Negative Inotropic Effects of ATP on the Isometric Contractions of Isolated Rat Heart Muscle

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The effects of ATP on the isometric contractions of isolated rat left ventricular papillary muscle were studied. Exogenously administered ATP had an immediate onset, an abrupt response, progressive recovery and produced dose-related depression in the peak developed tension, maximum rate of tension development and relaxation, which were statistically significant. There were no significant changes in the resting tension, time to peak tension and relaxation time, except for a significantly prolonged relaxation time at the highest concentration of ATP. In the studies of interactions of ATP and either epinephrine or Ca^{++}., we observed that ATP seemed to interfere with the inotropic effect of epinephrine, while Ca^{++} antagonized the negative inotropic action of ATP. We conclude that the site of negative inotropic action of ATP is most likely on the cell membrane, where ATP interferes with Ca^{++} flux, and that ATP interferes with the positive inotropic action of epinephrine. (Key words: adenosine triphosphate, rat heart muscle, negative inotropic action, epinephrine, Ca^{++})

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Since the original report of the effects of adenosine compounds on the mammalian heart by Drury and Szent-Gyorgyi[1], exogenously applied adenosine compounds have been studied and shown to depress the contractility of rat, rabbit, cat and guinea pig isolated atria[2–7]. There are, however, some conflicting results concerning the effects of adenosine compounds on the myocardial contractility[8–11]. It is also known that adenosine and ATP are potent coronary as well as systemic vasodilators[12–16] and decreases heart rate[12,16,17]. This study is designed to observe the direct myocardial effects of ATP on the isolated rat ventricular muscle and to find the possible mechanisms of action.

Methods

Isolated columns of rat left ventricular papillary muscle were prepared and examined in isometric contractions. Rats were 6–7 months old. Each preparation was placed in an oxygenated (Oxygen 95% and carbon dioxide 5%) 80 ml capacity muscle bath containing modified Krebs-bicarbonate solution, as described previously[18]. The temperature of the bath solution was held constant at 34°C by a thermoregulator (Precision Scientific Co.) and pH was stabilized at 7.40. One end of the muscle was fixed and the other was connected to a force displacement transducer (Grass FT03C) and its output recorded on one channel, while another output was connected to the differentiator (Optical Electronics Inc., model 9009) and recorded on another channel of the recorder (Gould Brush Recorder 2400). Each muscle

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Table 1. Effects of Adenosine Triphosphate on Isometric Contractions of Isolated Rat Heart Muscle (Means±SD)

ATP ($\times 10^{-4}$M)	Tpd	$+\frac{dP}{dt}$max	$-\frac{dP}{dt}$max	Tr	TPT	RT_{90}
2.06 × $\frac{1}{2}$	− 8.05±3.30*	− 6.53±3.42*	− 6.32±3.42*	−2.09±3.48	−0.70±2.50	+1.12±3.33
2.06 × 1	−12.33±6.27*	−13.66±7.67*	−15.21±6.46*	−2.64±3.71	+1.31±4.50	−0.35±5.54
2.06 × 2	−24.28±14.42*	−19.77±11.52*	−23.74±11.72*	−2.78±3.96	−2.38±5.56	+2.41±5.28
2.06 × 3	−29.97±9.88*	−29.10±10.96*	−31.18±13.28*	−4.38±5.97	−2.74±4.07	+5.01±4.61*

% depresson from control: Tpd, peak developed tension; $\pm\frac{dP}{dt}$max, maximum rate of tension development and relaxation; Tr, resting tension; TPT, time to peak tension; RT_{90}, relaxation time for tension to decay 90% of maximum; ATP, Adenosine Triphosphate; M, Molar concentration; *P <0.05.

was stimulated (Grass SD9 Stimulator) with 2 × 8 mm platinum plate electrodes, placed about 10 mm apart parallel to the muscle, at a frequency of 0.25 Hz with square wave pulse of 6.0 ms duration and voltages 10 percent above threshold. The length-tension curve of each preparation was determined after 30 min of isometric contractions.

Changes in muscle length measured with a micrometer transformer could be detected to 0.01 mm. The maximum muscle length (the length at the peak of the length-tension curve) was 5.79 ± 1.30 mm. The muscle was held at the peak of the length-tension curve for 30 min for stabilization and then peak developed tension (Tpd), resting tension (Tr), maximum rate of tension development ($+\frac{dP}{dt}$ max) and relaxation ($-\frac{dP}{dt}$ max), time to peak tension (TPT) and relaxation time (RT, time for Tpd to decay 90% of maximum) were recorded at a paper speed of 100 mm per second.

ATP (Sigma, from equine muscle disodium salt) was prepared in a solution of 10 mg/ml in normal saline (Travenol). Doses of 5, 10, 20 and 30 mg were chosen for dose response studies. The final concentrations in the 80 ml bath were $0.206 \times 10^{-3} \times 1/2$ (A), 0.206×10^{-3} (B), $0.206 \times 10^{-3} \times 2$ (C) and $0.206 \times 10^{-3} \times 3$ (D) M respectively. The dose selected was added directly to the muscle bath solution.

Epinephrine (Parke-Davis) was prepared in a solution of 50 μg/ml in normal saline. The final concentration of 1 ml of this diluted epinephrine in the 80 ml bath was 3.42×10^{-6}M.

The final concentration of 0.1 ml of 10% $CaCl_2$ (Elkins-Sinn, Inc.) in 80 ml bath was 1.14×10^{-3}M.

a) *ATP dose-response studies*

After control twitches were obtained, the four prepared concentrations of ATP were studied in random order. One selected dose was added directly to the muscle bath solution and the isometric contractions at the maximum depression which occurred within $1 \sim 1\frac{1}{2}$ min after administration of ATP were recorded. The bath solution was then drained, the muscle was washed twice with fresh solution and the bath was refilled. All isometric contractions were recorded at the paper speed of 100 mm/sec. Statistical significance was determined by paired t-test, p valued < 0.05 were considered significant.

b) *Interaction of ATP with epinephrine or Ca^{++}*

For these studies, concentration D of ATP, (0.206×10^{-3}M × 3), 3.42×10^{-6}M of epinephrine and 1.14×10^{-3}M of Ca^{++} were used. Two different recordings were made at a paper speed of 5×10^{-2} mm/sec. One was the effects of ATP on isometric contractions of heart muscle which was pretreated with either epinephrine or with Ca^{++}. The other was the effect of epinephrine or Ca^{++} with ATP pretreatment.

Results

a) *Direct effects of ATP on isometric contraction* (table 1)

All preparation of ATP in this study attenuated the Tpd, $+\frac{dP}{dt}$ max and $-\frac{dP}{dt}$ max in a dose-dependent fashion. The responses

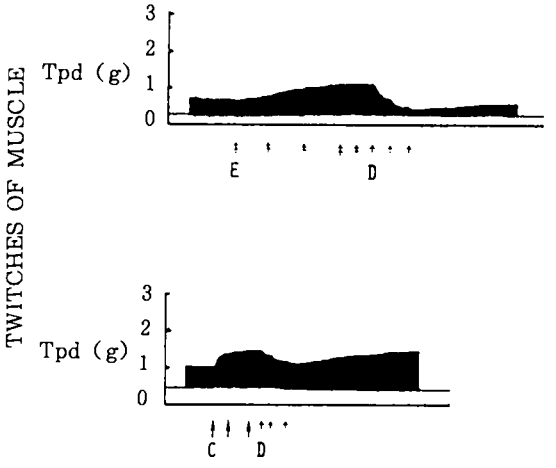

Fig. 1. Effects of adenosine triphosphate (ATP) on isometric contractions pretreated with either epinephrine or Ca^{++}, showing that ATP depressed the epinephrine-pretreated contractions asmuch as it did in the absence of epinephrine while Ca^{++}-pretreated contractions were much less depressed by ATP. ↑D: ATP 0.206 × 3 × 10^{-3}M. ↑ E: epinephrine 3.42 × 10^{-6}M, ↑ C: Ca^{++} 1.14 × 10^{-3}M. Tpd: peak developed tension.

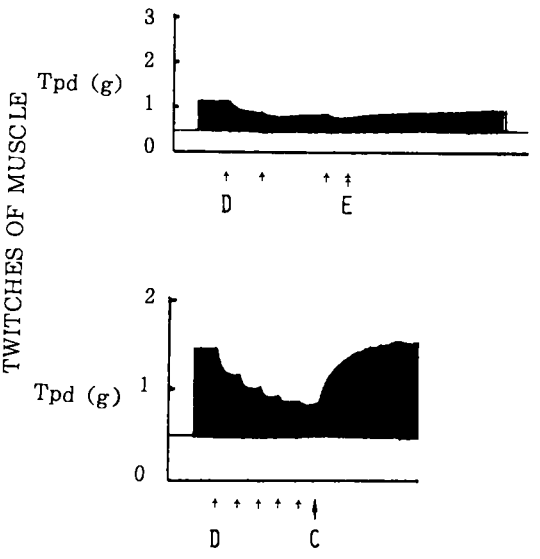

Fig. 2. Effects of epinephrine and Ca^{++} on isometric contractions pre-treated with ATP (adenosine triphosphate), demonstrating that epinephrine could not restore the contractions to normal while Ca^{++} did: ↑ D: ATP 0.206 × 3 × 10^{-3}M. ↑ E: epinephrine 3.42 × 10^{-6}M. ↑ C: Ca^{++} 1.14 × 10^{-3}M. Tpd: peak developed tension.

were immediate (within a few seconds), depressions were abrupt, with maximum depression occurring in 1 ~ $1\frac{1}{2}$ min after administration of ATP, and recoveries were steadily progressive.

There were no changes in Tr. There was a significant prolongation in RT at the highest concentration (D) of ATP, while no significant changes in TPT were observed.

b) Interaction of ATP with epinephrine or Ca^{++} (fig. 1 and 2)

As is shown in figure 1, in the presence of epinephrine, ATP (D) depressed the isometric contractions of rat muscle in the same manner that ATP did in the absence of epinephrine: However, ATP did not depress the contractions in the presence of Ca^{++}. In these instances, there was much less depression and more rapid recovery.

In the presence (near saturation) of ATP, depressed isometric contractions could not be restored to normal by epinephrine, while they were restored by Ca^{++}. All results were duplicated.

Discussion

Our experiments clearly demonstrated that ATP depressed the isometric contractions of isolated rat left ventricular papillary muscle in a dose-dependent fashion.

The immediate onset and abrupt depressions caused by ATP suggest that ATP may have an effect on the cell membrane. This is most likely due to a direct effect by ATP itself rather than to the products of any metabolic process. Our results agree with those of Hollander and Webb[3] who suggested that the site of action is the cell membrane because of the rapidity of action and the impermeability of cells to large molecular purine nucleotides. There is suggestive evidence, however, that ATP may enter the cell in the form of ADP[10]. It appears that the high-energy phosphate bonds of ATP are not associated with the activity of ATP since both adenosine[3] and GTP (guanosine triphosphate)[11] showed the same effect.

The fact that ATP demonstrated the same degree of depression of isometric con-

traction in the absence and in the presence of epinephrine strongly suggests that ATP does not directly involve the same site of action that epinephrine involves. Similar conclusions were reached by Yatani et al.[11] who observed that propranolol did not produce any significant effects on the contractile tension of the bull frog atrial muscle and, in the presence of propranolol, the dose-response curve for adrenaline shifted to the right but that for ATP did not.

It is not yet clear, however, why the depression produced by ATP could not be restored to control level by epinephrine. It seems reasonable, at present, to postulate that ATP may indirectly attenuate the β-receptor response to catecholamine. Schrader et al.[7,19] reported that adenosine attenuated the positive inotropic effect of catecholamine in isolated guinea pig heart and that this nucleoside effectively inhibited transmembrane Ca^{++} flux into the catecholamine-stimulatecd atrial muscle.

However, ATP did not depress the contractions in the presence of Ca^{++} as much as it did without Ca^{++} pretreatment. It is conceivable that Ca^{++} antagonizes the depressnat effect of ATP on the isolated rat ventricular papillary muscle.

It has been observed and suggested that the nucleoside effectively inhibits transmembrane Ca^{++} flux into atrial muscle[4,7]. In addition, adenosine derivatives decrease the force of contraction and the plateau phase of action potential which may largely depend on calcium current, and these nucleosides may be involved in modulation of the calcium channel[11]. This is suggested by our observation that Ca^{++} effectively restored the depressed contractions produced by ATP to the control level.

We found in this study that RT was significantly prolonged at the highest concentration of ATP. This may also suggest that the calcium sequestrating system (sarcolemma and sarcoplasmic reticulum) is involved and the rate of active re-uptake of Ca^{++} by sarcoplasmic reticulum is delayed. However, the basic mechanism of action of ATP on RT is not yet fully understood.

The well-known negative chronotropic action of ATP and related compounds may be due (1) to direct (vagal) action[20]; (2) to direct action[17] on the SA node since intravenous administration of atropine did not alter the effect of ATP; (3) to effects of ATP on cardiac muscle cells themselves, since recent observation indicated that adenosine may inhibit noradrenaline release in a number of tissues[15], and (4) to another action that affects the re-uptake of Ca^{++} by sarcoplasmic reticulum.

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