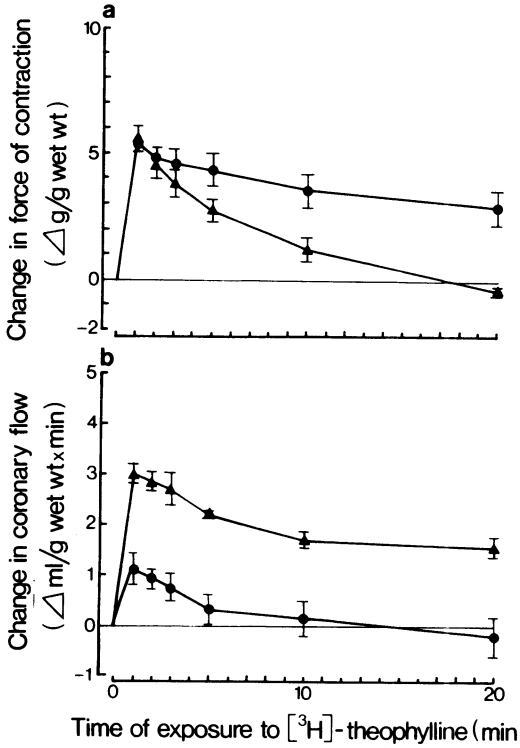

Figure 3 Effect of temperature (25° C: •, n = 3-26; 35° C: ▲, n = 3-34) upon (a) theophylline-induced changes in contractile force and (b) coronary flow of isolated perfused heart of guinea-pig. Ordinates: changes from base-line levels in force of contraction (Δ g/g wet weight) and in coronary flow (Δ ml/g wet weight x min). Abscissae: perfusion time (min). Drug addition (100 μg/ml) at zero time. Base-line values are listed in Table 3.

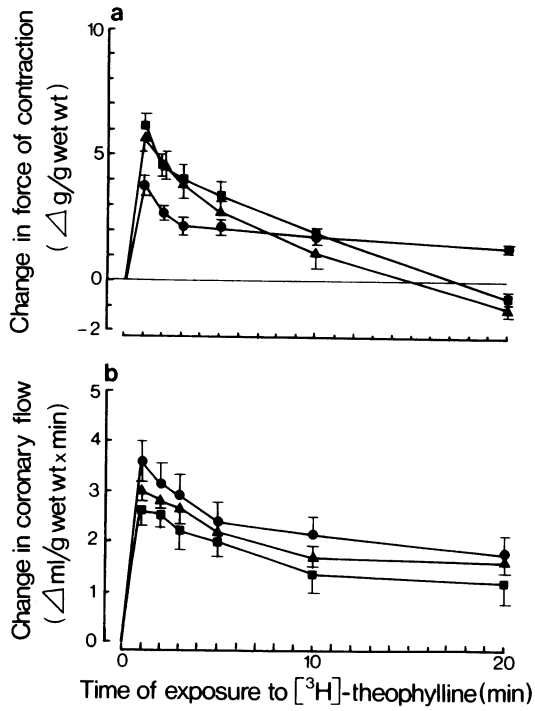

Figure 4 Effect of extracellular Ca^{++}-concentration ($[Ca]_0$ 0.45 mM: •, n = 3-18; $[Ca]_0$ 0.9 mM: ▲, n = 3-34; $[Ca]_0$ 1.8 mM: ■, n = 3-18) upon (a) theophylline-induced changes in contractile force and (b) coronary flow of isolated perfused hearts of guinea-pigs. Ordinates: changes from base-line levels in force of contraction (Δ g/g wet weight) and in coronary flow (Δ ml/g wet weight x min). Abscissae: perfusion time (min). Drug addition (100 μg/ml) at zero time. Base-line values are listed in Table 3.

Table 2 Effect of temperature (series A), pH (series B) and $[Ca]_0$ (series C) on the time to peak positive inotropic effect and on the half-time of the build-up of the positive inotropic effect in isolated electrically driven hearts of guinea-pigs treated with 100 μg/ml theophylline

Series	*Condition* $[Ca]_0$ *mM*	*Temperature* °C	*pH*	*Time to peak effect (s)*	*Half-time of tension increase (s)*	n
A	0.9	25	7.4	55.8 ± 2.6	8.4 ± 0.30	26
	0.9	35	7.4	49.7 ± 2.4	7.6 ± 0.29	34
B	0.9	35	6.6	64.6 ± 3.0^c	9.8 ± 0.55^b	19
	0.9	35	7.4	51.3 ± 3.5	7.8 ± 0.28	10
	0.9	35	8.2	55.9 ± 1.6	8.5 ± 0.60	20
C	0.45	35	7.4	59.3 ± 2.8^c	9.0 ± 0.36^a	18
	0.9	35	7.4	49.7 ± 2.4	7.6 ± 0.29	34
	1.8	35	7.4	37.3 ± 2.1^b	5.6 ± 0.26^b	18

Values represent means ± s.e. mean. n = number of preparations.
^{a,b,c} Significant difference from values measured under normal conditions (0.9 mM Ca^{++}, pH 7.4, 35° C).
$^a P < 0.001$; $^b P < 0.01$; $^c P < 0.02$.

with hypothermia (Figure 3), 1.8 mM Ca^{++} (Figure 4), and alkalosis (Figure 5) but was smaller at pH 6.6 (Figure 5) and 0.45 mM Ca^{++} (Figure 4). Finally, the decline in tension development between the peak inotropic effect and the end of theophylline perfusion was considerably slower with hypothermia (Figure 3) and in low calcium medium (Figure 4).

It is evident from Table 3 that the predrug force, i.e. the level before theophylline application, was also altered by hypothermia or variations of the extracellular calcium and hydrogen ion concentrations. These results are in accordance with previous work (e.g. Reiter, 1963; Kohlhardt, Wirth & Dudeck, 1967; Pannier & Leusen, 1968; Cingolani, Mattiazzi, Blesa & Gonzales, 1970; Scholz & de Yazikof, 1971; Reinhardt, Wagner & Schümann, 1972; Kammermeier & Rudroff, 1972; Schaer, 1974). Because of these variations in predrug force, the theophylline-induced changes in twitch tension given in Figures 3-5 are not expressed as a percentage of the base-line tension but, instead, as Δg/g wet weight, this is as absolute change from the respective control level (cf. Rall & West, 1963). The same applies to the coronary flow values described below.

Coronary flow

Under normal conditions, the predrug coronary flow amounted to 6.6 ± 0.2 ml/g wet weight x min (Table 3, series A, 2nd line). It was enhanced by hypothermia and acidosis, was diminished by alkalosis but was not altered by changes in $[Ca]_o$ (Table 3). Theophylline increased coronary flow in all series (Figures 3-5; lower panels); as compared

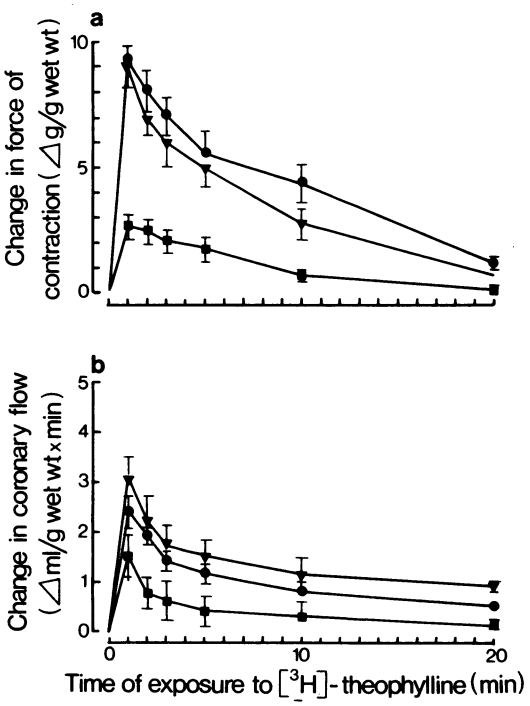

Figure 5 Effect of extracellular pH (pH 6.6 (■), n = 3-19; pH 7.4 (▼), n = 2-10; pH 8.2 (●), n = 3-20) upon (a) theophylline-induced changes in force of contraction and (b) coronary flow of isolated perfused hearts of guinea-pigs. Ordinates: changes from base-line levels in contractile force (Δ g/g wet weight) and in coronary flow (Δ ml/g wet weight x min). Abscissae: perfusion time (min). Drug addition (100 μg/ml) at zero time. Base-line values are listed in Table 3.

Table 3 Effect of temperature (series A), pH (series B) and $[Ca]_o$ (series C) on force of contraction and coronary flow of isolated electrically driven hearts of guinea-pigs as measured immediately before application of theophylline

Series	*Condition* $[Ca]_o$ *mM*	*Temperature °C*	*pH*	*Force of contraction g/g wet weight*	*Coronary flow ml/g wet weight x min*
A	0.9	25	7.4	13.4 ± 0.8 (31)^b	8.0 ± 0.2 (31)^a
	0.9	35	7.4	10.6 ± 0.6 (34)	6.6 ± 0.2 (34)
B	0.9	35	6.6	0.9 ± 0.1 (22)^a	10.0 ± 0.8 (22)^b
	0.9	35	7.4	5.9 ± 0.6 (10)	6.5 ± 0.5 (10)
	0.9	35	8.2	12.5 ± 1.2 (20)^a	5.1 ± 0.2 (20)^b
C	0.45	35	7.4	3.8 ± 0.3 (18)^a	6.6 ± 0.3 (18)
	0.9	35	7.4	10.6 ± 0.6 (34)	6.6 ± 0.2 (34)
	1.8	35	7.4	13.0 ± 0.7 (18)^c	6.5 ± 0.3 (18)

The number of preparations is given in parentheses. Values represent means ± s.e. mean.
^{a,b,c} Significant difference from values measured under normal conditions (0.9 mM Ca^{++}, pH 7.4, 35°C).
$^a P < 0.001$; $^b P < 0.01$; $^c P < 0.02$.

to normal conditions, the effect of theophylline was diminished by hypothermia (Figure 3) and, to a smaller extent, by acidosis (Figure 5) but remained unaltered by alkalosis (Figure 5) and the different calcium concentrations studied (Figure 4).

Discussion

We have previously shown that the uptake of theophylline into the mammalian heart proceeds very rapidly and that the steady-state concentration of theophylline in the myocardial tissue corresponds to that of the perfusion fluid (Bellemann & Scholz, 1974). These findings led us to suggest that the myocardial uptake of theophylline is mainly by rapid diffusion and that the drug distributes itself in the heart muscle as freely as in the Tyrode solution. The present results are in accord with this view and allow additional conclusions. With respect to the mechanism of the uptake process, the most meaningful observations of this study are that the myocardial uptake of theophylline is not affected by hypothermia (25° C) or by varying the extracellular pH within a range of 6.6-8.2. From the former finding it can be concluded that the theophylline uptake is unlikely to be mediated by an energy-dependent process. The latter result suggests that theophylline which is a weak acid (proton at N-7; pK_a 8.77; Merck Index, 8th ed., p. 1034) as well as a negligibly weak base (pK_b 13.72) is mainly taken up as an uncharged molecule, at least in the pH range studied. The present results thus confirm and extend our previous conclusions which can now be summarized thus: theophylline moves through the cardiac cell membrane mainly by nonspecific passive diffusion and not by an active process or by electrical forces.

The second point to be discussed is the lack of coincidence between theophylline uptake and inotropic effect. Such dissociation which is felt to be relevant with respect to the mechanism of the inotropic response to the drug applies firstly to the different time courses of both events, and secondly to their dissimilar responsiveness to variations in the experimental conditions.

In all series of experiments, the positive inotropic effect of the drug developed appreciably more rapidly than even the fast component of the theophylline uptake. This finding corroborates our previous results (Bellemann & Scholz, 1974) and suggests that the 'receptor' involved in the development of the inotropic effect is mainly located at a site which the drug reaches before it enters the intracellular space. It appears not unreasonable to assume that this site is the sarcolemma because previous electrophysiological work has provided evidence that theophylline increases the slow calcium inward current during the cardiac action potential and that this effect is closely related to the inotropic response to the drug (Scholz, 1971).

However, theophylline uptake and the inotropic effect were not only dissimilar with respect to their time course but also with respect to their different susceptibility to changes in the extracellular conditions. For instance, the rate of development and the peak magnitude of the positive inotropic effect of the drug were diminished by acidosis and by reducing the extracellular calcium concentration but the rate and amount of theophylline uptake were not affected by the same changes. On the other hand, elevation of the extracellular calcium concentration was found to accelerate the development of the inotropic response to theophylline but the theophylline uptake again remained unchanged. These dissociations further support the view that there is no direct cause and effect relationship between the uptake of theophylline into the myocardial cell and the inotropic response to the drug or, in other words, that the former event, although rapid and well pronounced, is not a prerequisite for the latter.

The effects of pH or $[Ca]_o$ changes upon the cardiac inotropic response to theophylline described here correspond to those previously observed with catecholamines (e.g. Reiter & Schöber, 1965; Schaer, 1974). The altered activity, e.g. with variations of pH, of the latter agents may be attributed to a changed sensitivity to catecholamines of transmembrane calcium fluxes (Morgenstern, Noack & Köhler, 1972), of calcium sequestration by the sarcoplasmic reticulum (Chimoskey & Gergely, 1968), of the cyclic AMP-system (Reynolds & Haugaard, 1967), or even to nonspecific drug-independent changes in myocardial contractility (Schaer, 1974). Unfortunately, similar data with theophylline are as yet not available so that the mechanism underlying the effects of variations of pH or $[Ca]_o$ upon theophylline action on cardiac mechanical behaviour remains to be elucidated. However, from the present results it can be concluded that changes in the access of theophylline to its receptor site or interference with metabolic degradation of the drug during incubation are unlikely to be significant factors.

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