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A23187: A Calcium Ionophore that Directly Increases Cardiac Contractility (38704)

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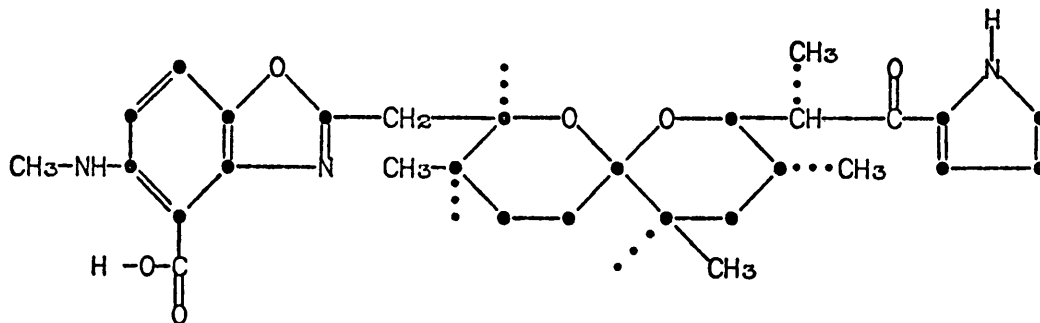
Contraction in cardiac muscle is initiated during the action potential by the movement of calcium ions to troponin binding sites. This activator calcium is made available by release of intracellular stores of calcium (1) as well as by influx of extracellular calcium (2). A juxtasarcolemmal binding site may mediate this influx by acting as a carrier for calcium ions from the extracellular space to troponin binding sites (3, 4).

The ionophore A23187[1] selectively binds divalent ions (6) and acts as a calcium ion carrier across a number of biological membranes (7–10). Such a calcium ion carrier might be expected to enhance the delivery of extracellular calcium to troponin binding sites in the myocardial cell and increase contractile force. Another calcium ionophore, X537A, has been extensively studied with cardiac preparations and has been found to increase contractile force (11–13). However, it is much less specific as an ion carrier than A23187 since it also effectively transports monovalent ions (14). Furthermore, X537A can form complexes with catecholamines (15) and can release them from adrenergic nerve terminals in the heart and vas deferens in concentrations that increase cardiac contractility (16). In contrast we have found that A23187, a specific divalent ionophore, increases myocardial contractility (17) but its effects are not dependent on the release of endogenous norepinephrine.

Materials and Methods. Female guinea pigs (250–350 g) were stunned by a blow on the head and the left atrium was dissected. The tissue was mounted horizontally in a 5 ml organ bath according to conventional techniques (18) and isometric tension was measured. Resting tension was set at a value predetermined to give half maximal contractile tension. Stimuli of one msec duration were continuously delivered at a rate of 2 Hz through bipolar silver electrodes.

In most experiments a modified Chenoweth-Koelle buffer (saturated with 95% oxygen, 5% carbon dioxide) was used. The composition of this buffer was: NaCl, 120 mM; KCl, 5.6 mM; $CaCl_2$, 2.2 mM; $MgCl_2$, 2.2 mM; $NaHCO_3$, 25 mM; dextrose, 10 mM. In studying the effect of external calcium ion concentration, however, the following buffer (Tris buffer) was used: NaCl, 148.2 mM; KCl, 5.6 mM; $CaCl_2$, 2 mM; $MgCl_2$, 0.5 mM; dextrose, 10 mM; Tris (hydroxymethyl) aminomethane, 5 mM. This buffer was saturated with 100%

[1] A23187 was isolated from a strain of Streptomyces chartreusis (5) and has the following chemical structure:



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oxygen. The tissue was equilibrated at 35° for 30–60 min prior to the experiment. During equilibration the bathing solution was changed every 5 min. A23187 was added to the organ bath as an opalescent, aqueous suspension made by diluting a stock solution of ionophore dissolved in 100% dimethylsulfoxide (DMSO). The maximal volume of DMSO added to the bath by this technique was 5 μl and this produced no inotropic effect in four control experiments.

In order to reduce the variability of the contractile response of atrial preparations to A23187 and to obtain an index of its intrinsic inotropic activity, results were expressed when possible as a percent of the maximal response elicited by a test concentration of norepinephrine (3×10^{-5} *M*). The test concentration of norepinephrine was added after removal of the ionophore and at a time when the tissue had returned to control values. The maximum attainable contractile force (±SEM) without prior exposure of the tissue to the ionophore was 204 ± 28% increase from control. That attainable after exposure to ionophore was 228 ± 15% increase from control.

Results and Discussion. Following addition of A23187 to the organ bath, both the force of contraction (Fig. 1a) and the rate of tension development (Fig. 1b) increased in a concentration dependent manner. The onset of the inotropic effect was clearly evident within 1 minute and peak response was achieved within 2–5 min depending on ionophore concentration. A positive inotropic effect was evident until the conclusion of the experiments which extended up to 30 min.

Pretreatment with reserpine did not alter the effects of the ionophore (Fig. 1a, b). In contrast, we found that pretreatment of guinea pigs with reserpine greatly reduced the inotropic effect of tyramine, which is known to act by releasing endogenous stores of norepinephrine (19). Propranolol, which is known to block β-receptor agonists (20), also failed to diminish the positive inotropism of A23187 (Fig. 2). The increase in contractile force produced by norepinephrine, however, was reduced by propranolol in our system.

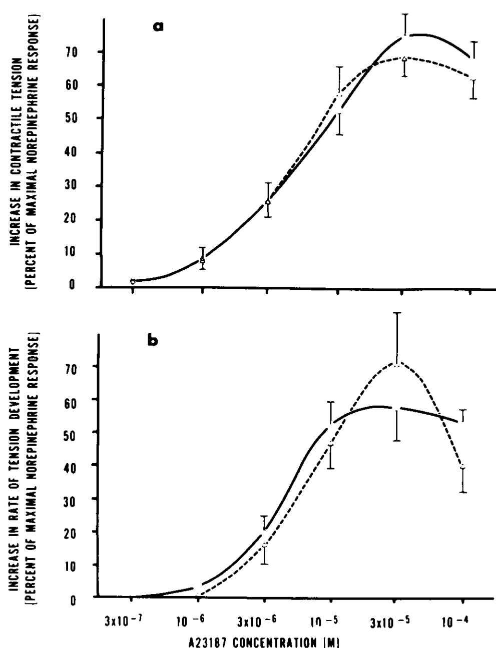

FIG. 1. Increases in contractile tension (a) and maximal rate of tension development (b) produced by A23187. Single concentrations of A23187 were added to each atrium. Individual points on the graph represent the means of 5–11 experiments and reference bars represent standard errors of the mean. Values for control tissue, o, and reserpine pretreated tissue, Δ, were computed as a percentage of the maximal increases obtained with norepinephrine. Maximal contractile tension was determined after the tissue was washed three times with buffer and the contractile force was allowed to return to the initial equilibrated value. EDTA ($4 \times 10^{-5}M$) was added to the bath and five minutes later, *l*-norepinephrine bitartrate ($3 \times 10^{-5}M$) was added. Reserpine (5 mg/kg) dissolved in 20% ascorbic acid was injected intraperitoneally 24 hr prior to the experiments. Mean control values (± SEM) of F and *dF/dt* were 0.396 ± 0.021 and 17.1 ± 1.2 g/sec, respectively. Maximal values of *F* and *dF/dt* were 1.159 ± 0.049 g and 52.1 ± 3.5 g/sec.

The positive inotropic effect of A23187 therefore, is not mediated either by activation of β-receptors or by the release of endogenous catecholamines. The findings of Thoa *et al.* (16) appear to support our data since norepinephrine was not released from rat atria by concentrations of A23187 below 3×10^{-5} *M*. Another ionophore X537A is less specific than A23187 in that it binds not only divalent ions but also binds

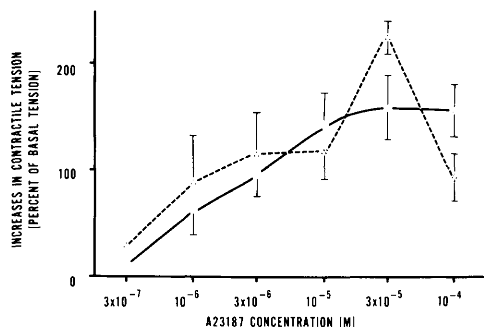

FIG. 2. Increases in contractile tension produced by A23187 in the presence, Δ, and absence, o, of propranolol (3 × 10^{-6} *M*). Values were expressed as a percent increase over basal tension just prior to addition of the ionophore. Propranolol HCl was added to the bath in 0.9% saline 15 min before A23187. The mean basal tension (±SEM) was 0.380 ± 0.019.

monovalent ions and catecholamines (15). This ionophore also increases cardiac contractility but, unlike A23187, it apparently acts, at least in part, by the release of endogenous stores of norepinephrine since propranolol and reserpine pretreatment markedly attenuate or abolish the response (11–13).

Earlier investigations failed to show a positive inotropic activity of A23187 in cardiac muscle. Schaffer *et al.* (11) reported that A23187 (10^{-7} *M*–10^{-6} *M*) was without significant effect on cardiac contractility but that it increased coronary flow. Schwartz and coworkers (12) reported that in guinea pig left atria, A23187 (4.5 × 10^{-6} *M*) had no effect on contractility. By contrast, our data show that A23187 (10^{-6} *M*–10^{-4} *M*) increases contractility in isolated guinea pig left atria (Fig. 1a). We have not attempted a rigorous evaluation of the source of the discrepancy between our results and those previously reported by others, however, several factors which may contribute to this discrepancy emerged from the following observations: (a) Preliminary experiments revealed that the negative inotropic effects of certain solvents such as ethanol can effectively mask the positive inotropic action of the ionophore. (b) We observed flocculent precipitation when the ionophore, dissolved in ethanol or DMSO, was added directly to buffer solutions. The preparation of a finely divided aqueous suspension as described above appeared to prevent this. (c) Lack of stability of stock solutions may be a source of variance inasmuch as DMSO stock solutions darken with time due to an apparently light dependent reaction. (d) The use of concentrations below the range that might be expected to produce substantial increases in contractility is also a possible cause of discrepancy. This may explain the negative findings of Schaffer *et al.* (11).

Scholtz (21) showed that the contractile force of guinea pig atrium is a function of external calcium concentration. We obtained similar data (Fig. 3). When A23187 was added to the bathing solution, the curve relating contractile tension to calcium concentration was shifted to the left so that less calcium was required to produce a given amount of tension (Fig. 3). The enhancing effect of the ionophore on contractility was statistically significant at the 0.02 level in the external calcium concentration range of 0.7 m*M*–4 m*M*. Above 4 m*M* calcium, the differences shown in Fig. 3 were not significant at the 0.05 level suggesting that both curves approach the same plateau.

Chapman and Tunstall (22) analyzed the effect of external calcium concentration on myocardial contractility. Their data support the conclusion that the cube root of the contractile force is a function of external calcium concentration. Using this relationship we have calculated the maximum force of contraction (F_{max}) and the calcium concentration at half maximal force (K_m) by the method of Wilkinson (23). The results are shown in Table I. It is apparent that, at each concentration used, the ionophore significantly reduced the K_m ($P < .01$). F_{max}, however, was not significantly changed except at the highest concentration of ionophore (3 × 10^{-5} *M*).

Our studies of the dependence of contractile force on external calcium indicate that A23187 enhances the response of atrial muscle to external calcium ions. The contractile machinery of the muscle does not appear to be altered by A23187 since the maximum contractile force was unchanged

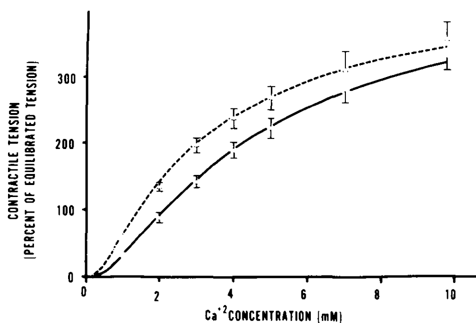

FIG. 3. Influence of A23187 on contractile response of guinea pig left atria to increasing concentrations of external $CaCl_2$. Contractile tension was calculated in both the presence, Δ, and absence, o, of A23187 (10^{-5} *M*) as a percentage of the force following equilibration in TRIS buffer containing 2 m*M* $CaCl_2$. The bathing solution was then changed to a TRIS buffer containing 0.7 m*M* $CaCl_2$. Five minutes later we added either A23187 or solvent control. The contractile force in each atrium was determined over the full range of external calcium concentrations by cumulative addition of $CaCl_2$. Both curves in the figure were plotted from F_{max} and K_m values derived from 13 experiments as described in the text. Standard errors of the mean are indicated as reference bars. Mean tension (±SEM) after equilibration in TRIS buffer containing 2 m*M* $CaCl_2$ was 0.441 ± 0.031 g.

TABLE I. INFLUENCE OF A23187 ON K_m AND F_{max} OF THE CONTRACTILE RESPONSE TO INCREASING CONCENTRATIONS OF EXTERNAL $CaCl_2$.[a]

A23187 concentration (*M*)	K_m (m*M*)	P[b]	F_{max} (% of initial contractile force)	P
0	1.455±0.075		489±23	
3×10^{-6}	1.189±0.045	<0.01	518±16	ns[c]
10^{-5}	0.932±0.052	<0.001	455±18	ns
3×10^{-5}	1.014±0.032	<0.001	559±10	<0.05

[a] Each value ± standard error of the mean represents from 8 to 13 experiments.

[b] Determined by Student's *t* test.

[c] Not significant at the 0.05 level.

at ionophore concentrations that produced marked positive inotropism (3×10^{-6} *M*, 10^{-5} *M*). The decrease in apparent K_m suggests that the ionophore altered the transmembrane or intracellular translocation of calcium required for contraction.

Since A23187 is known to facilitate the movement of calcium across biological membranes (7–10), we believe that the ionophore may act by increasing calcium influx across the myocardial cell membrane. Alternatively, the ionophore may enter the cell and facilitate calcium release from sarcoplasmic reticulum. This ionophore-induced release of calcium has been shown to occur in fragmented sarcoplasmic reticulum (8). Either of these mechanisms could increase the basal intracellular calcium activity at the troponin binding sites and potentiate the effect of activator calcium entering the myoplasm during the action potential. X537A, the only other calcium ionophore whose effects on cardiac contractility (as far as we know) have, been systematically investigated, possesses multiple pharmacologic actions which could account for its inotropic activity. These include alteration of sodium potassium-ATPase activity in the myocardial cell membrane (12) and the release of endogenous stores of catecholamines (11–13). A23187 may, therefore, be the prototype for a class of cardiotonic agents that produce a positive inotropic effect by directly altering intracellular calcium activity.

Summary. The effect of the calcium ionophore A23187 on contractility in guinea pig left atria was studied. This ionophore increased both the force of contraction and the rate of tension development in a concentration dependent manner. The positive inotropic effect of A23187 is not mediated by activation of β-receptors or by the release of endogenous catecholamines since neither propranolol nor pretreatment with reserpine altered the inotropic effect of this agent. Our studies of the dependence of contractile force on external calcium indicate that A23187 enhances the response of atrial muscle to external calcium ions. Since A23187 is known to facilitate the movement of calcium across biological membranes, we believe the ionophore may be acting either by increasing calcium influx across the myocardial cell membrane or by facilitating calcium release from sarcoplasmic reticulum.

1. Morad, M., and Goldman, Y., *in* "Progress in Biophysics and Molecular Biology" (J. A. V.

Butler and D. Noble, eds.), Vol. 27, p. 257. Pergamon Press, New York (1973).
2. Sandow, A., Yale J. Biol. Med. **25**, 176 (1952).
3. Lüttgau, H. C., *in* "International Symposium on the Electrophysiology of the Heart" (B. Taccardi and G. Marchetti, eds.), p. 87. Pergamon Press, New York (1965).
4. Langer, G. A., Ann. Rev. Physiol. **35**, 55 (1973).
5. Hamill, R. L., Gorman, M., Gale, R. M., Higgens, C. E., and Hoehn, M. M., Twelfth Intersci. Conf. Antimicrobial Agents and Chemotherapy, Abstr. 65 (1972).
6. Reed, P. W., Fed. Proc. Fed. Amer. Soc. Exp. Biol. **31**, 432 (1972).
7. Caswell, A. H., and Pressman, B. C., Biochem. Biophys. Res. Commun. **49**, 292 (1972).
8. Scarpa, A., Baldassare, J., and Inesi, G., J. Gen. Physiol. **60**, 735 (1972).
9. Foreman, J. C., Mongar, J. L., and Gomperts, B. D., Nature (London) **245**, 249 (1973).
10. Wong, D. T., Wilkinson, J. R., Hamill, R. L., and Horng, J., Arch. Biochem. Biophys. **156**, 578 (1973).
11. Schaffer, S. W., Safer, B., Scarpa, A., and Williamson, J. R., Biochem. Pharmacol. **23**, 1609 (1974).
12. Schwartz, A., Lewis, R. M., Hanley, H. G., Munson, R. G., Dial, F. D., and Ray, M. V., Circ. Res. **34**, 102 (1974).
13. Levy, J. V., Cohen, J. A., and Inesi, G., Nature (London) **242**, 461 (1973).
14. Pressman, B., Fed. Proc. Fed. Amer. Soc. Exp. Biol. **32**, 1698 (1973).
15. Pressman, B. C., *in* "The Role of Membranes in Metabolic Regulation" (M. A. Mehlman and R. W. Hanson, eds.) p. 149. Academic Press, New York (1972).
16. Thoa, N. B., Costa, J. L., Moss, J., and Kopin, I. J., Life Sci. **14**, 1705 (1974).
17. Holland, D. R., Steinberg, M. I., and Armstrong, W. McD., Pharmacologist **16**, 245 (1974).
18. Levy, J. V., *in* "Methods in Pharmacology" (A. Schwartz, ed.), Vol. 1, p. 77. Meredith, New York (1971).
19. Trendelenburg, U., Pharmacol. Rev. **15**, 225 (1963).
20. Epstein, S. E., and Braunwald, E., New Engl. J. Med. **275**, 1106 (1966).
21. Scholz, H., Pflüegers Arch. **308**, 315 (1969).
22. Chapman, R. A. and Tunstall, J., J. Physiol. (London) **215**, 139 (1971).
23. Wilkinson, G. N., Biochem. J. **80**, 324 (1961).

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