MOLECULAR PHARMACOLOGY, 19:525–528

Effect of Adenosine Triphosphate on Contractility and Adenosine Triphosphatase Activity of the Rabbit Urinary Bladder

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Received July 28, 1980; Accepted January 26,1981

SUMMARY

LEVIN, R. M., R. JACOBY, AND A. J. WEIN. Effect of adenosine triphosphate on contractility and adenosine triphosphatase activity of the rabbit urinary bladder. *Mol. Pharmacol.* **19**:525–528, (1981).

There is circumstantial evidence that ATP may be an excitatory neurohumoral transmitter in the urinary bladder of several species. Muscle bath studies *in vitro* demonstrated that exogenously applied ATP can produce a dose-dependent contraction of the rabbit urinary bladder. These studies indicate that the concentrations of ATP necessary to stimulate bladder contraction are significantly greater than one would expect if ATP were acting through a neurohumoral receptor system. The purpose of our study was to compare the contractile action of ATP on isolated strips of urinary bladder with the ATP hydrolysis activity of these strips. The results from these studies demonstrate that, over the time course of the contractile effect of ATP, less than 0.1% of the ATP present in the bath is hydrolyzed by the tissue. Thus, the requirement for high concentrations of ATP for contractile stimulation cannot be ascribed to ATP hydrolysis by the tissue. Additionally, it is interesting to note that β,γ-methylene ATP was approximately 100 times as potent as disodium ATP in its ability to stimulate contraction in the rabbit urinary tract. This difference in potency is probably a function of the structure of β,γ-methylene ATP rather than its resistance to hydrolysis by ATPase.

There is circumstantial evidence that ATP is a neurohumoral transmitter in certain specific smooth muscle organ systems (1–3). In the rabbit urinary bladder, ATP has been demonstrated to produce a direct stimulation of contraction, and there is evidence that ATP may mediate the contractile effect of low-frequency field stimulation (3). One of the major difficulties in establishing ATP as a specific neurohumoral transmitter concerns the relatively high concentrations of ATP which are required to stimulate contraction (3, 4). These concentrations are significantly greater than one might expect if it were working through a neurohumoral receptor system. One explanation might be that the tissue rapidly hydrolyzes the ATP, thus requiring high concentrations of ATP in order to stimulate contraction (5, 6).

ATPase, the enzyme responsible for hydrolyzing ATP, and the production of energy, is a membrane-bound enzyme and has been shown to exist in both external and internal cellular membranes. It is possible that the addition of extracellular ATP may affect intracellular processes by the stimulation of an "ecto-ATPase" (an ATPase present in the external cell membrane) (7, 8). In this manner, the enzyme ATPase may provide a binding site for extracellular ATP independent of any "purinergic" receptor site.

The purpose of this study was to compare the contractile effect of ATP with the rate of disappearance of ATP from the muscle bath, and to characterize the ATP hydrolysis of the smooth muscle strips.

Urinary bladders of 12 adult, male New Zealand White rabbits were removed under light sodium pentobarbital anesthesia. The bladders were dissected free of fat and serosa. Longitudinal smooth muscle strips of bladder detrusor (1 cm × 0.5 cm) were mounted in a 30-ml glass chamber containing Tyrode's solution equilibrated with a gas mixture of 95% O_2/5% CO_2, and maintained at 37°. Contractility was monitored with a Grass force displacement transducer connected to a four-channel Beckman recorder.

After equilibrating for approximately 1 hr, a tension of 1 g was placed on each strip. Disodium ATP, β,γ-methylene ATP, or α,β-methylene ATP was dissolved in deionized water and added in 500-μl aliquots. Dose-response curves were performed by discontinuous addition of ATP. After the response to one ATP concentration, the tissues were washed thoroughly and allowed to re-

This work was supported in part by research grants from the Veterans Administration and the McCabe Fund, and by National Institutes of Health Grant 1RO1-AM-26508-01.

0026-895X/81/03525-04$02.00/0

equilibrate for approximately 20 min prior to subsequent dose-response trials. ATP concentrations presented are the total concentrations in the bath. A minimum of six concentrations was used for each curve.

Urinary bladder strips were mounted as previously described. ATP (1 mM or 5 mM final concentration) was added to the baths and the contractile effect was monitored; 100-μl samples of bath were removed at times ranging between 5 sec and 48 min and analyzed for ATP by using firefly luciferase and a DuPont luminescence biometer. Additionally, the disappearance of ATP from bladder strips incubated in 5 ml of buffer containing 1 or 5 mM ATP was determined.

The urinary bladders of 22 adult male New Zealand White rabbits were removed under light pentobarbital anesthesia. The bladders were prepared in a manner identical with that for the sections utilized for the contractility studies.

The tissue sections were incubated in 15 ml of phosphate-free Tyrode's solution equilibrated with a gas mixture of 95% O_2 and 5% CO_2 and maintained at 37°. The tissues were allowed to equilibrate for approximately 1 hr. The enzyme reaction was initiated with the addition of ATP. Disodium ATP was utilized in the enzymatic studies. At various times following ATP addition, 500 μl of bath medium was removed and added to 500 μl of 15% trichloroacetic acid. The inorganic phosphate present was measured spectrophotometrically by the method of Akera and Brody (9). In all experiments, less than 10% of the substrate was utilized during the incubation.

Figure 1 shows the time course of the contractile effect of ATP (5 mM). The maximal contractile effect occurred within seconds of the addition of ATP and the effect terminated within 3 min. With the use of the firefly luciferase methodology to measure ATP directly, no significant decrease in the concentration of ATP present in the smooth muscle bath occurred within 48 min. Additionally, incubation of muscle strips in 5-ml baths (instead of the 30-ml baths utilized for the contractile studies) in the presence of 1 or 5 mM ATP also failed to show any significant decrease in the concentration of ATP for incubation times up to 48 min.

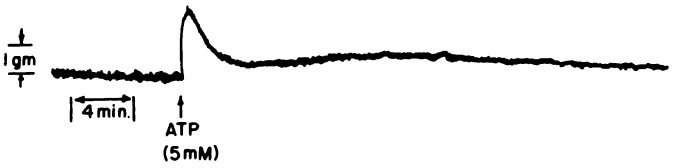

FIG. 1. *Time course of the effect of ATP on rabbit urinary bladder*
Representative tracing of the effect of 5 mM ATP on the contractility of an isolated strip.

Figure 2 demonstrates that both ATP and β-γ-methylene ATP produced a dose-dependent increase in contraction of the bladder detrusor. β,γ-Methylene ATP was approximately 100 times as potent as disodium ATP in its ability to stimulate contraction. Additionally, α,β-methylene ATP was tested for its ability to stimulate contraction in the rabbit urinary bladder. These studies (data not shown) demonstrated that α,β-methylene ATP was equipotent with β,γ-methylene ATP.

Figure 3 demonstrates that ATP (2 mM) is hydrolyzed by the intact bladder strip, and that this hydrolysis is linear up to 40 min. To determine whether the hydrolysis of ATP that we were measuring was a function of the damaged muscle around the cut areas, experiments were performed utilizing the whole bladders (having a mass equal to that of the muscle strips) of small rabbits. In these studies the whole bladders hydrolyzed ATP at a rate of 4.92 ± 1.15 nmoles of Pi per minute as compared with 3.33 ± 0.55 nmoles of Pi per minute for bladder strips of larger rabbits at an ATP concentration of 4 mM. Thus, we feel that the ATP hydrolysis which we were measuring was a function of an ATPase located within the external cellular membrane.

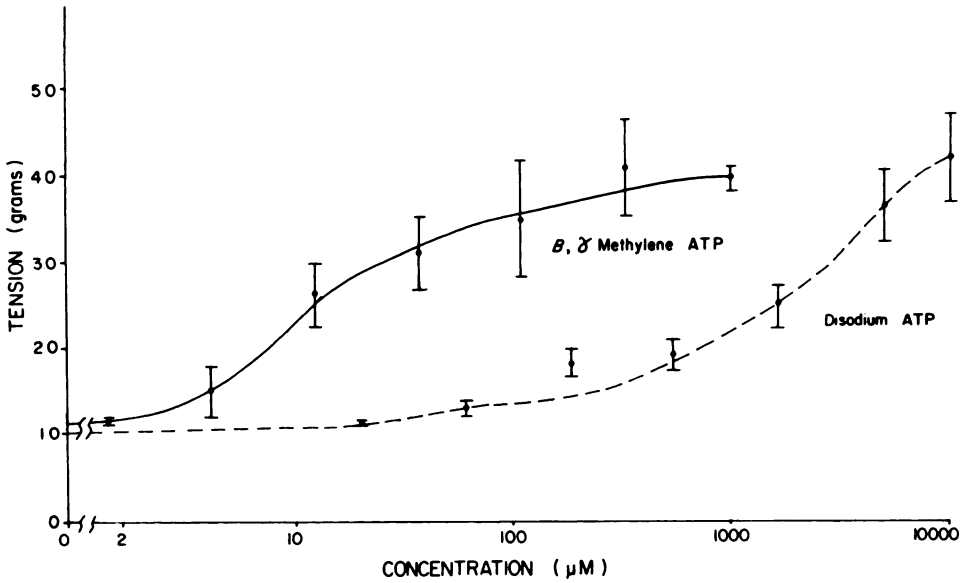

FIG. 2. *Effect of ATP and β,γ-methylene ATP on bladder contractility*
The response of strips isolated from bladder body to ATP and β,γ-methylene ATP was determined as described in text. Each value represents the mean of three to six separate determinations; vertical brackets indicate the standard error.

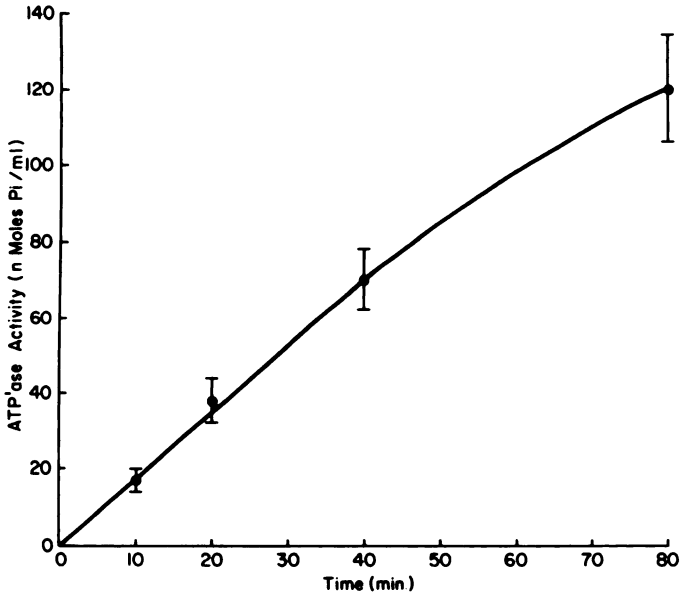

FIG. 3. *ATP hydrolysis activity of the urinary bladder as a function of time*

The ATP hydrolysis activity of isolated strips of bladder body (200 mg, wet weight) were determined at 2 mM ATP as described in text. Each value represents the mean of eight separate determinations. Vertical brackets indicate the standard error.

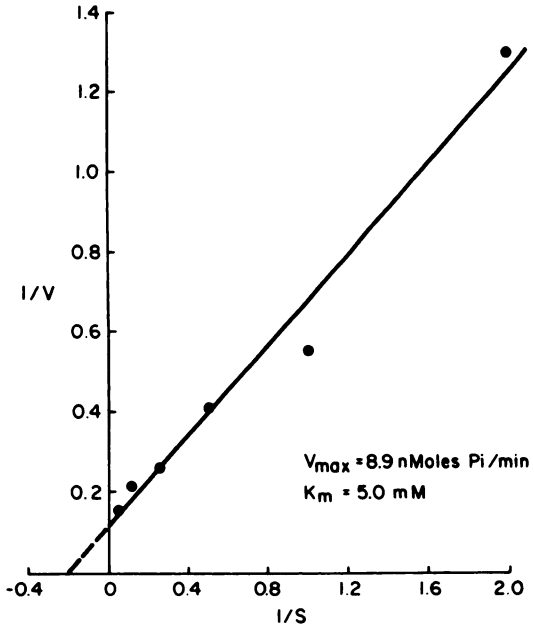

FIG. 5. *Kinetic analysis of ATP hydrolysis activity*

The mean values displayed in Fig. 3 were plotted according to the Lineweaver-Burk procedure, i.e., 1/velocity against 1/substrate concentration. The line of best fit was calculated by linear regression analysis.

Figure 4 shows the effect of increasing the concentration of ATP on ATP hydrolysis. Kinetic determination of the enzymatic activity by Lineweaver-Burk graphical analysis (Fig. 5) demonstrated that the dissociation constant (K_m) was 5 mM and the maximal velocity was 8.9 nmoles of Pi per minute per tissue strip.

It is clear from these studies that the relatively high concentrations of ATP necessary to stimulate contraction in the rabbit urinary bladder are not a function of the disappearance of ATP from the muscle bath. The maximal velocity of ATPase activity observed in our muscle strips was 9 nmoles of Pi per minute. Figure 1 demonstrates that the contractile effect of ATP reaches a maximum within seconds and terminates within 3 min. At an ATP concentration of 5 mM ATP, there are 150 μmoles of ATP within the 30-ml bath; of these 150 μmoles of ATP, only 27 nmoles (using the V_{max} for ATP hydrolysis) would be hydrolyzed in 3 min. Thus, when we measured the concentration of ATP within the bath directly, there was a negligible loss of ATP.

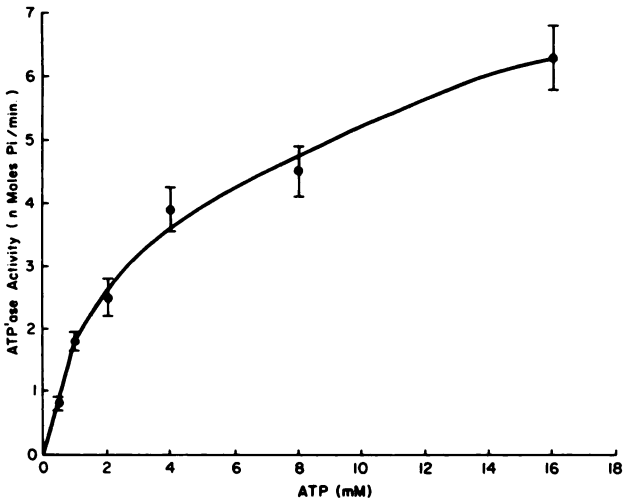

FIG. 4. *ATP hydrolysis activity of the urinary bladder as a function of the concentration of ATP*

The ATP hydrolysis activity of isolated strips of bladder body (200 mg, wet weight) were determined at various concentrations of ATP as described in text. Each value represents the mean of eight separate determinations. Vertical brackets indicate the standard error.

Our results differ from those of Brown *et al.* (5) and Burnstock (6), who theorized that rapid hydrolysis of ATP could explain why such high concentrations of ATP are required to stimulate contractility. Burnstock (6) used a completely different tissue and method of analysis than we utilized; thus we cannot comment directly on their conclusions. Our direct measurement of the concentration of ATP in our bath and our characterization of the ATPase activity of our muscle strips offer clear and strong evidence that the hydrolysis of ATP is much too slow to account for the high concentrations of ATP required to stimulate contractility.

As others have demonstrated (5), we show that β,γ-methylene ATP is approximately 100 times more potent than disodium ATP in stimulating contraction. Unlike these other investigators, we feel that the reason for this difference in potency does not involve the resistance of β,γ-methylene ATP for hydrolysis by ATPase. Evidence for this conclusion includes results of our studies on the rate of hydrolysis of ATP by the tissue and the fact that α,β-methylene ATP was just as potent as β,γ-methylene ATP. We prefer the theory that configurational differences between disodium ATP and its methylated analogues are responsible for the difference in potency of these compounds.

One of the major objections to the theory that ATP is a neurohumoral transmitter in the urinary bladder is the

fact that it requires millimolar concentrations to stimulate contraction. Whereas the concentrations of ATP required to stimulate contractility are greater than one would expect for a transmitter, the concentrations of β,γ-methylene ATP are not. If there is "purinergic" transmission in the urinary bladder, perhaps the transmitter is not ATP but an analogue of ATP which, like β-γ-methylene ATP, would be much more potent than ATP itself. Although these studies demonstrate a marked difference in the potency of disodium ATP and methylene ATP in their ability to stimulate contractility, it is not known whether these analogues are acting via the same receptor system.

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