Two ATP-activated Conductances in Bullfrog Atrial Cells

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ABSTRACT Currents activated by extracellular ATP were studied in single voltage-clamped bullfrog atrial cells. Rapid application of ATP elicited currents carried through two different conductance pathways: a rapidly desensitizing conductance reversing near −10 mV, and a maintained, inwardly rectifying conductance reversing near −85 mV. ATP activated the desensitizing component of current with a $K_{1/2}$ of ~50 μM and the maintained component with a $K_{1/2}$ of ~10 μM. Both types of current were activated by ATP but not by adenosine, AMP, or ADP. The desensitizing current was selectively inhibited by α,β-methylene ATP, and the maintained, inwardly rectifying current was selectively suppressed by extracellular Cs. The desensitizing component of current was greatly reduced when extracellular Na was replaced by *N*-methylglucamine, but was slightly augmented when Na was replaced by Cs. GTP, ITP, and UTP were all ineffective in activating the desensitizing current, and of a variety of ATP analogues, only ATP-γ-S was effective. Addition of EGTA or BAPTA to the intracellular solution did not obviously affect the desensitizing current. Fluctuation analysis of currents through the desensitizing conductance suggested that current is carried through ionic channels with a small (<1 pS) unitary conductance.

INTRODUCTION

There is increasing evidence that ATP may be important as an extracellular chemical transmitter (reviewed by Burnstock, 1979; Stone, 1981; Su, 1983). Externally applied ATP produces contraction of some visceral and vascular smooth muscle (e.g., Fedan et al., 1982; Kennedy and Burnstock, 1985; Stone, 1985), excites some types of neurons (Jahr and Jessell, 1983; Krishtal et al., 1983; Salt and Hill, 1983; Fyffe and Perl, 1984), and stimulates secretion by gland cells (Gallacher, 1982). As with other transmitters, it seems likely that many of ATP's actions are mediated by control of ionic channels in the cell membranes of target cells. However, little is known about the types of channels that might be controlled by ATP in various tissues. So far, probably the best-characterized mechanism is the excitation of sensory neurons, where voltage-

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clamp experiments have shown that ATP induces a cation-selective conductance with a reversal potential near zero (Krishtal et al., 1983).

In the heart, ATP has complicated effects that, depending on animal species and ATP concentration, can include tachycardia, bradycardia, positive inotropy, and negative inotropy (Drury and Szent-Gyorgyi, 1929; Hollander and Webb, 1957; James, 1965; Vesprille and Van Duyn, 1966). In frog hearts, at least, ATP may be released by the sympathetic neurons innervating the heart (Hoyle and Burnstock, 1986). In many species, the variety of ATP effects on the intact heart undoubtedly arises from a mixture of actions on the coronary vasculature and on heart muscle itself. However, even in preparations of isolated cardiac muscle (Goto et al., 1977; Yatani et al., 1978; Burnstock and Meghji, 1983), changes in tension and action potentials tend to have complicated time courses and are difficult to interpret; it seems very likely that a variety of cellular mechanisms underlie ATP's effects. Sorting out the various effects of ATP has been especially difficult because, in application to multicellular preparations, it is likely that some effects are not due to ATP itself, but to adenosine produced by breakdown of ATP by ecto-enzymes.

In the last decade or so, preparations of single cardiac muscle cells have helped greatly in understanding the cellular actions of better-understood cardiac neurotransmitters like acetylcholine (ACh) and norepinephrine. We have used the preparation of single bullfrog atrial cells developed by Hume and Giles (1981) to look for possible electrophysiological effects of ATP on heart cells. We have found that ATP appears to activate two distinct types of current in these cells, with different kinetics, ionic selectivity, and pharmacology. One conductance, which desensitizes quickly, has a linear current-voltage relation that reverses near −10 mV, while the other is a longer-lasting, inwardly rectifying conductance that reverses near the K equilibrium potential. Both conductances are highly selective for ATP as a ligand, with adenosine, AMP, and ADP being completely ineffective. The desensitizing conductance seems to be cation selective, but with little discrimination between Na, K, and Cs ions. Fluctuation analysis suggests that this conductance is mediated by channels with a small unitary conductance.

A preliminary report of some of this work has appeared (Friel and Bean, 1986).

METHODS

Cell Isolation

Cells were isolated from bullfrog (*Rana catesbeiana*) atria by the method of Hume and Giles (1981). In most experiments, we used a slightly modified procedure that avoided the use of trypsin: atria were removed and minced in oxygenated, nominally Ca-free Tyrode's solution (in millimolar: 150 NaCl, 4 KCl, 2 $MgCl_2$, 10 glucose, 10 HEPES, pH 7.40) and then digested in an enzyme solution of 1 mg/ml collagenase (Type I, Sigma Chemical Co., St. Louis, MO) and 1 mg/ml albumin (Sigma Chemical Co.; essentially fatty-acid free) in nominally Ca-free Tyrode's. After incubation at room temperature, with gentle stirring for 1 h, the enzyme solution was exchanged for fresh solution of the same composition and digestion was continued. The fragments were triturated with a broken-off Pasteur pipette every 30 min or so, and cells appeared within ~1 h. After several more hours, the suspension was diluted 1:5 into 1 mM Ca Tyrode's and stored in the refrigerator.

In about one-third of the preparations, cells gave either very small responses to ATP or no response at all. Generally, cells isolated from a single frog gave similar responses, although in every preparation there might be a few cells that failed to respond. Omitting trypsin from the isolation procedure (sometimes with trypsin inhibitor added) had no obvious effect on the fraction of responsive preparations, nor were unresponsive preparations obviously correlated with individual lots of collagenase. Since unresponsive preparations tended to be grouped together in individual batches of frogs, we favor the idea that the variability probably arose from differences in the animals, but there were both successful and unsuccessful preparations at all times of the year. We kept the frogs at 5°C; we noticed no obvious differences in responsiveness in frogs used without cold adaptation.

Cells isolated from both left and right atria responded to ATP, so we usually pooled the atria. In a number of experiments, we also isolated cells from the sinus venosus (cf. Shibata and Giles, 1985), and these also responded to ATP (e.g., Fig. 8*B*).

Solutions and Electrical Recording

Cells were voltage-clamped using patch pipettes as described by Hamill et al. (1981). Pipettes (Boralex glass, Rochester Scientific Co., Rochester, NY) contained an internal solution consisting of (in millimolar) 120 K-glutamate, 10 K_2 EGTA, 5 $MgCl_2$, 5 ATP (Na or Mg salt), 1 GTP (Na or Tris salt), 10 HEPES, buffered to pH 7.40 with KOH. Internal solutions were prepared daily from frozen (−70°C) stocks of ATP and GTP solutions and were kept on ice during the experiments. In the absence of internal ATP, cells developed a large, nonrectifying conductance reversing near E_K, as previously described in mammalian cardiac cells (Kakei and Noma, 1984; Trube and Hescheler, 1984). Internal GTP was added after early experiments suggested that the maintained, inwardly rectifying ATP-elicited conductance was GTP dependent, like the ACh-activated K current in heart cells (Bretweiser and Szabo, 1985; Pfaffinger et al., 1985). (In contrast, the transient ATP-elicited conductance was not affected by omission of GTP or replacement by GTP-γ-S.) The Tyrode's solution normally used as the external solution was (in millimolar) 2 $CaCl_2$, 150 NaCl, 4 KCl, 2 $MgCl_2$, and 10 mM HEPES, adjusted to pH 7.40 with NaOH. All experiments were performed at room temperature (20–25°C).

Currents were filtered at 1–3 kHz (−3 dB, eight-pole Bessel filter), digitized (usually at 5 kHz), and stored on a laboratory computer. During the application of ATP solutions, a command voltage protocol was used that made it possible to follow the time course of membrane current at two different membrane potentials: the membrane potential was rapidly switched between two potentials (usually −50 and −130 mV), being held for ~80 ms at each potential, and the current over the last 3 ms of each interval was averaged and digitized. This protocol was used for most of the records shown in this article (Figs. 2, 3, and 6–12), although, for clarity, usually only the current at −130 mV is shown.

In 61 typical cells, the parallel combination of seal and membrane resistance was 2.5 ± 0.4 GΩ, the resting potential was −86 ± 2 mV, the cell capacitance was 41 ± 3 pF, the pipette resistance was 13 ± 1 MΩ, and the series resistance was 24 ± 7 MΩ (mean ± SEM). We generally used rather small-tipped, high-resistance pipettes and no series resistance compensation, since the ATP-induced currents were small (20–500 pA).

All potentials are corrected for a junction potential of −10 mV between the K-glutamate internal solution and the Tyrode's solution in which the pipette current was zeroed before sealing onto a cell.

Application of ATP Solutions

ATP and other drug solutions were freshly prepared every day and, after addition of ATP, were readjusted to pH 7.40 with NaOH. Solution changes were made using a

modification of the "sewer-pipe" technique described by Yellen (1982). 10 microcapillary tubes (1 μl, 64 mm special length, 145 μm i.d.; Microcaps, Drummond Scientific Co., Broomall, PA) were glued together into a linear array and bent so that their ends were parallel to the bottom of the recording chamber. Solutions were delivered by gravity from reservoirs mounted ~500 cm above the capillaries. After a whole-cell clamp was established, the patch pipette and attached cell were lifted and the cell was placed in front of a tube, within ~100–150 μm of the mouth. Rapid solution changes were effected by shifting the pipette horizontally using a coarse micromanipulator. To measure the speed of solution changes made in this way, voltage-clamped atrial cells were moved from a solution containing 4 mM K^+ to a K^+-free solution and the time course of the decline in the inward rectifier K current at −110 mV was followed (Fig. 1). Measured in this way, solution changes were found to be complete within 100 ms; similar values were found by applying ACh to bullfrog sympathetic ganglion cells and following the rise of current through nicotinic ACh receptor channels. As the cell is moved from one capillary mouth to the next, it is transiently exposed to the bulk chamber solution; to avoid accumulation of test substances in the bulk solution, the chamber was continuously perfused at ~5 ml/min.

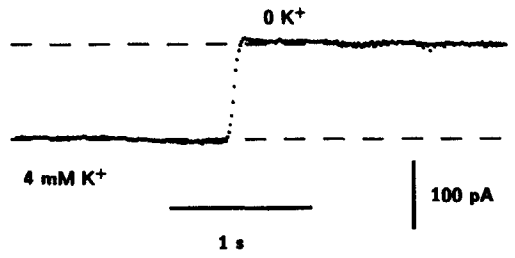

FIGURE 1. Time course of solution change. Holding current at −110 mV (largely through background ["IK_1"] inward rectifier channels) is shown as a cell was abruptly moved from the flow of a capillary containing standard 4 mM K^+ Tyrode's to an adjacent capillary emitting K^+-free Tyrode's. Current was sampled at 10-ms intervals. The time course was identical for solution changes in the opposite direction. Cell C95A.

RESULTS

Fig. 2 shows the ionic currents elicited in a bullfrog atrial cell by 200 μM ATP. Currents at membrane potentials of −50 and −130 mV were monitored concurrently by rapidly switching the membrane potential between the two voltages. The current elicited by ATP consisted of two kinetic components: a transient component of current that was inward at both −50 and −130 mV and decayed within ~1 s, followed by a maintained current that was inward at −130 mV but outward at −50 mV. On removal of ATP, the maintained component of current declined, with a time constant of a few seconds.

Desensitization

Fig. 3*A* shows, in another cell, current at −130 mV elicited by two applications of ATP ~30 s apart. The first application of ATP elicited both transient and maintained components of current, but the second application elicited only a maintained component of current, as if the transient component had somehow been desensitized or inactivated during the first application and did not have time to recover in the 30 s in the control solution. The maintained component of current was virtually identical in the two applications. The selective desensi-

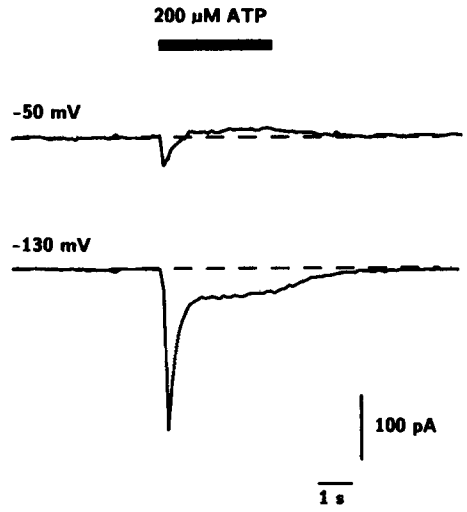

FIGURE 2. Currents induced by rapid application of 200 μM ATP. Membrane potential was rapidly switched between −50 and −130 mV (spending 80 ms at each potential). Current at each potential (measured as the average over the final 3 ms of each step) is plotted as a function of time; individual current points (at 160-ms intervals) are connected by solid lines for clarity. Cell C76B.

tization of the transient component of ATP-induced current was always seen. With longer resting periods between applications of ATP, the transient component recovered from desensitization. Fig. 3*B* shows the time course of recovery of the transient component; half-recovery took ~2 min, and recovery was nearly complete after 4–6 min. In all further experiments, applications of ATP were at least 4 min apart to allow recovery of the transient component.

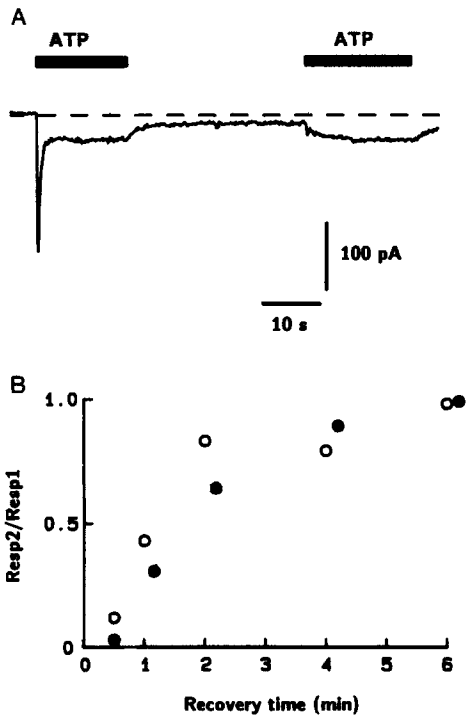

FIGURE 3. Desensitization and resensitization of the transient component of current. (*A*) Current at −130 mV during two applications of 200 μM ATP separated by 30 s. Cell C78E. (*B*) Time course of recovery from desensitization in two cells. In each trial, an initial 15-s application of 200 μM ATP was followed by a variable recovery time and the extent of recovery was tested with a second application of ATP. Cells were allowed to recover for 6 min between trials. Filled circles: cell C78E. Open circles: cell C79B.

Current-Voltage Relationships

The relative magnitudes of the transient and maintained components of the ATP-induced current varied from cell to cell. Fig. 4 shows results from a cell that exhibited little or no maintained current, making it possible to study the transient component in near isolation. Multiple applications of ATP (separated by 5 min) allowed a definition of the current-voltage relation for this component of current. (For each application of ATP, currents at two potentials were

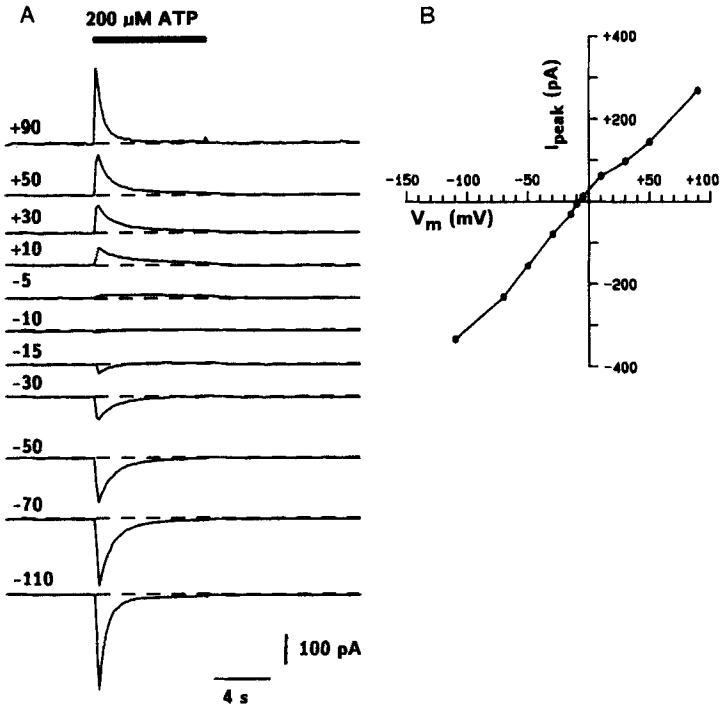

FIGURE 4. Voltage dependence of transient ATP-induced current. (*A*) Currents were monitored at two different potentials for each application of ATP, as in Fig. 2 (pairs were +90 and −110, +50 and −70, +30 and −50, +10 and −30, −5 and −15), except for the application at −10 mV, where the potential was held steadily at −10 mV. ATP was applied for 8 s in each case. The cell was held at −50 mV between applications (except before the +90, −110 mV pair, where it was held at −70 mV). (*B*) Peak current vs. membrane potential. Cell C88B.

monitored by rapid switching of the command potential, as in Fig. 2.) The transient component of the ATP-elicited current reverses at −8 mV and has a roughly linear current-voltage curve.

The current-voltage relation for the maintained component of current is very different. This current-voltage relation was determined in a cell that had both transient and maintained components of current by recording currents over a wide voltage range before application of ATP and ~7 s after applying ATP, when the transient component had decayed and only the maintained component

was left. The currents before ATP application were subtracted from those after application to yield the current-voltage relation for the maintained ATP-elicited current, shown in Fig. 5. The maintained current elicited by ATP reverses at −85 mV and shows pronounced inward rectification.

Differential Block by Cs

The very different reversal potentials and current-voltage relations for the two kinetic components of ATP-elicted current suggest that the two components arise from two different conductance pathways, rather than reflecting the complex kinetics of a single conductance. We tested this hypothesis by searching for other ways to distinguish the two components. The reversal potential and inward

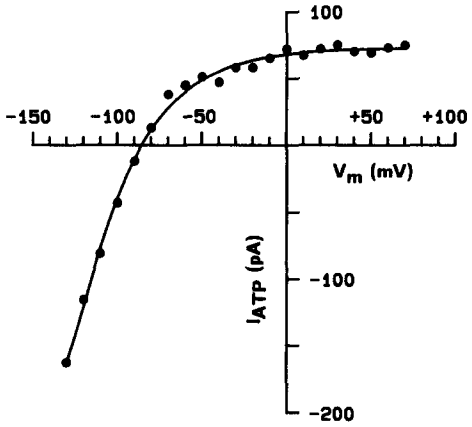

FIGURE 5. Current-voltage relationship for the steady component of ATP-elicited current. ATP-induced current at each potential was measured as the difference between current just before application of ATP and current 6–8 s after application of 100 μM ATP (when the transient component had completely decayed). Complete current-voltage curves were obtained every 2 s using a train of 20 steps from −130 to +70 mV in 10-mV increments, with steps 30 ms long given every 100 ms from a holding potential of −50 mV. Current at each potential was calculated as the average over the last 3 ms of the pulse. The cell's current-voltage relationship before application of ATP was stable, and the current-voltage relationship measured between 6 and 8 s after ATP application was virtually unchanged over the next 8 s. Cell C37A.

rectification of the maintained component of current are strongly reminiscent of a variety of resting and ACh-induced inwardly rectifying K currents in cardiac muscle, many of which are sensitive to block by external Cs (Argibay et al., 1983). We therefore investigated the ability of Cs to affect the currents induced by ATP. As shown in Fig. 6, addition of 10 mM Cs to the external solution inhibited the maintained component of current without inhibiting the transient component. The block of the maintained component was reversible on removal of Cs.

Differential Inhibition by α,β-Methylene ATP

We found that α,β-methylene ATP, a stable ATP analogue that has been reported to inhibit some actions of ATP on smooth muscle (e.g., Sneddon and Burnstock, 1984), specifically depressed the transient component of ATP-elicited current with little effect on the maintained component of current (Fig. 7). In three experiments, 200 μM α,β-methylene ATP reduced the transient compo-

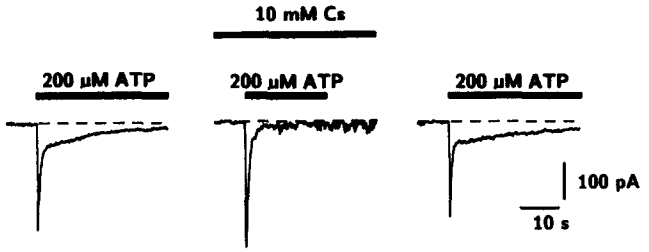

FIGURE 6. Selective inhibition of the steady component of the ATP-elicited current by external Cs. Current at −130 mV is shown. The response to 200 μM ATP in normal Tyrode's solution was first determined (left). After a 5-min rest, the cell was transferred to Tyrode's with 10 mM CsCl added; after 20 s in this solution, the cell was moved to this solution plus 200 μM ATP (middle). The cell was then returned to normal Tyrode's and after another 5-min rest, ATP was applied again (right), showing that the effects of Cs are completely reversible. (To facilitate comparison of the ATP-evoked currents, the currents are plotted with the currents before ATP aligned; the absolute current level in the Cs-containing solution was shifted outward owing to block of the inward rectifier current.) The increase in current noise during the application of ATP in the Cs-containing solution was not seen in other experiments. Cell C76C.

nent of current at −130 mV to 30 ± 10% of control, but left the maintained component at 98 ± 5% of control. Block of the transient component by α,β-methylene ATP required exposure of at least several minutes; there was little effect with exposures of ~20 s. In some studies of smooth muscle contraction, α,β-methylene ATP has been reported to act also as a weak agonist, but in our experiments, application of the analogue alone had no effect on background current (see Fig. 11*A*).

Ionic Selectivity of the Transient Conductance

The results so far suggest that the transient and maintained components of ATP-induced current arise from distinct conductance pathways, with different reversal

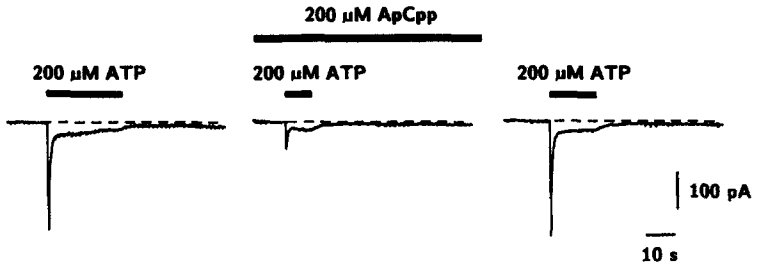

FIGURE 7. Selective inhibition of the transient component of current by α,β-methylene ATP. Current at −130 mV is shown. After an initial application of ATP in normal Tyrode's (left), the cell was transferred to Tyrode's containing 200 μM α,β-methylene ATP. After equilibration for 4 min in this solution, 200 μM ATP was applied again, with α,β-methylene ATP continuously present (middle). The right-hand panel shows an ATP response in normal Tyrode's later in the experiment, ~5 min after returning to normal Tyrode's following a second trial in α,β-methylene ATP. Cell C78C.

potentials, different sensitivities to block by Cs, and different susceptibilities to inhibition by α,β-methylene ATP. In the remainder of this article, we focus on the properties of the transient conductance activated by ATP. As this work was being done, we found that the maintained conductance pathway could be studied more easily in atrial cells isolated from calf hearts, where the maintained component of ATP-elicited current could often be recorded in isolation, with no interference from a transient component; a separate study of the properties of maintained ATP-induced current in calf cells is in progress. Most of our further experiments on ATP responses in frog atrial cells investigated the properties of the transient component of current, often in cells that exhibited little or no maintained component of current.

We tried to determine which ions carry the ATP-induced transient current by changing the ionic composition of the external solution. Replacing external Na (150 mM) with an equimolar concentration of the large cation *N*-methyl-D-glucamine (NMDG) results in a substantial decrease in the size of the current elicited by ATP. In the experiment of Fig. 8*A*, substitution of NMDG for Na resulted in a decrease in the ATP-elicited current to ~40% of control, which suggests that the inward current is largely carried by Na ions. Together with the reversal potential near −10 mV, this result suggests that the transient ATP-elicited conductance is cation selective, but with little discrimination between Na and K ions, the major external and internal cations. The inward current remaining when Na is replaced by NMDG might be carried by the K (4 mM), Mg (2 mM), or Ca (2 mM) in the external solution; this interpretation is supported by the finding that the ATP-elicited current is reduced further by NMDG substitution for K and Mg as well as Na (Fig. 8*B*). It is interesting that there was a measurable, though very small, transient current remaining with an external solution containing only NMDG (159 mM) and Ca (2 mM) as cations. It is unclear whether this current is carried by Ca or NMDG. We were unable to remove Ca since the cells (or electrode seals) became too leaky to carry out the experiment. We tested more directly for Ca permeability in the ATP-elicited conductance by applying 200 μM ATP in a solution of 110 mM $CaCl_2$. ATP consistently failed to elicit any current in this solution (in cells showing responses with normal external solutions). This may be because Ca ions are impermeant, but another possibility is that only ATP in the free-acid form is able to activate the conductance, and that in such high $CaCl_2$, the free-acid form is reduced to subthreshold levels owing to Ca chelation (cf. Cockcroft and Gomperts, 1979). Preliminary experiments show that increasing Ca or Mg in the external solution, with 156 mM Na present throughout, does reduce the ATP-elicited conductance in a manner generally consistent with the free-acid form of ATP being the active species. However, it is difficult to rule out the alternative possibility that Ca or Mg ions might reduce the current because of ionic interactions in the conductance pathway itself. Further work will be needed to resolve the question of whether Ca is permeant in the ATP-elicited conductance pathway.

In the experiment shown in Fig. 6, the transient component of current actually seemed to be slightly larger after addition of 10 mM Cs to the external solution. The suggestion that Cs might be permeant through the transient conductance

was tested directly in the experiment shown in Fig. 8*C*. With equimolar substitution of Cs for Na and K, the transient ATP-induced current was slightly larger than with Na as the major cation. The ability of Cs to carry large currents through the conductance pathway fits well with a picture of this conductance being cation selective, but with little discrimination between (at least) Na, K, and Cs ions.

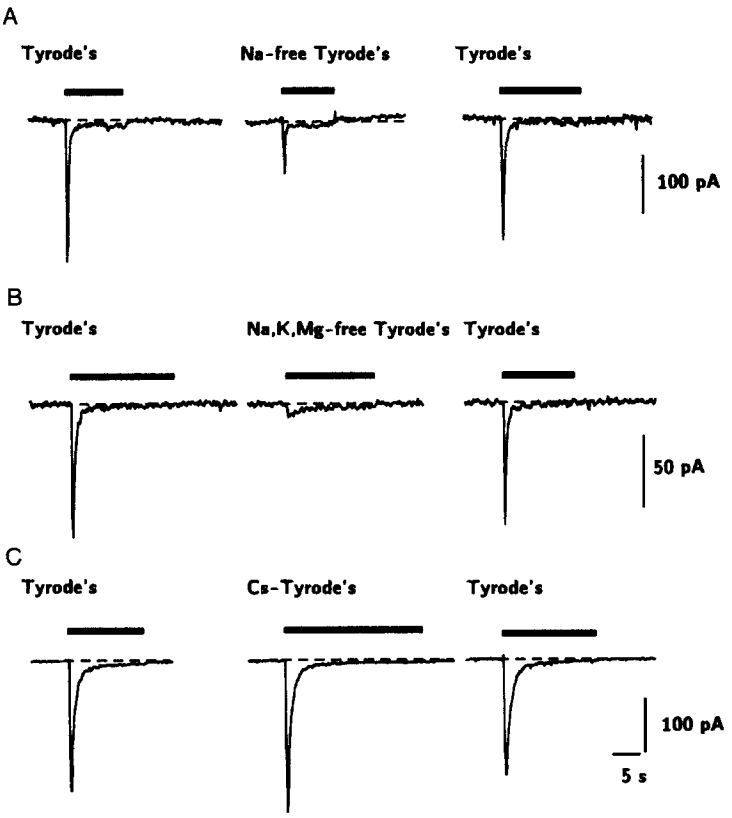

FIGURE 8. Effect of cation substitutions on the transient component of ATP-induced current. (*A*) After an initial test with 200 μM ATP in normal Tyrode's (left), the cell was transferred to a solution in which NMDG replaced Na (in millimolar: 150 NMDG, 4 KCl, 2 $CaCl_2$, 2 $MgCl_2$, 10 mM HEPES, pH adjusted to 7.40 with HCl). After 4 min, 200 μM ATP was applied in this solution (middle). After this application, a trial was made in a solution with NMDG replacing K; the ATP-induced transient current was little changed from that in control solution (not shown). After returning to control Tyrode's and resting 4 min, ATP was applied again (right). Cell D23A. (*B*) A similar experiment in a different cell, except that NMDG replaced Na, K, and Mg (159 NMDG-HCl, 2 $CaCl_2$, 10 HEPES, pH 7.4) in the middle trial. Cell D26B, isolated from the sinus venosus. (*C*) A similar experiment, but with Cs replacing Na and K (154 CsCl, 2 $CaCl_2$, 2 $MgCl_2$, 10 mM HEPES, pH 7.4) in the middle trial. Cell D33C. In all cases, the solution in the chamber was changed to match that in the sewer pipe without ATP before the cell was transferred to the flow from the ATP-containing sewer pipe.

Selectivity for ATP over Other Adenosine Compounds

The ability of other phosphorylated derivatives of adenosine to activate the transient ATP-activated conductance was tested in the experiment shown in Fig. 9. Adenosine itself failed to elicit any current in the cells. Fig. 9*A* shows a typical cell in which 200 μM adenosine had no effect on background current, while 200 μM ATP applied a few seconds later elicited both transient and maintained components of current. Adenosine had no effect on background current in nine cells (isolated from nine different hearts). Both AMP and ADP, tested at 200 μM, also failed to activate either the transient or maintained component of current (Fig. 9*B*).

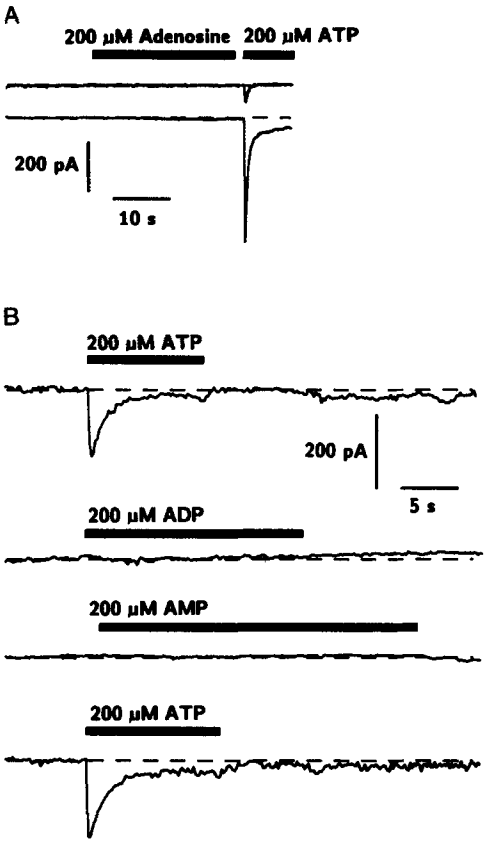

FIGURE 9. Lack of effect of other adenosine derivatives. (*A*) Current at −50 mV (top trace) and −130 mV (bottom trace) during application of adenosine and ATP. Cell C78D. (*B*) Data from a single cell, showing lack of effect of ADP and AMP on current at −130 mV, with bracketing applications of ATP. Cell D09A.

Nucleotide Selectivity

Fig. 10 shows that the transient ATP-activated current is also highly selective for ATP over other nucleoside triphosphates. In this experiment, GTP, ITP, and UTP all had no effect on background current, although large responses to ATP were seen both before and after testing the other nucleoside triphosphates. The same result was obtained in another cell. In both cells, the response to ATP consisted almost entirely of the transient component of current, so it remains

undetermined whether the receptor mediating the maintained conductance pathway shows the same high selectivity for ATP over the other nucleoside triphosphates.

Nonhydrolyzable ATP Analogues

The finding that ATP is the only phosphorylated adenosine derivative, and the only nucleoside triphosphate, to be effective in activating the transient conductance pathway raises the possibility that ATP might actually be used as a substrate. For example, ecto-kinases may exist that can transfer a high-energy phosphate group to some membrane protein (cf. Ehrlich et al., 1986). We therefore tested the ability of various nonhydrolyzable ATP analogues to activate the conductance. The nonhydrolyzable analogue α,β-methylene ATP (ApCpp) does not mimic the effect of ATP (Fig. 11*A*), though, as already shown, it does inhibit ATP activation of the transient conductance. We found that two other nonhydrolyzable analogues, β,γ-methylene ATP (AppCp) (Fig. 11*B*) and 5′-adenylylimidodiphosphate (AppNHp) (Fig. 11*C*), were also ineffective in activating the transient conductance. The only compound that we found to be capable of mimicking the action of ATP was adenosine 5′-*O*-3-thiotriphosphate (ATP-γ-S) (Fig. 11*D*), which elicited a current much like that induced by ATP, though slightly smaller and with a more slowly decaying time course. Since ATP-γ-S can substitute for ATP in some kinase reactions, donating a thiophosphate group (Gratecos and Fischer, 1974), our results with analogues are not necessarily inconsistent with the idea that ATP is used as a substrate in a phosphorylation reaction. Of course, it is equally possible that the conductance pathway is governed by a ligand receptor with very high specificity for ATP but not involving hydrolysis of the ATP (or ATP-γ-S) molecule.

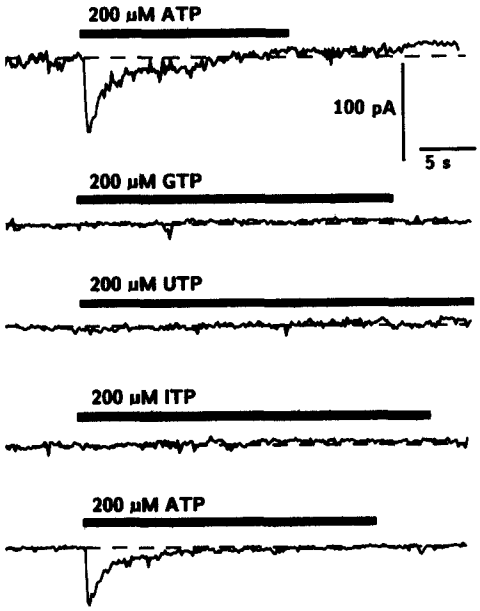

FIGURE 10. Lack of effect of other nucleoside triphosphates. Data are from a single cell; current at −130 mV is shown, with application of the various nucleoside triphosphates made in the order shown. Cell D11A.

Internal Ca Buffers

Does the ATP activation of the transient conductance involve a second messenger pathway? One possibility is that ATP could produce release of Ca from internal stores and that the conductance could be activated by this Ca. The ability of ATP to release internal Ca has been suggested for a variety of noncardiac cells, and Ca transients in response to ATP have recently been reported in guinea pig ventricular cells (Sharma and Sheu, 1986). The slow recovery of the transient ATP-elicited current could plausibly be consistent with refilling of sarcoplasmic

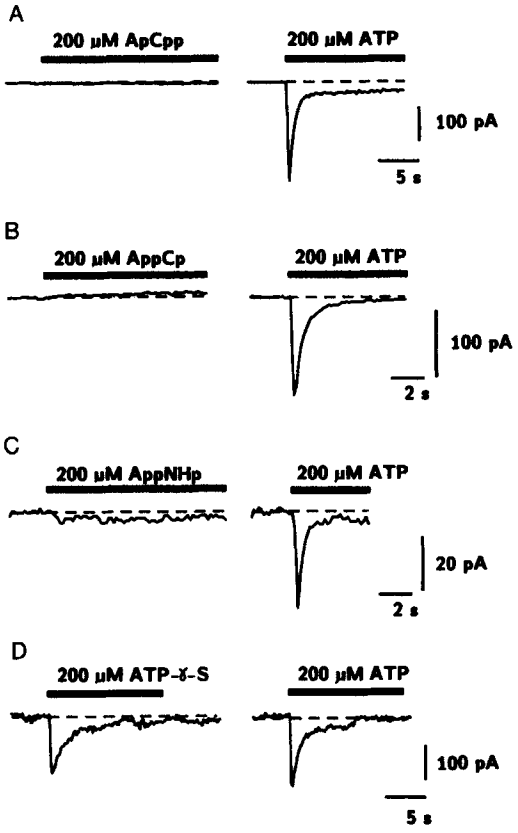

FIGURE 11. Effects of ATP analogues on current at −130 mV. Each part is from a different cell, showing application of the analogue on the left and a subsequent application of ATP on the right. In each case, ATP was applied at least 4 min after application of the analogue. (*A*) Cell C78C. (*B*) Cell D32B. The small outward shift in current at −130 mV with β,γ-methylene ATP was also seen in two other cells. Current at −50 mV was concurrently shifted inward. (*C*) Cell D30A. The small steady inward current elicited by AppNHp in this cell was also seen in a repetition of the experiment on another cell. (*D*) Cell D06B.

reticulum stores. Our internal solution routinely contained 10 mM EGTA, which would be expected to buffer internal Ca transients; however, the rather slow Ca binding by EGTA (Hellam and Podolsky, 1969; Tsien, 1980) might be consistent with the decay of the conductance in a few seconds. We therefore tested whether omitting EGTA from the internal solution altered the time course of the transient ATP-induced current. It did not. Fig. 12*A* shows ATP-induced current at −130 mV in a cell with no Ca buffer inside. There was no striking difference in the time course of the ATP-elicited conductance compared to cells with EGTA inside; Fig. 12*B* shows the ATP-induced current in an EGTA-containing cell from the same batch of cells. In several other experiments, we replaced the usual

internal EGTA with the much faster Ca buffer BAPTA (Tsien, 1980); the ATP responses with internal BAPTA (e.g., Fig. 12*C*) were not obviously different from those with EGTA or with no Ca buffer. These results make it seem unlikely that the ATP-induced current is mediated by internal Ca.

Voltage Dependence of Decay

The time course of the decay of the transient ATP-elicited current depended on membrane potential. Fig. 13 shows the currents elicited by applying ATP to the same cell held steadily at various membrane potentials, with applications of ATP

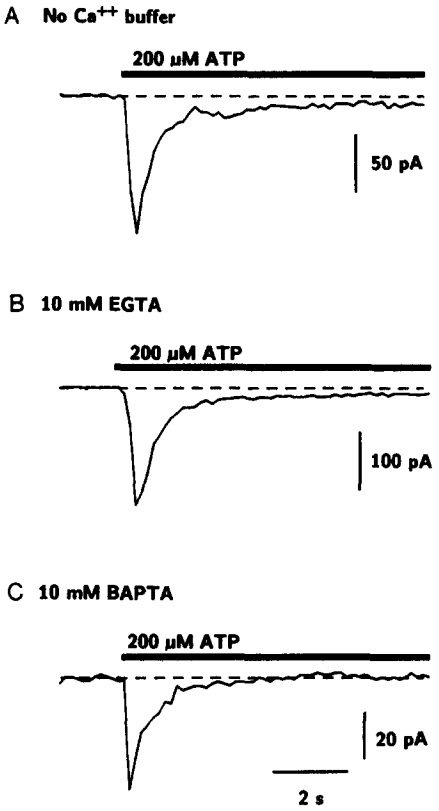

FIGURE 12. Time course of transient ATP-induced current with and without internal Ca buffers. Currents at −130 mV are shown. Each panel is from a different cell. (*A*) ATP-elicited current in a cell with no Ca buffer in the internal solution. The external solution contained 10 mM Cs to block maintained current. Cell C76G. (*B*) ATP elicited current in a cell from the same dispersion as that in *A*, with the normal EGTA-containing internal solution. 10 mM Cs in the external solution. Cell C76F. (*C*) Current elicited by ATP 10 min after the establishment of whole-cell recording with a BAPTA-containing internal solution (identical to the standard internal solution but with BAPTA replacing EGTA); virtually identical ATP-elicited currents were also recorded at 6 and 15 min after beginning whole-cell recording. No Cs was added to the external solution since there was little, if any, maintained current. Cell D31D.

separated by at least 5 min to allow for complete recovery from desensitization. The decay of the current was faster at more negative potentials. At each potential, the decay could be reasonably well fitted by a single exponential (plus a very small constant current); the time constant varied from ~0.5 s at −90 mV to ~1 s at +50 mV.

Dose-Response Relationship

Fig. 14 shows the dose-response relationship for the activation of the transient current by ATP. In this cell, typical of four cells in which various concentrations of ATP were applied, ATP at 1 μM or below elicited no response, 10 μM

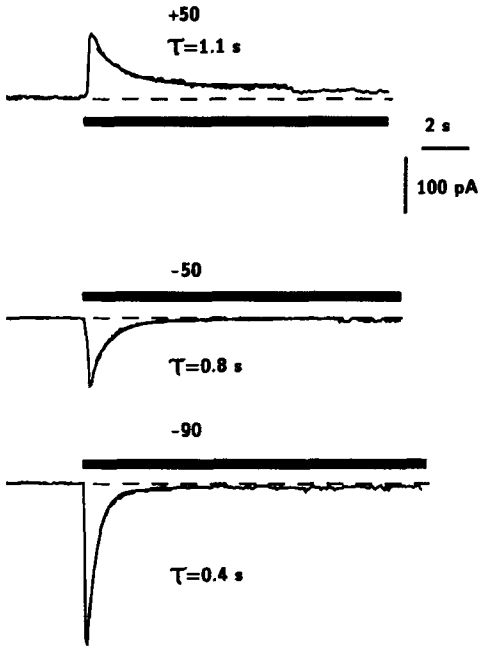

FIGURE 13. Voltage dependence of the decay of transient ATP-induced current. 200 μM ATP was applied (for the times indicated by the bars) to a cell held steadily at various potentials. Least-squares fits of an exponential plus a constant are shown superimposed on the current records; fits were made beginning at the time the current had decayed by ~30%. Cell C88B.

produced a barely detectable response, 100 μM elicited a sizeable current, and 1 mM was only slightly more effective than 100 μM. The experimental data can be fitted well by assuming that a receptor needs to bind two ATP molecules at identical, noninteracting binding sites in order to activate current; however, more data points would be needed for us to feel very confident about the

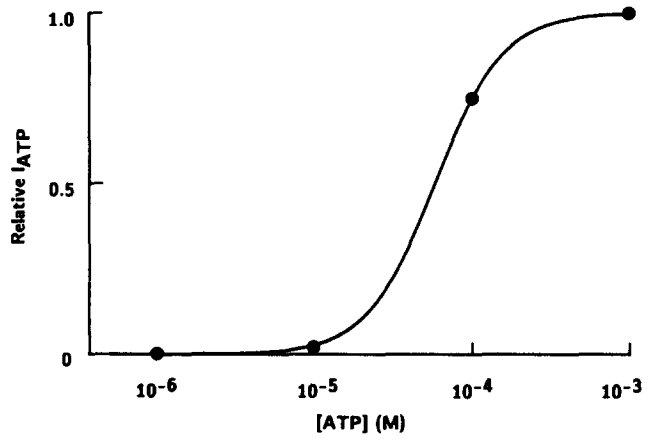

FIGURE 14. Dose-response for the transient ATP-induced current, determined in a cell with no maintained component of ATP-induced current. Sequence of applications and actual peak current sizes: 100 nM (no response), 1 μM (no response), 1 mM (159 pA), 10 μM (3 pA), 100 μM (109 pA), 1 mM (127 pA). The cell was rested at least 4 min between applications. The solid curve is $1/\{1 + (56\ \mu M/[ATP])^2\}$. Cell C92E.

meaningfulness of the fit. From the fitted curve, the interpolated concentration for half-activation of the conductance is 56 μM.

The results in Fig. 14 were obtained in a cell that had no maintained component of current. In cells that did have a maintained component in addition to the transient component, it was clear that the maintained component was activated by lower concentrations of ATP: 1 μM ATP was enough for substantial activation of the maintained current, while 10 μM was enough for activation of about half of the current elicited by 1 mM ATP. The difference in sensitivity to ATP fits well with the other evidence that the two components of current represent distinct conductances.

Fluctuations in the Transient ATP-induced Current

We attempted to determine whether the transient ATP-induced current is carried by channels, and to estimate the single-channel conductance, by examining fluctuations in the ATP-induced current. For these purposes, we recorded the current elicited by ATP applied in an external solution containing Cs as the primary cation. This solution was chosen to optimize the measurement of any excess noise in the ATP-induced current: the ATP-induced current is slightly larger in this solution than in normal Tyrode's (Fig. 8*C*), and Cs blocks both normal background current at −130 mV and any maintained component of ATP-induced current. Fig. 15*A* shows currents elicited by hyperpolarizations to −130 mV before ATP, at two times during the application of ATP, and after the washout of ATP. The current induced by ATP was accompanied by a clear increase in current noise, as expected if current is carried through channels. During the application of ATP, as the current declined (owing to desensitization), the excess noise in the current also declined. Fig. 15*B* shows the ATP-induced variance as a function of the ATP-induced current; the variance is a roughly linear function of current, as expected if the current is carried through a population of homogeneous, independently gated channels with a fairly low probability of opening. In such a case, the single-channel current is given by the slope of the variance-current relationship; in Fig. 15*B*, this was 0.046 pA. Assuming a reversal potential of −10 mV, this corresponds to a single-channel conductance of 0.35 pS. A repetition of the experiment in a different cell yielded an estimate of 0.033 pA at −130 mV.

The experiment of Fig. 15*A* suggests that the ATP-induced transient current flows through channels with a rather low single-channel conductance. The increase in noise produced by ATP is clear, but the estimate of single-channel conductance is only a rough one. A number of factors probably introduce errors into the estimate. The ATP-induced current is not truly stationary; to correct for steady drift in the current owing to desensitization, the variance was calculated after subtracting the current during a 50-ms interval from a fitted straight line. The error introduced by this procedure probably was not large, since the results were virtually unchanged if the linear fit and variance were calculated over 20-ms rather than 50-ms intervals. A larger error might result from filtering of high-frequency fluctuations in the current. Although the current signal was nominally filtered at 1 kHz before being digitized, the actual filtering of the

current by the R-C combination of series resistance and cell capacitance (with a time constant of 700 μs, from the decay of the capacity transient) is more severe. However, while further experiments would be necessary to establish more precisely the value of the single-channel conductance, the experiment suggests that the unitary currents may be too small to be easily resolved in single-channel patch recording. Even if the single-channel current were underestimated by as much as a factor of 3 or 4 in the experiment shown in Fig. 15, the single-channel current of <0.2 pA (at −130 mV) would be difficult to resolve in patch recordings.

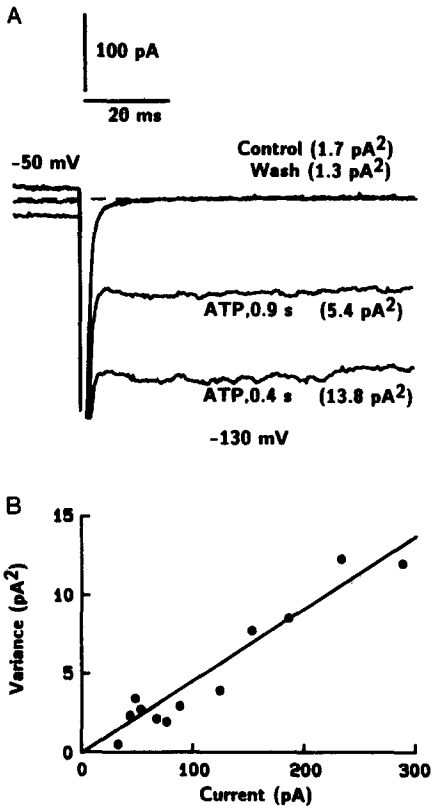

FIGURE 15. Fluctuations in ATP-induced current. ATP (200 μM) was applied to a cell bathed in 154 CsCl, 2 $CaCl_2$, 2 mM $MgCl_2$, 10 HEPES, pH 7.4. 80-ms steps to −130 mV were given every 120 ms from a holding potential of −50 mV. (*A*) Traces shown are immediately before ATP, near the peak of the ATP response (0.5 s), after some desensitization had occurred (0.9 s), and 4 s after returning to ATP-free solution; ATP was applied for a total of 20 s before beginning the washout. (*B*) Excess variance at −130 mV induced by ATP vs. current induced by ATP. The points were calculated from the final 50 ms of various sweeps after application of ATP; the decline in current is due to desensitization. The line was drawn by eye. Cell D33C.

DISCUSSION

Two Conductances Activated by External ATP

The main conclusion from our experiments is that externally applied ATP activates two different ionic conductances in bullfrog atrial cells. One conductance is transient and has a linear current-voltage relation with a reversal potential near −10 mV; the other is maintained (at least for seconds) and has an inwardly rectifying current-voltage relation with a reversal potential of −85 mV. Besides their different desensitization properties and current-voltage relationships, evidence that the two current components reflect different pathways includes the

selective block of the maintained current by external Cs and the selective inhibition of the transient current by α,β-methylene ATP.

We have interpreted both components of ATP-elicited current as reflecting activation of *de novo* conductances, rather than modulation of existing conductances. While it is difficult to be certain about this point, all of the evidence seems consistent with this view. The transient ATP-activated conductance was most often studied in a potential range (−50 to −130 mV) where there is no activation of voltage-dependent Ca or K channels. When transient currents were also studied at more positive potentials (e.g., Figs. 4 and 13), the kinetics of the current seemed to change smoothly with voltage, with no obvious evidence of extra components at potentials where Ca or K channels might be activated (for example, the transient ATP-activated current reversed at −10 mV, where Ca current is largest). Possible changes in voltage-dependent Ca or K currents cannot be ruled out from our data, but they seem unlikely to contribute much to the currents we recorded; different protocols designed specifically to check for such changes would be needed. It is also unlikely that the transient ATP-activated current represents a modification of the inward rectifier current since the two currents have very different reversal potentials (and different susceptibilities to block by Cs). Whether the maintained component of ATP-elicited current might represent modulation of existing inward rectifier channels remains to be determined.

Both the transient and maintained conductance pathways show great specificity for ATP as an activator; adenosine, AMP, and ADP were completely ineffective in activating either conductance in concentrations at least as high as 200 μM. The lack of effect of adenosine surprised us, since it has previously been found that both adenosine and ATP are effective in hyperpolarizing cells in the intact frog sinus venosus (Hartzell, 1979). One possibility is that adenosine receptors have been destroyed during the enzymatic isolation of the cells. However, we found that elimination of trypsin from the enzyme solutions used in isolating the cells did not affect the lack of sensitivity to adenosine. Using identical internal and external recording solutions (and the same batches of adenosine), we saw large adenosine-activated inwardly rectifying currents in atrial cells that were isolated from rabbit hearts using treatment with collagenase concentrations similar to those used for the frog cells; this makes it seem less likely that the lack of response to adenosine is due to proteolysis of receptors or to our recording conditions. In fact, previous voltage-clamp recordings from multicellular preparations of bullfrog atrial muscle have also failed to show any background current activated by adenosine (Goto et al., 1978). One possibility is that grass frog (*Rana pipiens*) sinus venosus cells have adenosine receptors but bullfrog (*Rana catesbeiana*) atrial muscle proper does not; differences owing to seasonal variability also cannot be excluded. Whatever the reason for the lack of response to adenosine, the result is very useful, since it makes clear the existence of cellular responses to ATP that are definitely not due to adenosine produced as a breakdown product.

We have also done some experiments with atrial cells from mammalian species. In atrial cells from both calf and rabbit hearts, two components of ATP-induced current were seen, with properties broadly similar to those in bullfrog atrial cells.

As already mentioned, most calf atrial cells show primarily the maintained, inwardly rectifying current induced by ATP, with only a few showing a clear transient component. Our preliminary studies on the maintained current in calf cells suggest that it is identical to the maintained component of current in bullfrog cells; both are Cs sensitive and inwardly rectifying, and reverse near the K equilibrium potential. The maintained ATP-activated current is much like the K current activated by muscarinic ACh receptors in atrial cells; in fact, our preliminary data suggest that the K channels activated by ATP are the same channels that are activated by ACh. A more transient current reversing near −10 mV was seen in a few calf atrial cells and also in several rabbit atrial cells. While further work will be needed to fully characterize the ATP-induced currents in the atria from various species, it seems quite likely that the currents we have described are not peculiar to amphibian heart.

Ligand Specificity and Pharmacology of the Transient Conductance

There is clearly a variety of purinergic receptor types in various tissues, classified according to several different schemes (Burnstock, 1978; Stone, 1981; Burnstock and Kennedy, 1985). The ligand specificity and pharmacology of the transient ATP-elicited conductance we have described is most similar to the "P_2" type of receptor characterized by Burnstock and colleagues (reviewed by Burnstock and Kennedy, 1985). Thus, ATP is the most potent adenosine derivative, and the response is sensitive to inhibition by α,β-methylene ATP. However, unlike other responses mediated by P_2 receptors, the transient ATP-elicited conductance in atrial cells is absolutely selective for ATP over adenosine, AMP, or ADP (at least at 200 μM). Also, β,γ-methylene ATP is ineffective, and α,β-methylene ATP appears to inhibit the transient ATP-evoked conductance without itself acting as an agonist, as it does in some other P_2 responses.

Mechanism of ATP Control of the Transient Conductance

Our experiments leave open many possible mechanisms by which ATP might open the transient cationic conductance. Among the possibilities to be considered are direct control of a channel closely linked to a ligand receptor, control of a Ca-sensitive channel by release of internal Ca stores, and generation of a chemical second messenger.

One trivial possibility can probably be eliminated. ATP could act simply by chelating extracellular Ca ions, thereby inducing some sort of leakiness in the membrane. A number of observations make this possibility unlikely. First, the concentration of ATP usually employed (200 μM) could not reduce the external Ca concentration (2 mM) by any more than 200 μM; in control experiments, we found that abruptly transferring a cell from 2 to 1.8 mM Ca had no effect on the background current in the cell. Second, ITP and UTP have divalent binding properties nearly identical to those of ATP (Walaas, 1958), but were completely ineffective in activating the ATP-elicited conductance (Fig. 10). Third, the inhibition of the ATP response by α,β-methylene ATP implies an interaction of ATP with a specific receptor and seems inconsistent with the hypothesis of Ca chelation. ATP does not seem to simply "permeabilize" heart cells the way it does mast cells (Cockcroft and Gomperts, 1979) or transformed cells (Heppel et

al., 1985) since the conductance in heart cells excludes molecules as small as *N*-methylglucamine (Fig. 8), while those in "permeabilized" cells pass molecules as large as nucleotides or even proteins.

A more plausible explanation is that ATP might act by releasing Ca from internal stores and that the increase in internal Ca directly activates a cation-passing channel sensitive to internal Ca. There is some evidence that ATP can release Ca from internal stores in a variety of cell types (Charest et al., 1985; Dubyak and De Young, 1985; Sung et al., 1985). ATP may modulate Ca release by the sarcoplasmic reticulum in frog heart (Niedergerke and Page, 1981), and transiently raises free Ca in guinea pig ventricular cells (Sharma and Sheu, 1986). There is good evidence for a poorly selective cation-passing channel in cardiac tissue that can be activated by internal Ca (Colquhoun et al., 1981; Hill et al., 1986). Such a mechanism might fit well with the many minutes it takes the ATP-induced conductance to recover from desensitization; this time it could represent replenishment of the internal stores. However, an argument against such a mechanism is that the ATP-induced conductance seems little changed by 10 mM EGTA or 10 mM BAPTA in the internal solution; nor is the recovery from desensitization obviously prolonged with internal Ca buffers present. It seems certain that at least some EGTA or BAPTA was introduced into the cells in these experiments, since inclusion of Ca buffer in the pipette solution rapidly (within seconds) prevented contractions elicited by depolarization and also prevented contraction in response to the Na-free solutions used in experiments like that in Fig. 8. Nevertheless, it is difficult to rule out the Ca-release hypothesis completely; for example, it is possible that sarcoplasmic reticulum near the internal surface of the sarcolemma could release Ca into a restricted space into which diffusion of EGTA or BAPTA is very slow.

One specific example of internal Ca release activating the transient current can be eliminated with more confidence. An increase in internal Ca could elicit a transient current carried by the Na/Ca exchanger. According to this model, the electrogenicity of the exchanger would result in a net inward current (carried by Na) when the activity of the exchanger was stimulated by the rise in internal Ca. Such currents, superficially similar to those activated by ATP, have been reported to be activated by application of caffeine to cardiac cells (Mechmann and Pott, 1986). However, a number of facts about the ATP-controlled current do not fit with this model. First, the ATP-elicited conductance pathway can pass large outward currents at potentials positive to the reversal potential of −10 mV (Figs. 4 and 13). In contrast, an exchanger current stimulated by internal Ca would be expected to be smaller at increasingly positive potentials, but would not show a genuine reversal of current: extrusion of increased internal Ca would always result in an inward current. Second, external Cs is incapable of substituting for Na in the Na/Ca exchanger, but the ATP-elicited current is larger when Cs replaces Na in the external solution (Fig. 8). Third, the presence of fluctuations in the ATP-stimulated current (Fig. 15) suggests that the current is carried through low-conductance channels rather than by a carrier.

It is quite possible that the ATP-induced current is mediated by some chemical second messenger. A second messenger system would have to be fast, since the

ATP-induced current reaches a peak within several hundred milliseconds. This probably rules out cyclic AMP as a second messenger, since cAMP-mediated processes (for example, the adrenergic increase in voltage-activated cardiac Ca current) take at least seconds to activate. A protein kinase C–mediated pathway might be fast enough: such a pathway apparently mediates the norepinephrine inhibition of Ca current in sensory neurons (Rane and Dunlap, 1986), which is also complete in several hundred milliseconds (Bean, B. P., unpublished observations). However, we found that the kinase C activator 1-oleyl-2-acetyl-glycerol was incapable of mimicking ATP in eliciting the transient conductance. Other possible second messenger pathways with some precedent in mediating ATP actions are production of inositol trisphosphate (Haggblad and Heilbronn, 1987) or of prostaglandins (Brown and Burnstock, 1981). These possibilities remain to be explored.

Comparison with Nicotinic ACh Receptor Channels

Although a second messenger mechanism cannot be ruled out, all of our data are also consistent with the much simpler possibility that ATP binds to a specific receptor closely associated with an ionic channel. There are many similarities between the transient current induced by ATP and the current that flows through the nicotinic ACh receptor channel at the endplate, one of the few transmitter-controlled channels for which such a simple mechanism is clearly established. Both currents reverse near 0 mV and are cation selective, but show little discrimination between small monovalent cations and undergo desensitization with maintained agonist exposure. In both cases, substantial desensitization occurs within 1 s and recovery can take up to several minutes. The primary difference is that the ATP-elicited current in bullfrog atrial cells completely desensitizes in a few seconds and takes several minutes to recover even after short applications of agonist, while the rapid desensitization of the ACh response is only partial, and slow recovery occurs only after more prolonged applications of ACh (Feltz and Trautmann, 1982). It is interesting that in both cases the rate of desensitization depends on membrane potential, being slower at more depolarized potentials (Fig. 13; Magazanik and Vyskocil, 1970; Scubon-Mulieri and Parsons, 1977). The stoichiometry of ligand activation of the conductance is also similar in the two cases, with dose-response curves with a Hill coefficient of ~2 (Fig. 14; Dionne et al., 1978). The most obvious difference between the two conductances is the much larger unitary conductance of the nicotinic endplate channel.

Comparison with ATP-activated Conductances in Other Excitable Cells

There are broad similarities in membrane conductances activated by ATP in a variety of excitable cells. In rat sensory neurons (Krishtal et al., 1983), chick skeletal muscle myotubes (Hume and Honig, 1986), and in smooth muscle cells from the vas deferens (Friel, D. D., manuscript submitted for publication) and from rabbit ear artery (Benham and Tsien, 1987), ATP activates a conductance that reverses near 0 mV, much like that which we have recorded in heart cells. In all cases (except the myotube conductance, where ionic selectivity has not yet

been studied), the conductance is cation selective, with little discrimination between small monovalent cations. In at least sensory neurons and vas deferens smooth muscle, the ligand specificity is also similar to that in atrial cells, with very strong selectivity for ATP over other adenosine derivatives. Also, all of the conductances show desensitization, with recovery taking at least minutes. Despite the broad similarities, however, it is already clear that the ATP-activated conductances are not identical in the various cells. The current-voltage relationship shows prominent inward rectification in sensory neurons and vas deferens smooth muscle cells but is nearly linear in atrial cells. The channels carrying the current also seem to have different unitary conductances in the various cells. In both chick myotubes and bullfrog atrial cells, the unitary conductance is very small: Hume and Honig (1986) were unable to resolve single-channel events or fluctuations in macroscopic currents in myotubes (despite the earlier report by Kolb and Wakelam [1983] of large-conductance ATP-activated channels); we were able to detect excess noise in the ATP-elicited current in atrial cells, but the unitary conductance seemed very small (<1 pS). In contrast, very similar experiments in vas deferens muscle cells suggest a unitary conductance ~10 times larger (Friel, D. D., manuscript submitted for publication), and recent patch recordings have shown similarly large unitary conductances of the ATP-operated channel in smooth muscle cells from rabbit ear artery (Benham and Tsien, 1987). The selectivity of the channels is probably somewhat different in the various cells as well, since the channels in ear artery cells, unlike those in atrial cells, can pass large currents with isotonic $CaCl_2$ solutions.

While in all cases the receptors show strong specificity for ATP, there are also clear differences in detailed ligand specificity and receptor pharmacology. The strongest ligand specificity is seen in myotubes and atrial cells: in both cases, ATP and ATP-γ-S are the only effective adenosine derivatives or ATP analogues. In vas deferens muscle, ADP and α,β-methylene ATP elicit some current (though much less effectively than ATP), and in sensory neurons, ADP and β,γ-methylene ATP are weak agonists. So far, the only clear antagonistic effect of an ATP analogue on a membrane conductance is the inhibition by α,β-methylene ATP that we observed in atrial cells (Fig. 7). Taken all together, the currently available results suggest a family of related ATP-activated conductances in excitable cells, with similar but not identical receptors, and similar but not identical conductance pathways.

ATP has recently been found to evoke three different currents in frog oocytes (Lotan et al., 1986); none seems to correspond to either of the currents we have found in frog heart cells. Two of the ATP-activated currents in oocytes are Cl^- currents; the third, a K^+ current, is mediated by adenosine-sensitive P_1 receptors, in contrast to the P_2 receptors in frog atrial cells.

Possible Functional Roles of ATP-evoked Conductances in the Control of the Heart

It is possible that ATP is released from nerves supplying the heart. It has frequently been reported that ATP is co-stored (and perhaps co-released) with catecholamines in sympathetic nerves, as well as with ACh in a variety of cholinergic nerves (see Stone, 1981, for a review). So far, the best evidence for

ATP actually being used as a cardiac neurotransmitter comes from studies on the frog heart. In frog atria, a positive inotropic response to intramural nerve stimulation persists even after block of cholinergic and adrenergic responses (Donald, 1985), and this response can be blocked by α,β-methylene ATP (Hoyle and Burnstock, 1986). This effect of nerve stimulation is quite similar to the most immediate effect of applying exogenous ATP to frog heart muscle, an increase in the force of contraction (Flitney and Singh, 1980; Niedergerke and Page, 1981). The α,β-methylene block of the nonadrenergic, noncholinergic response to nerve stimulation raises the possibility that the transient ATP-elicited current we have described, which is also inhibited by α,β-methylene ATP, could contribute to this positive inotropic effect. One possibility, suggested to us by Prof. Harald Reuter, is that Na entry through the transient conductance could, by raising internal Na, produce an accompanying increase in internal Ca (by decreasing the Na gradient driving the Na/Ca exchanger) and thus increase the force of contraction. It seems plausible that the transient ATP-elicited conductance could produce substantial extra Na entry. Although currents through the ATP-activated conductance (20–500 pA) are smaller than the voltage-activated Na currents flowing during action potentials (~1–5 nA), the ATP-activated currents last perhaps 1,000 times longer than the rapidly inactivating voltage-dependent Na currents. The possibility also remains open that Ca can enter directly through the ATP-evoked channels.

The maintained, K-selective conductance could also play a role in the response to nerve-released ATP. In frog atria, the initial phase of positive inotropy is followed by a later phase of negative inotropy and slowed beating (Hoyle and Burnstock, 1986), which is not sensitive to block by α,β-methylene ATP. In mammals, the usual response to exogenous ATP is slowed beating and negative inotropy without a preceding phase of positive inotropy. The maintained K conductance activated by ATP could very well underlie the slowed beating, by hyperpolarizing pacemaker cells exactly as does the K conductance activated by ACh. In some species, however, ATP may also act (or only act) after being broken down to adenosine. Adenosine hyperpolarizes atrial cells from rats, rabbits, and guinea pigs by activating a background K^+ current much like the maintained current activated by ATP in bullfrog cells or the K^+ current activated by ACh in all atrial cells (Belardinelli and Isenberg, 1983; Furshpan et al., 1986; Bean, B. P., and D. D. Friel, unpublished data). This effect of adenosine is probably mediated through P_1 purinergic receptors (Burnstock and Meghji, 1983; Furshpan et al., 1986).

Extracellular ATP may also be released by heart muscle itself, especially hypoxic muscle. ATP is present in the normal effluent of frog heart and increases with workload (Doyle and Forrester, 1985); ATP is also detectable in the effluent of hypoxic rat hearts (Clemens and Forrester, 1981) and is released by isolated myocytes in response to hypoxia (Forrester and Williams, 1977). Either of the conductances we have described could be activated by muscle-released ATP; however, it is still uncertain whether concentrations of ATP from this source can approach the concentrations needed to activate the currents we have described (>1 μM for the maintained conductance, >10 μM for the transient conductance).

Whether or not the two ATP-evoked conductances actually play a physiologically important role in the control of the heart, it is very likely that they are only a part of the response of cardiac muscle cells to ATP. It is well established that ATP can also produce an increase in voltage-dependent Ca currents (Yatani et al., 1978), and it may also augment Ca release from the sarcoplasmic reticulum (Niedergerke and Page, 1981). Either or both of these effects may be secondary to an ATP-induced rise in internal cAMP (Flitney and Singh, 1980), which undoubtedly has consequences for cellular function beyond those involving sarcolemmal channels.

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REFERENCES

Argibay, J. A., P. Dutey, M. Ildefonse, C. Ojeda, O. Rougier, and Y. Torneur. 1983. Block by Cs of K current i_{K1} and of carbachol induced K current i_{Cch} in frog atrium. *Pflügers Archiv.* 397:295–299.

Belardinelli, L., and G. Isenberg. 1983. Isolated atrial myocytes: adenosine and acetylcholine increase potassium conductance. *American Journal of Physiology.* 244:H734–H737.

Benham, C. D., and R. W. Tsien. 1987. A novel receptor-operated Ca^{2+}-permeable channel activated by ATP in smooth muscle. *Nature.* 328:275–278.

Bretweiser, G. E., and G. Szabo. 1985. Uncoupling of cardiac muscarinic receptors and beta-adrenergic receptors from ion channels by a guanine nucleotide analogue. *Nature.* 317:538–540.

Brown, C. M., and G. Burnstock. 1981. The structural conformation of the polyphosphate chain of the ATP molecule is critical for its promotion of prostaglandin synthesis. *European Journal of Pharmacology.* 69:81–86.

Burnstock, G. 1978. A basis for distinguishing two types of purinergic receptor. *In* Cell Membrane Receptors for Drugs and Hormones: a Multidisciplinary Approach. L. Bolis and R. W. Straub, editors. Raven Press, New York. 107–118.

Burnstock, G. 1979. Past and current evidence for the purinergic nerve hypothesis. *In* Physiological and Regulatory Functions of Adenosine and Adenine Nucleotides. H. P. Baer and G. I. Drummond, editors. Raven Press, New York. 3–32.

Burnstock, G., and C. Kennedy. 1985. Is there a basis for distinguishing two types of P_2-purinoceptor? *General Pharmacology.* 16:433–440.

Burnstock, G., and P. Meghji. 1983. The effect of adenyl compounds on the rat heart. *British Journal of Pharmacology.* 79:211–218.

Charest, R., P. F. Blackmore, and J. H. Exton. 1985. Characterization of responses of isolated rat hepatocytes to ATP and ADP. *Journal of Biological Chemistry.* 260:15789–15794.

Clemens, M. G., and T. Forrester. 1981. Appearance of adenosine triphosphate in the coronary sinus effluent from isolated working rat heart in response to hypoxia. *Journal of Physiology.* 312:143–158.

Cockcroft, S., and B. D. Gomperts. 1979. Activation and inhibition of calcium-dependent histamine secretion by ATP ions applied to rat mast cells. *Journal of Physiology.* 296:229–243.

Colquhoun, D., E. Neher, H. Reuter, and C. F. Stevens. 1981. Inward current activated by intracellular Ca in cultured cardiac cells. *Nature.* 294:752–754.

Dionne, V. E., J. H. Steinbach, and C. F. Stevens. 1978. An analysis of the dose-response relationship at voltage-clamped frog neuromuscular junctions. *Journal of Physiology.* 281:421–444.

Donald, F. M. S. 1985. A non-adrenergic, non-cholinergic (NANC) excitatory response in frog atria. *British Journal of Pharmacology.* 85:211P. (Abstr.)

Doyle, T. B., and T. Forrester. 1985. Appearance of adenosine triphosphate in the perfusate from working frog heart. *Pflügers Achiv.* 405:80–82.

Drury, A. N., and A. Szent-Gyorgyi. 1929. The physiological activity of adenine compounds with especial reference to their action upon the mammalian heart. *Journal of Physiology.* 68:213–237.

Dubyak, G. R., and M. B. De Young. 1985. Intracellular Ca mobilization by extracellular ATP in Ehrlich ascites tumor cells. *Journal of Biological Chemistry.* 260:10653–10661.

Ehrlich, Y. H., T. B. Davis, E. Bock, E. Kornecki, and R. H. Lenox. 1986. Ecto-protein kinase activity on the external surface of neural cells. *Nature.* 320:67–70.

Fedan, J. S., G. K. Hogaboom, D. P. Westfall, and J. P. O'Donnell. 1982. Comparison of contractions of the smooth muscle of the guinea-pig vas deferens induced by ATP and related nucleotides. *European Journal of Pharmacology.* 81:193–204.

Feltz, A., and A. Trautmann. 1982. Desensitization at the frog neuromuscular junction: a biphasic process. *Journal of Physiology.* 322:257–272.

Flitney, F. W., and J. Singh. 1980. Inotropic responses of the frog ventricle to adenosine triphosphate and related changes in endogenous cyclic nucleotides. *Journal of Physiology.* 304:21–42.

Forrester, T., and C. A. Williams. 1977. Release of adenosine triphosphate from isolated heart cells in response to hypoxia. *Journal of Physiology.* 268:371–390.

Friel, D. D., and B. P. Bean. 1986. External ATP-activated currents in cardiac atrial cells. *Biophysical Journal.* 49:402*a*. (Abstr.)

Furshpan, E. J., D. D. Potter, and S. G. Matsumoto. 1986. Synaptic function in rat sympathetic neurons in microcultures. III. A purinergic effect on cardiac myocytes. *Journal of Neuroscience.* 6:1099–1107.

Fyffe, R. W., and E. R. Perl. 1984. Is ATP a central synaptic mediator for certain primary afferent fibers from mammalian skin? *Proceedings of the National Academy of Sciences.* 81:6890–6893.

Gallacher, D. V. 1982. Are there purinergic receptors on parotid acinar cells? *Nature.* 296:83–86.

Goto, M., A. Yatani, and Y. Tsuda. 1977. An analysis of the effects of ATP and related compounds on membrane current and tension components in bullfrog atrial muscle. *Japanese Journal of Physiology.* 27:81–94.

Goto, M., A. Yatani, and Y. Tsuda. 1978. Stabilizing effects of adenosine on the membrane currents and tension components of the bullfrog atrium. *Japanese Journal of Physiology.* 28:611–625.

Gratecos, D., and E. H. Fischer. 1974. Adenosine 5′-*O*(3-thiotriphosphate) in the control of phosphorylase activity. *Biochemical and Biophysical Research Communications.* 58:960–967.

Haggblad, J., and E. Heilbronn. 1987. Externally applied adenosine 5′-triphosphate causes

inositol triphosphate accumulation in cultured chick myotubes. *Neuroscience Letters.* 74:199–204.

Hamill, O., A. Marty, E. Neher, B. Sakmann, and F. J. Sigworth. 1981. Improved patch-clamp techniques for high-resolution current recording from cells and cell-free membrane patches. *Pflügers Archiv.* 391:85–100.

Hartzell, H. C. 1979. Adenosine receptors in frog sinus venosus: slow inhibitory potentials produced by adenine compounds and acetylcholine. *Journal of Physiology.* 293:23–49.

Hellam, D. C., and R. J. Podolsky. 1969. Force measurements in skinned muscle fibres. *Journal of Physiology.* 200:807–819.

Heppel, L. A., G. A. Weisman, and I. Friedberg. 1985. Permeabilization of transformed cells in culture by external ATP. *Journal of Membrane Biology.* 86:189–196.

Hill, J. A., Jr., R. Coronado, and H. C. Strauss. 1986. Reconstitution of a Ca-activated channel from ventricular sarcolemma. *Circulation.* 74(Suppl. II):II–28. (Abstr.)

Hollander, P. B., and J. L. Webb. 1957. Effects of adenine nucleotides on the contractility and membrane potentials of rat atrium. *Circulation Research.* 5:349–353.

Hoyle, C. H. V., and G. Burnstock. 1986. Evidence that ATP is a neurotransmitter in the frog heart. *European Journal of Pharmacology.* 124:285–289.

Hume, J. R., and W. Giles. 1981. Active and passive properties of single bullfrog atrial cells. *Journal of General Physiology.* 78:19–42.

Hume, R. I., and M. G. Honig. 1986. Excitatory action of ATP on embryonic chick muscle. *Journal of Neuroscience.* 6:681–690.

Jahr, C. E., and T. M. Jessell. 1983. ATP excites a subpopulation of rat dorsal horn neurons. *Nature.* 304:730–733.

James, T. N. 1965. The chronotropic effect of ATP and related compounds studied by direct perfusion of the sinus node. *Journal of Pharmacology and Experimental Therapeutics.* 149:233–247.

Kakei, M., and A. Noma. 1984. Adenosine-5′-triphosphate-sensitive single potassium channel in the atrioventricular node cell of the rabbit heart. *Journal of Physiology.* 352:265–284.

Kennedy, C., and G. Burnstock. 1985. ATP produces vasodilation via P_1 purinoceptors and vasoconstriction via P_2 purinoceptors in the isolated rabbit central ear artery. *Blood Vessels.* 22:145–155.

Kolb, H.-A., and M. J. O. Wakelam. 1983. Transmitter-like action of ATP on patched membranes of cultured myoblasts and myotubes. *Nature.* 303:621–623.

Krishtal, O. A., S. M. Marchenko, and V. I. Pidoplichko. 1983. Receptor for ATP in the membrane of mammalian sensory neurones. *Neuroscience Letters.* 35:41–45.

Lotan, I., N. Dascal, S. Cohen, and Y. Lass. 1986. ATP-evoked membrane responses in *Xenopus* oocytes. *Pflügers Archiv.* 406:158–162.

Magazanik, L. G., and F. Vyskocil. 1970. Dependence of acetylcholine desensitization on the membrane potential of frog muscle fibre and on the ionic changes in the medium. *Journal of Physiology.* 210:507–518.

Mechmann, S., and L. Pott. 1986. Identification of Na-Ca exchange current in single cardiac myocytes. *Nature.* 319:597–599.

Niedergerke, R., and S. Page. 1981. Two physiological agents that appear to facilitate calcium discharge from the sarcoplasmic reticulum in frog heart cells: adrenalin and ATP. *Proceedings of the Royal Society of London, Series B.* 213:325–344.

Pfaffinger, P. J., J. M. Martin, D. D. Hunter, N. M. Nathanson, and B. Hille. 1985. GTP-binding proteins couple cardiac muscarinic receptors to a K channel. *Nature.* 317:536–538.

Rane, S. G., and K. Dunlap. 1986. Kinase C activator 1,2-oleoylacetylglycerol attenuates voltage-dependent calcium current in sensory neurons. *Proceedings of the National Academy of Sciences.* 83:184–188.

Salt, T. E., and R. G. Hill. 1983. Excitation of single sensory neurones in the rat caudal trigeminal nucleus by iontophoretically applied adenosine 5′-triphosphate. *Neuroscience Letters.* 35:53–57.

Scubon-Mulieri, B., and R. L. Parsons. 1977. Desensitization onset and recovery at the potassium depolarized frog neuromuscular junction are voltage sensitive. *Journal of General Physiology.* 71:285–299.

Sharma, V. K., and S.-S. Sheu. 1986. Micromolar extracellular ATP increases intracellular calcium concentration in isolated rat ventricular myocytes. *Biophysical Journal.* 49:351*a*. (Abstr.)

Shibata, E., and W. R. Giles. 1985. Ionic currents that generate the spontaneous diastolic depolarization in individual cardiac pacemaker cells. *Proceedings of the National Academy of Sciences.* 82:7796–7800.

Sneddon, P., and G. Burnstock. 1984. Inhibition of excitatory junction potentials in guinea pig vas deferens by α,β-methylene ATP: further evidence for ATP and noradrenaline as cotransmitters. *European Journal of Pharmacology.* 100:85–90.

Stone, T. W. 1981. Physiological roles for adenosine and adenosine 5′-triphosphate in the nervous system. *Neuroscience.* 6:523–555.

Stone, T. W. 1985. The activity of phosphorothioate analogues of ATP in various smooth muscle systems. *British Journal of Pharmacology.* 84:165–173.

Su, C. 1983. Purinergic neurotransmission and neuromodulation. *Annual Review of Pharmacology and Toxicology.* 23:397–411.

Sung, S.-S. J., J. D.-E. Young, A. M. Origlio, J. M. Heiple, H. R. Kaback, and S. C. Silverstein. 1985. Extracellular ATP perturbs transmembrane ion fluxes, elevates cytosolic [Ca^{2+}], and inhibits phagocytosis in mouse macrophages. *Journal of Biological Chemistry.* 260:13442–13449.

Trube, G., and Hescheler. 1984. Inward-rectifying channels in isolated patches of the heart cell membrane: ATP-dependence and comparison with cell attached patches. *Pflügers Archiv.* 401:178–184.

Tsien, R. Y. 1980. New calcium indicators and buffers with high selectivity against magnesium and protons: design, synthesis, and properties of prototype structures. *Biochemistry.* 19:2396–2404.

Vesprille, A., and C. D. Van Duyn. 1966. The negative chronotropic effect of adenine derivatives and acetylcholine on frog and rat hearts. *Pflügers Archiv.* 292:288–296.

Walaas, E. 1958. Stability constants of metal complexes with mononucleotides. *Acta Chemica Scandinavica.* 12:528–536.

Yatani, A., M. Goto, and Y. Tsuda. 1978. Nature of catecholamine-like actions of ATP and other energy-rich nucleotides on the bullfrog atrial muscle. *Japanese Journal of Physiology.* 28:47–61.

Yellen, G. 1982. Single Ca^{2+}-activated nonselective cation channels in neuroblastoma. *Nature.* 296:357–359.