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THEOPHYLLINE AND PHENYLEPHRINE EFFECTS ON CARDIAC RELAXATION

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1 In the driven isolated left atrium of the rabbit theophylline shortened relaxation time in a similar manner to isoprenaline and histamine.

2 Phenylephrine lengthened relaxation time in a similar manner to calcium.

3 Theophylline caused phenylephrine to shorten relaxation time, which was inhibited by a β-adrenoceptor blocking drug, but theophylline did not potentiate the effect of phenylephrine on peak tension.

4 Theophylline separated drug effects on cardiac relaxation and contraction: in the presence of theophylline at a low calcium concentration, phenylephrine shortened relaxation time by β-adrenoceptor stimulation and increased peak tension by α-adrenoceptor stimulation. At a high calcium concentration, theophylline potentiated the effect of isoprenaline, histamine and phenylephrine on relaxation time but inhibited the effect on peak tension.

Introduction

The positive inotropic effect of agents such as catecholamines and histamine is accompanied by a rise in the myocardial concentration of cyclic adenosine 3′,5′-monophosphate (cyclic AMP), and it has been suggested that this nucleotide mediates the effect of the amines on contractility. An exact definition of the function of cyclic AMP may not be possible until the basic steps in contractile activation are completely understood. This study describes mechanical effects of two inotropic drugs in which an involvement of cyclic AMP remains controversial.

Catecholamines and histamine which stimulate the enzyme adenylate cyclase typically shorten relaxation time and abbreviate cardiac systole. Usually these agents increase relaxation velocity (or relaxation rate) more than agents which do not stimulate adenylate cyclase. The effect is presumably related to the stimulation by catecholamines of calcium sequestration in the sarcoplasmic reticulum (Katz, Taka & Kirchberger, 1975).

Theophylline and the related methylxanthine, caffeine, are believed to owe part or all of their effects to an inhibition of the enzyme cyclic nucleotide phosphodiesterase and the resulting increase in tissue cyclic AMP concentration (Sutherland & Rall, 1958; 1960). However, methylxanthines generally slow cardiac relaxation, which is compatible with the fact that these drugs inhibit calcium uptake by the sarcoplasmic reticulum (Nayler, Dunnett & Berry, 1975). Thus methylxanthines prolonged relaxation time in cat atrium (Blinks, Olson, Jewell & Braveny, 1972), cat papillary muscle (Blinks *et al.*, 1972; Henderson, Brutsaert, Forman & Sonnenblick, 1974), and rabbit interventricular septum (Shine & Langer, 1971). Also, caffeine inhibited the catecholamine effect on relaxation in rabbit papillary muscle (Gibbs, 1967) and cat papillary muscle (Blinks *et al.*, 1972). It has not been reported before that a methylxanthine can stimulate relaxation and potentiate effects of catecholamines and histamine on relaxation.

Methylxanthine effects on peak tension differ quantitatively in different species. Thus in cat papillary muscle, theophylline had no significant effect on peak tension below a concentration of 2 mmol/l, exerted its greatest effect on peak tension at 20 mmol/l, and prolonged systole in both of these concentrations (Blinks *et al.*, 1972). In guinea-pig atrium theophylline increased peak tension in concentrations as low as 0.17 and 1.7 mmol/l which did not prolong systole; 11 mM theophylline reduced peak tension and prolonged systole (Scholz & de Yazikof, 1971). Also, caffeine had a greater effect on peak tension in rabbit papillary muscle than in cat or dog papillary muscle (Bodem & Sonnenblick, 1975).

Agents which increase myocardial cyclic AMP concentration do not have a special effect on time to peak tension. Methylxanthine effects on time to peak tension differ qualitatively in different species. Methylxanthines shortened time to peak tension in rabbit interventricular septum (Shine & Langer, 1971) and rabbit papillary muscle (Bodem & Sonnenblick, 1975), did not change time to peak tension in guinea-pig

atrium (Scholz & de Yazikof, 1971), and prolonged time to peak tension in cat atrium (Blinks *et al.*, 1972), cat papillary muscle (Skelton, Karch, Hougen, Marcus & Epstein, 1971; Marcus, Skelton, Grauer & Epstein, 1972; Blinks *et al.*, 1972; Henderson *et al.*, 1974; Bodem & Sonnenblick, 1975), dog papillary muscle (Bodem & Sonnenblick, 1975), and guinea-pig papillary muscle (Nayler *et al.*, 1975). It may be noted that caffeine shortened the action potential duration in rabbit atrium (Yanaga & Holland, 1969) but prolonged it in heart preparations of other species, e.g., the guinea-pig atrium (De Gubareff & Sleator, 1965).

Phenylephrine increases peak tension in isolated mammalian heart preparations without stimulating adenylate cyclase or raising the cyclic AMP concentration (Benfey, 1971; Osnes & Øye, 1975; Picken & Jarrott, 1975). In the driven left atrium of the rabbit phenylephrine increased peak tension and prolonged refractory period through α-adrenoceptor stimulation; in the spontaneously beating right atrium of the rabbit phenylephrine increased rate through β-adrenoceptor stimulation (Benfey, 1973). Effects of phenylephrine on the duration of cardiac systole have not been reported before.

Methods

Strips of rabbit left atrium were suspended at 31°C in a solution containing (mM): Na^+ 139.8, K^+ 5.9, Mg^{2+} 1.2, Ca^{2+} 0.9 or 3.2, Cl^- 120.5 or 122.8, HCO_3^- 24.9, $H_2PO_4^-$ 1.2, SO_4^{2-} 1.2, glucose 10, and disodium edetate 0.03, which was aerated with 5% CO_2 in O_2. The muscles were stimulated with square-wave pulses of 1 ms duration and a voltage just above threshold at a rate of 1 Hz. Tension was recorded isometrically, and times to peak tension and to complete relaxation were estimated from oscilloscope recordings. Incubation with theophylline was for 20 minutes. Dose-response curves for isoprenaline, phenylephrine, and histamine were obtained cumulatively.

The drugs included theophylline, (–)-phenylephrine hydrochloride (K & K Laboratories), (–)-isoprenaline bitartrate dihydrate (Winthrop), histamine dihydrochloride (Hoffman-La Roche), and propranolol hydrochloride (Ayerst, McKenna & Harrison).

Results

Theophylline

In concentrations of 0.1, 0.3, and 1 mmol/l theophylline increased peak tension, contraction velocity (or rate of tension rise), and relaxation velocity, and decreased time to peak tension and time to complete relaxation (Figures 1 and 2). There were

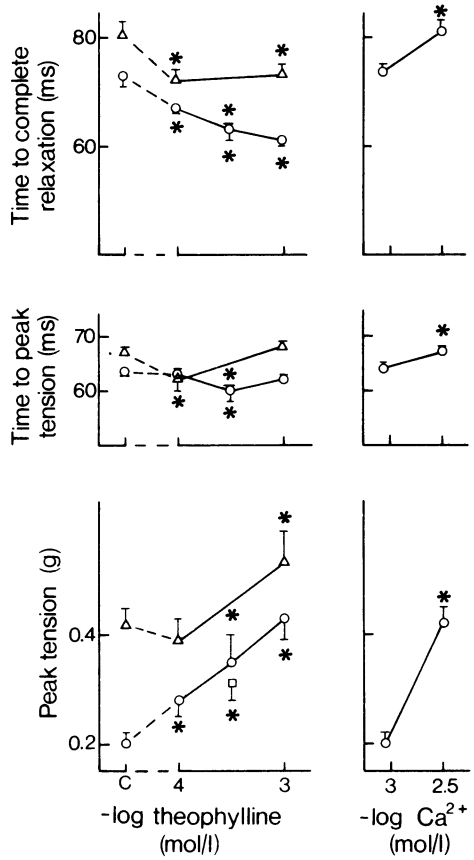

Figure 1 Effects of theophylline at 0.9 mmol/l $[Ca^{2+}]_o$ in the absence (○) and presence of 1 μmol/l propranolol (□) and at 3.2 mmol/l $[Ca^{2+}]_o$ (△), and effects of calcium. Means of 9–24 experiments. Vertical lines show s.e. $^*P<0.05$, compared to control (C or 0.9 mmol/l $[Ca^{2+}]_o$).

some exceptions: at the lowest concentration (0.1 mmol/l) theophylline did not shorten time to peak tension at 0.9 mmol/l $[Ca^{2+}]_o$, did not increase peak tension at 3.2 mmol/l $[Ca^{2+}]_o$, and did not increase contraction velocity or relaxation velocity; at the highest concentration (1 mmol/l) theophylline did not shorten time to peak tension.

The effects of theophylline were not inhibited by the β-adrenoceptor blocking drug propranolol (Figures 1 and 2).

Calcium

Increasing $[Ca^{2+}]_o$ from 0.9 to 3.2 mmol/l increased peak tension, time to peak tension, relaxation time, contraction velocity, and relaxation velocity (Figures 1 and 2).

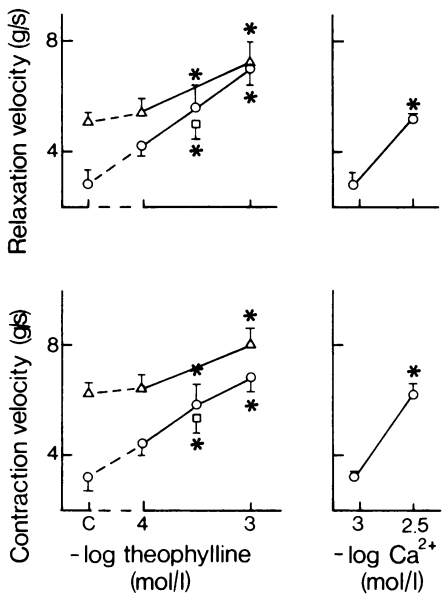

Figure 2 As for Figure 1.

Isoprenaline

Isoprenaline increased peak tension and reduced time to peak tension and relaxation time (Figure 3). All these effects were potentiated by theophylline at 0.9 mmol/l $[Ca^{2+}]_0$. Thus at 0.9 mmol/l $[Ca^{2+}]_0$ the ED_{50} of isoprenaline for the effect on peak tension was 6.3 nmol/l in the absence, and 1.1 nmol/l in the presence, of 1 mmol/l theophylline.

At 3.2 mmol/l $[Ca^{2+}]_0$ the ED_{50} of isoprenaline for the effect on peak tension was 2.2 nmol/litre. Theophylline potentiated the effects of isoprenaline on time to peak tension and relaxation time at the higher calcium concentration but inhibited the effect of isoprenaline on peak tension.

Effects of histamine were similar to those of isoprenaline, both in the absence and presence of theophylline, and at 0.9 and 3.2 mmol/l $[Ca^{2+}]_0$. The ED_{50} of histamine for the effect on peak tension was 8.9 μmol/l in the absence, and 1.9 μmol/l in the presence, of 1 mmol/l theophylline at 0.9 mmol/l $[Ca^{2+}]_0$, and 5.6 μmol/l at 3.2 mmol/l $[Ca^{2+}]_0$.

Phenylephrine

Phenylephrine increased peak tension and time to complete relaxation and did not significantly change time to peak tension (Figure 4).

Theophylline caused phenylephrine to shorten relaxation time, but theophylline did not potentiate the phenylephrine effect on peak tension. The ED_{50} of phenylephrine for the effect on peak tension at 0.9 mmol/l $[Ca^{2+}]_0$ was 1.4 μmol/l in the absence, and 1.7 μmol/l in the presence, of 1 mmol/l theophylline. In the presence of theophylline and propranolol, phenylephrine did not shorten relaxation time. Propranolol did not inhibit the effect of phenylephrine on peak tension; the ED_{50} of phenylephrine for the inotropic effect was 2.0 μmol/l in the presence of 0.3 mmol/l theophylline and 1 μmol/l propranolol.

At 3.2 mmol/l $[Ca^{2+}]_0$ the ED_{50} of phenylephrine for the effect on peak tension was 0.93 μmol/litre. Theophylline caused phenylephrine to shorten time to peak tension and relaxation time at the high calcium concentration and inhibited the phenylephrine effect on peak tension.

Discussion

It is not known why theophylline shortens relaxation time in rabbit atrium and lengthens relaxation time in rabbit ventricle. It may be assumed that theophylline stimulates calcium sequestration in rabbit atrium in a similar manner to isoprenaline and histamine. Whether this effect is due to inhibition of cyclic nucleotide phosphodiesterase and an increase in myocardial cyclic AMP concentration is not known. Theophylline has been reported both to increase cyclic AMP in guinea-pig heart (Kukovetz & Poch, 1971; Watanabe & Besch, 1974; Kukovetz, Poch & Wurm, 1975) and not to increase cyclic AMP in guinea-pig and rat heart (McNeill, Coutinho & Verma, 1974), rat heart slices (LaRaja & Reddy, 1969), and rat atrium (Birnbaum, Abel, Amidon & Buckner, 1975).

Theophylline potentiated the effect of noradrenaline (Rall & West, 1963) and histamine on peak tension (Kukovetz, Poch & Wurm, 1973), but theophylline did not potentiate the effect of phenylephrine on peak tension (Hamakawa, Shimizu & Toda, 1973). Thus there is a difference between agents that stimulate adenylate cyclase and phenylephrine which does not stimulate this enzyme. But there is no evidence that potentiation by methylxanthines is associated with an increase in cyclic AMP concentration. The potentiation of the inotropic effect of noradrenaline and histamine by theophylline was not accompanied by a rise in cyclic AMP (McNeill *et al.*, 1974).

It should be noted that inhibition of cyclic nucleotide phosphodiesterase(s) by methylxanthines interferes with the breakdown not only of cyclic AMP but also of cyclic guanosine 3',5'-monophosphate (cyclic GMP) (Hardman & Sutherland, 1969; Goldberg, O'Dea & Haddox, 1973). Stimulation of guanylate cyclase by muscarinic agents increases tissue cyclic GMP levels (Goldberg, Haddox, Nicol, Glass, Sanford, Kuehl & Estensen, 1975). Acetylcholine increased the cyclic GMP concentration in guinea-pig ventricle, antagonized the inotropic effect

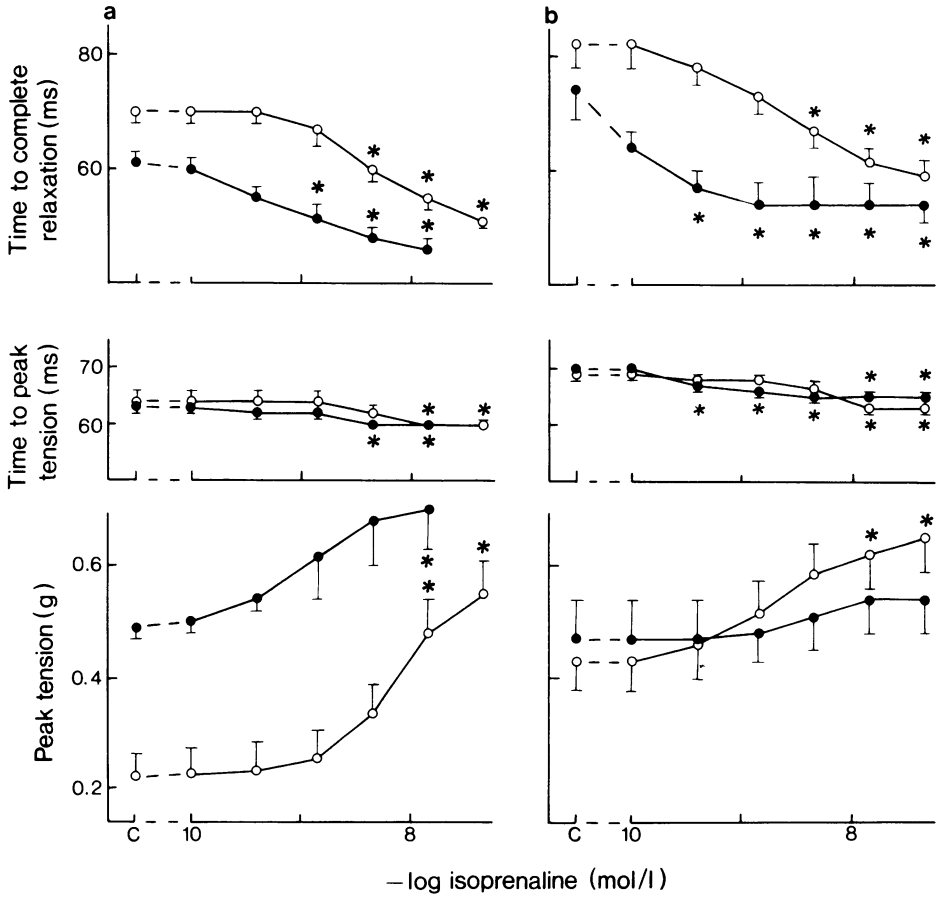

Figure 3 Effects of isoprenaline (a) at 0.9 mmol/l $[Ca^{2+}]_0$ and (b) at 3.2 mmol/l $[Ca^{2+}]_0$ in the absence (○) and presence of 1 mmol/l theophylline (●). Means of 4–10 experiments. Vertical lines show s.e. $^*P < 0.05$, compared to control (C).

of isoprenaline and histamine, and did not inhibit the rise in cyclic AMP concentration produced by isoprenaline and histamine; it was suggested that cyclic GMP inhibits the inotropic effect of cyclic AMP (Watanabe & Besch, 1975). Can we assume that in the presence of theophylline the positive inotropic effect of cyclic AMP outweighs the negative inotropic effect of cyclic GMP? We have no data on myocardial cyclic GMP levels in the presence of theophylline.

There is controversy about the existence of calcium currents and the involvement of calcium-triggered calcium release from the sarcoplasmic reticulum in contractile activation. Catecholamines, histamine, and methylxanthines which increased cyclic AMP in guinea-pig hearts increased the rate of calcium entry via a slow inward current; ouabain or glucagon which did not increase cyclic AMP had no effect on the slow inward current (Thyrum, 1974; Watanabe & Besch, 1974).

It has been suggested that in cat, dog and rat heart contraction might be initiated largely by calcium released from stores near the myofilaments, most probably the sarcoplasmic reticulum, whereas in rabbit heart activating calcium might originate from superficial sites in the cell (Henderson *et al.*, 1974; Bodem & Sonnenblick, 1975). A methylxanthine might reduce the amount of calcium available to be released from internal stores by impairing sequestration, but might allow an increased calcium influx to exceed the concurrent sequestration rate during contraction so that the free calcium at the active sites rises. The effect of the methylxanthine on peak tension would then depend upon the relative contribution of calcium influx and calcium released from internal stores, and upon the extent to which these are affected by the methylxanthine.

Theophylline inhibited the effect of isoprenaline, histamine and phenylephrine on peak tension in rabbit

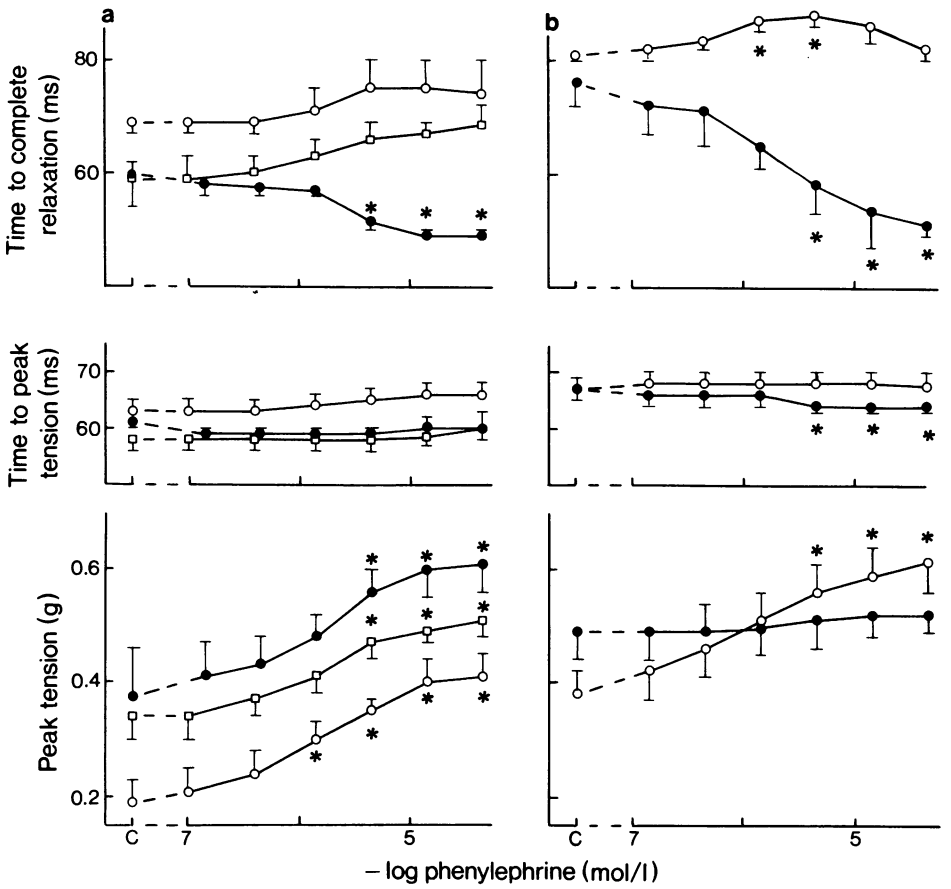

Figure 4 Effects of phenylephrine (a) at 0.9 mmol/l $[Ca^{2+}]_0$ and (b) at 3.2 mmol/l $[Ca^{2+}]_0$ in the absence (○) and presence of 1 mmol/l theophylline (●) and 1 μmol/l propranolol and 0.3 mmol/l theophylline (□). Means of 4–9 experiments. Vertical lines show s.e. $*P < 0.05$, compared to control (C).

atrium when the calcium concentration was high (3.2 mmol/litre). It could be suggested that the strong effect of the drugs on relaxation interfered with the effect on tension and terminated contraction prematurely (Reiter & Schober, 1965; Kavaler & Morad, 1966).

Contracture did not occur in rabbit atrium in the presence of theophylline at 3.2 mmol/l $[Ca^{2+}]_0$. In guinea-pig atrium 2.8 mmol/l theophylline, which produced the maximal increase in peak tension at 1.8 mmol/l $[Ca^{2+}]_0$, caused contracture when $[Ca^{2+}]_0$ was increased to 3.6 mmol/l (Scholz & de Yazikof, 1971).

In conclusion, it is evident that there are species differences in the mechanism of the inotropic effect of theophylline. Also, it appears that drugs exert their effect on relaxation independently of their effect on contraction, which has been proposed for catecholamines (Morad & Rolett, 1972).

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