Adenosine 5′-Triphosphate: a P2-Purinergic Agonist in the Myocardium

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I. Introduction
II. Extracellular Adenosine 5′-Triphosphate: Sources, Metabolism, and Receptors
   A. Sources of extracellular ATP
   B. Mechanisms of ATP release
   C. ATP degradation by ecto-ATPases
   D. Sources and metabolism of UTP and diadenosine polyphosphates
   E. ATP binding sites
   F. Ionotropic P2X receptors
   G. Metabotropic P2Y receptors
   H. Adenine dinucleotide receptors
   I. Pharmacological concerns
III. Physiological and Physiopathological Effects of Extracellular Adenosine 5′-Triphosphate
   A. Contractile effects
   B. Chronotropic effects
   C. Pro- and anti-arrhythmic effects of ATP
   D. Hypertrophy
   E. Other general effects of ATP in cardiac cells
   F. Effects of diadenosine polyphosphates on heart
IV. Molecular and Cellular Mechanisms
   A. Modulation of cardiac transmembrane ionic currents
   B. Signal transduction pathways
V. Concluding Remarks

Vassort, Guy. Adenosine 5′-Triphosphate: a P2-Purinergic Agonist in the Myocardium. Physiol Rev 81: 767–806, 2001.—ATP, besides an intracellular energy source, is an agonist when applied to a variety of different cells including cardiomyocytes. Sources of ATP in the extracellular milieu are multiple. Extracellular ATP is rapidly degraded by ectonucleotidases. Today ionotropic P2X1–7 receptors and metabotropic P2Y1,2,4,6,11 receptors have been cloned and their mRNA found in cardiomyocytes. On a single cardiomyocyte, micromolar ATP induces nonspecific cationic and Cl− currents that depolarize the cells. ATP both increases directly via a Gs protein and decreases Ca2+ current. ATP activates the inward-rectifying currents (ACh- and ATP-activated K+ currents) and outward K+ currents. P2-purinergic stimulation increases cAMP by activating adenylyl cyclase isoform V. It also involves tyrosine kinases to activate phospholipase C-γ to produce inositol 1,4,5-trisphosphate and Cl−/HCO3− exchange to induce a large transient acidosis. No clear correlation is presently possible between an effect and the activation of a given P2-receptor subtype in cardiomyocytes. ATP itself is generally a positive inotropic agent. Upon rapid application to cells, ATP induces various forms of arrhythmia. At the tissue level, arrhythmia could be due to slowing of electrical spread after both Na+ current decrease and cell-to-cell uncoupling as well as cell depolarization and Ca2+ current increase. In as much as the information is available, this review also reports analog effects of UTP and diadenosine polyphosphates.

I. INTRODUCTION

Interest in ATP as a molecule was for many years devoted to the concept of the “high-energy phosphate bond” introduced by Lippman (292); this despite the fact that roles for extracellular purines had been described by Drury and Szent-Györgyi in 1929 (137) soon after the discovery of ATP (144, 295). In fact, the crude tissue extracts used by Drury and Szent-Györgyi contained mostly AMP and induced a negative chronotropic effect
and a decrease in blood pressure. ATP was later shown to produce transient tachycardia at low doses or to slow heart and to induce atrioventricular block at higher doses (207, 454). These effects were associated with vasodilatation of coronary vessels and led Berne (36) to postulate that adenosine was the mediator. Adenosine then became the topic of most studies concerning purines and the cardiovascular system.

ATP is an amphiphilic compound showing both hydrophilic and strong hydrophobic interaction. Adenine, its 6-aminopurine base, has $pK_a < 1, 4.1, and 9.8$. Like free fatty acids, ATP binds to BSA, which is generally used to mimic protein solutions and establish an oncotic pressure. Up to 94%, but not all, ATP could be liberated from BSA by the addition of palmitate. Interaction of BSA with fatty acids, ATP binds to BSA, which is generally used to mimic protein solutions and establish an oncotic pressure.

Excitatory and P2Y for inhibitory, then P2U for those also activated by UTP and $P_{2T}$ at which ADP induces platelet aggregation, finally $P_{2Z}$ for a large molecular pore activated by ATP$^-$ and P2Y, which is activated by dinucleotides. The second system is based on the cloning of P2 receptors where, the IUPHAR, Committee on Receptor Nomenclature (164, 165), has defined as $P_{2X}$, the ligand-gated ion channel and as $P_{2Y}$, the G protein-coupled receptors. Very slow progress has been made to establish the correspondence of the cloned to the pharmacologically defined P2 subtypes. This has proven difficult to realize in part because some P2 purinoceptors are functional heteroligomers and in part because the molecule binding to a given receptor is often a hydrolysis product of that which was added to the bath. Moreover, this field is characterized by the paucity of receptor subtype-selective agonists and antagonists that have their own propensity to undergo rapid hydrolysis and interconversion just like the natural agonists. It is also a problem that commercially available compounds might already contain breakdown products that act as effective agonists (42, 198, 342, 513), a point already considered in 1934 by Gillespie (180).

After a characterization of the sources and extracellular metabolism pathways of ATP and the various purinergic receptors known today, the present review concentrates on the mechanical, chronotropic, and arrhythmogenic effects of ATP at the cellular level and under the whole heart as well as in pathological conditions. These pieces of information are then interpreted on the basis of our present knowledge concerning the electrophysiological consequences and signal transduction pathways activated by applying ATP to isolated cardiomyocytes. Inasmuch as it is possible, similar pieces of information are given for UTP and diadenosine polyphosphates. Much more is known about the effects of ATP in wider aspects of cardiovascular system, and this has been extensively reviewed elsewhere (59, 143, 191, 272, 355, 402, 488).

### II. EXTRACELLULAR ADENOSINE 5'-TRIPHOSPHATE: SOURCES, METABOLISM, AND RECEPTORS

#### A. Sources of Extracellular ATP

Adenine nucleotides are continually present in quite variable amounts in the extracellular space of the heart. More precise knowledge of ATP exocytosis could be expected with the development of new bioluminescence technologies (464) involving cell surface-attached firefly luciferase (28) or atomic force microscopy in combination with myosin functional cantilevers (431). Basal ATP found in the coronary effluent from isolated, saline-perfused hearts had been shown to range below 1 nM (54,
491). This rather low value reflects the rapid degradation of ATP, which is over 95% during a single coronary passage. With the use of microdialysis, ATP in the interstitial space has been estimated to be 40 nM (276). These levels increase markedly during electrical stimulation as already noted in 1962 (1): hypoxia or ischemia (36, 163, 276, 359, 491, 519), challenge by cardiotonic agents (54, 117, 244, 491), increased blood flow (117, 492), mechanical stretch (483), and even work load in frog heart (136).

There is strong evidence supporting the notion that ATP is a cotransmitter in perivascular sympathetic nerves (68). Other likely sources of the nucleotide include the following: (1) ischemic myocytes (36, 163, 359, 519), (2) activated platelets (118, 209, 328), (3) nerve terminals (70, 210, 408), (4) inflammatory cells (133), (5) erythrocytes (33, 141, 449), (6) endothelial cells (46, 401, 526), (7) smooth muscle cells (45, 245, 369), and (8) exercising muscle cells (162, 364).

### B. Mechanisms of ATP Release

The mechanism by which ATP is transported across the cell membrane or released from muscle cells is not fully understood. In living cells, the electrochemical gradient favoring ATP efflux is near nine orders of magnitude (versus 6 for Ca$^{2+}$) considering intra- and extracellular ATP concentrations to be 10 mM and 10 nM, respectively, a cell resting potential of −90 mV and MgATP$^{2−}$ as the preferred flowing anion. Thus membrane permeability to ATP should be very low, which is in agreement with the molecular size of ATP.

ATP can be released by exocytosis from platelets and nerves like other neurotransmitters. It can also leak out during cell lysis (139, 182). In recent years, the cystic fibrosis transmembrane conductance regulator (CFTR) has been suggested to act as an ATP channel and enables intracellular ATP to cross the cell membrane (2, 3, 6, 366, 432, 509). This is strongly disputed (see Ref. 123 for review), and it has been shown that mechanical stimulation of the cell surface, rather than CFTR activation, is sufficient to release ATP (510). A recent work (457) suggests that the permeation pathways for Cl$^{−}$ and ATP are distinct, in which case CFTR would be a regulator of the ATP channel. Identification of the molecular basis of the CFTR-associated ATP channel is of critical interest.

Supporting the idea that an anion channel can allow for ATP to cross through membranes, it has been recently reported that the mitochondrial voltage-dependent anion channel (412) as well as the 116-pS Cl$^{−}$ channel in cardiac sarcoplasmic reticulum (SR) conduct ATP and adenine nucleotides (247).

### C. ATP Degradation by Ecto-ATPases

The breakdown of ATP and ADP to form adenosine was first reported nearly 50 years ago (235), and it is long recognized that this susceptibility to degradation does limit the potency of ATP and its degradable analogs (515). In considering the biological responses to extracellular ATP in multicellular tissues and even isolated cells, it should be noted that such effects are not only complicated by the presence of multiple P2-purinergic receptors but also by the rapid catabolism of ATP to produce adenosine. This cascade of surface-located enzymes converts P2 into P1 signaling. The extracellularly formed adenosine can itself modulate cardiac functions and also serve purine salvage after reuptake via plasma membrane-located adenosine transporters (471). The majority of the ATP perfused into the heart is dephosphorylated during a single passage through the coronary vasculature (29, 158, 359). The half-life of ATP is 0.2 s when perfused in blood through the lung vasculature (413) instead of ~10 min in whole blood ex vivo (479). It is likely that this catabolism is mostly due to endothelial cell ectonucleotidases. This hydrolysis of ATP, ADP, and AMP was first demonstrated in isolated rat myocytes by Bowditch et al. (56), but the nature and characteristics of the enzymes were not studied. Kinetic properties of the extracellular reaction sequences ATP → ADP → AMP → adenosine catalyzed by ectonucleotidases has been investigated at the surface of adult rat cardiomyocytes by following the catabolism of $^3$H-labeled nucleotides (158, 324) and by NMR (35) or in the ventricular muscle by microdialysis (276). It is worth noting that a significant ATP catabolism occurs even on isolated cardiomyocytes. Thus ATP is rapidly hydrolyzed to 65 and 33% after 3 and 15 min, respectively, by plasma membrane at 20°C (42).

A variety of extracellular enzymes utilize ATP to induce biological responses (256, 324, 325, 368, 373, 541, 542) (Table 1). In many tissues including heart, extracellular ATP as well as other nucleotide tri- and diphosphates appear to be hydrolyzed by E-type enzymes. They include ecto-ATPases and ecto-ATP diphosphohydrolases or ecto-ATP Dases. All (but the $\alpha$-sarcoglycan or Adhalin) lack the Walker consensus ATP binding motif; instead, they have several apyrase conserved domains, a putative ATP $\beta$-phosphate 1 binding motif, found in proteins as diverse as actins, 70-kDa heat shock protein, and sugar kinases. Ecto-ATPases are insensitive to known inhibitors of various intracellular ATPases such as ouabain and vanadate (P-type) or Na$^{+}$-K$^{+}$-ATPase and two orders of magnitude higher than the one of the SR Ca$^{2+}$-ATPase (380). These enzymes are activated by either Ca$^{2+}$ or Mg$^{2+}$ and inhibited however at
Ecto-ATPase is a highly glycosylated protein with six potential N-linked glycosylation, five apyrase-conserved regions, and two potential transmembrane domains. Both NH₂ and COOH terminals are short with COOH terminals demonstrating phosphorylation consensus sites (92, 304). This 82-kDa protein is identical to a lymphoid cell activation antigen CD39, an ecto-apyrase (368). The first vertebrate ecto-ATPase was cloned and sequenced from the chicken muscle (257). It has a molecular mass of 54.4 kDa. Ecto-ATPases share some homologies with CD39 ecto-ATPDases in that they have two transmembrane domains, four external putative glycosylation sites, and four apyrase-conserved regions. Like ecto-ATPDases, they do not show Walker consensus sequence. The COOH terminal contains a single putative cAMP/cGMP-dependent protein kinase phosphorylation site as well as a single putative tyrosine kinase phosphorylation site (257).

A Ca²⁺/Mg²⁺ ecto-ATPase activity has long been reported in the plasma membrane of cardiac myocytes (128, 414, 480, 531). Rat cardiac sarcolemmal Ca²⁺/Mg²⁺ ecto-ATPase (Myoglobin) requires millimolar concentrations of Ca²⁺ or Mg²⁺ for maximal ATP hydrolysis. It has been purified to apparent homogeneity, and a partial cDNA clone has been produced that revealed 100% homology to human platelet CD36 (241). In its native form, this enzyme has a molecular mass of 180 kDa and two subunits of 90 kDa each (240). The limited sequence information indicates that there is no sequence similarity with any of the previously reported ecto-ATPases, chicken gizzard smooth muscle ecto-ATPase (257), or CD39 (242, 507), could substitute for Mg²⁺, but Ca²⁺, Sr²⁺, Ba²⁺, or Be²⁺ could not fulfill the bivalent cation requirement (26). They can be distinguished from ecto-ATPDases by their inability to hydrolyze ADP and other nucleotide diphosphates at a rate >1–2% that of their ATP hydrolysis rate. Also, they are insensitive to azide. However, 8-azido-ATP is a good substrate of ecto-ATPase activity while it is an irreversible partial inhibitor after photoactivation (410). Another difference with ecto-ATPDases is fast inactivation of their ATP hydrolysis activity, an effect prevented by concanavalin A, a known inhibitor of 5′-nucleotidase (368). The first vertebrate ecto-ATPase was cloned and sequenced from the chicken muscle (257). It has a molecular mass of 54.4 kDa. Ecto-ATPases share some homologies with CD39 ecto-ATPDases in that they have two transmembrane domains, four external putative glycosylation sites, and four apyrase-conserved regions. Like ecto-ATPDases, they do not show Walker consensus sequence. The COOH terminal contains a single putative cAMP/cGMP-dependent protein kinase phosphorylation site as well as a single putative tyrosine kinase phosphorylation site (257).

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but partial homology with SR and sarcolemmal pump Ca\(^{2+}\)-ATPases.

\(\alpha\)-Sarcoglycan (Adhalin) has recently been shown to have ecto-ATPase activity in skeletal muscle (38). It is different from the t-tubule ecto-ATPase of 56 kDa (119). Its deduced amino acid sequence for ATP binding reveals two Walter consensus sequences present in several intracellular ATPases. It binds ATP in a Mg\(^{2+}\)-dependent, Ca\(^{2+}\)-independent manner. ATP binding is inhibited by 3’-O-(4-benzoyl)benzoyl ATP (BzATP). The authors thus suggest that \(\alpha\)-sarcoglycan also modulates the activity of P2X (P2X\(_7\)) receptor by buffering the extracellular ATP concentration (38).

An ecto-nucleoside diphosphokinase (NDPK) has also been described but is, as yet, not reported in cardiac muscle. It controls the interconversion of P2-receptor agonists with a rate of extracellular transphosphorylation up to 20-fold the rate of nucleotide hydrolysis (279). The latter can just couple ATP- and UTP-dependent stimulations. Other ecto-enzymes that also catalyze the hydrolysis of ATP such as alkaline phosphatase (518) or autotaxin, a nucleotide phosphodiesterase/ATP pyrophosphatase (93, 181), have not yet been studied in cardiac tissues.

Furthermore, the ATP degradation product AMP will be hydrolyzed to adenosine by 5’-nucleotidase, a phosphatase active mostly at alkaline pH (25, 146, 326, 377, 380, 433).

The kinetic properties of the ATP hydrolysis catalyzed by ectonucleotidases at the surface of cardiomyocytes were characterized (324). It is worthy of noting that the activity of these ecto-nucleotidases is modulated by Mg\(^{2+}\) (305), protein kinase C (PKC), and \(\alpha\)-adrenergic stimulation (260, 262, 347, 420) as well as during preconditioning or postmyocardial infarction (263, 276, 320) and also differs in isolated perfused versus in situ hearts (276). The activity of the ecto-ATPase is not affected by treadmill exercise training or by exhaustive exercise (120). In isolated cardiomyocytes, exogenous adenosine, via A\(_1\)-receptor activation and a G\(_i\) protein, decreases both ectosolic and cytosolic 5’-nucleotidase activity (261).

Intracellular protein kinases are important in the regulation of cellular functions. Protein kinases that use extracellular ATP to phosphorylate proteins localized at the external surface of the plasma membrane (ecto-protein kinases) have now been demonstrated in a variety of tissues. Several surface proteins are reported to be phosphorylated in nerve cells and in aortic endothelial cells (140, 142; see Ref. 139 for review). In addition to an ecto-protein kinase with catalytic properties of atypical PKC-\(\zeta\) implicated in hippocampal long-term potentiation (85), a cAMP-dependent kinase has been shown to phosphorylate the atrial natriuretic peptides (270, 271). In view of the fact that ATP and cAMP are released in the extracellular space, one should expect a role for these ectokinases in the cardiac tissues.

D. Sources and Metabolism of UTP and Diadenosine Polyphosphates

Most P2Y receptors (P2Y\(_{2, 4, 6}\)) and, maybe, some P2X receptors are also activated by uracil nucleotides. This suggests the presence of extracellular UTP and derivatives. Recently, it has been demonstrated that mechanical stimulation induces a 15-fold increase in UTP release from 1321 N1 human astrocytoma (278). A recent review has appeared (10). Presently, little is known in heart. UTP stimulates cardiomyocytes. Most studies deal with the mechanisms of nucleoside plasma salvage leading to uracil nucleotide synthesis (175, 297). Total uracil nucleotides is 1.7 \(\mu\)mol/g protein in myocardium and in freshly isolated cardiomyocytes much less than the ATP content (27 \(\mu\)mol/g protein) in myocardium (176, 354).

Adenosine polyphosphates are a group of adenosine dinucleotides that consist of two adenosine molecules bridged by a variable number of phosphates thus abbreviated Ap\(_n\)A (\(n = 2–6\)). They are ubiquitous molecules found in prokaryotes and eukaryotes. Intracellular Ap\(_n\)As appear to alert the cell under stress conditions, thus their name “alarmones.” The potency and efficacy of Ap\(_n\)As to inhibit ATP-activated K\(^+\) channel activity were described in cardiac cells and appeared similar to those of intracellular ATP (236). Also, Ap\(_n\)As stimulate a Ca\(^{2+}\)-induced Ca\(^{2+}\) release channel from skeletal muscle SR as well as increasing the binding of ryanodine to the calcium release channel by, respectively, nine- and threefold in skeletal and cardiac muscles (206). Another alarmon receptor is hemoglobin. Ap\(_n\)As bind preferentially with high affinity to the deoxy conformation of hemoglobin in a ratio of one per tetramer. This binding markedly enhances the Bohr effect (52). Recently, it has been reported that the mammalian myocardium contains abundant amounts of Ap\(_5\)A (237) or of Ap\(_4\)A in humans (485). In mammalian tissues, Ap\(_n\)As are produced and released from platelets or chro-maffin cells where it is stored with ATP and catecholamines. Ischemia induces a 10-fold decrease in the Ap\(_n\)A myocardial concentration (237, 264, 338). Ap\(_n\)As are degraded in rat plasma to ATP (from Ap\(_5\)A) and AMP, and these nucleotides are further degraded to adenosine. However, compared with ATP, Ap\(_n\)A and Ap\(_4\)A have a relatively long half-life (300). The rate of degradation is dose dependent: the biological half-life was ~3 s at 1 mg/kg after intravenous infusion (253). The endothelial ecto-diadenosine polyphosphate hydrolase of cultured adrenomedullary vascular endothelial cells is activated by Mg\(^{2+}\) and Mn\(^{2+}\) and inhibited by Ca\(^{2+}\), adenosine 5’-O-(3-thiotriphosphate) (ATP\(_{3S}\)), and suramin (308). These compounds are considered to act as extra- and intracellular signaling molecules, and many of their properties are close to those of ATP (22, 259, 353).
E. ATP Binding Sites

Purified heart sarcolemma membranes were found to bind a slowly hydrolyzable analog of ATP, $[^{35}S]ATP\gamma S$, in a specific manner and exhibited two apparent affinity sites (352). The high-affinity site, which may represent the ATP receptor according to the authors, had a dissociation constant ($K_d$) of 4.7–8.3 nM and a maximal binding ($B_{\text{max}}$) of 9.5–18.4 pmol/mg protein, whereas the low-affinity site had a $K_d$ of 655–1.227 nM and a $B_{\text{max}}$ of 812–2,955 pmol/mg protein. $[^{35}S]ATP\gamma S$ binding was displaced by GTP, UTP, CTP, and ITP. The number of high-affinity binding sites decreases during oxidative stress (334). Similar high- and low-affinity binding sites for $\alpha,\beta$-[H]$\text{met-ATP}$, then considered to be a P2X agonist, were also reported in rat heart membranes (327). In another study, 8-azido-ATP (8-Az-ATP) was used for labeling intact rat ventricular myocytes. After minimizing background labeling by large concentrations of UTP, two bands, one near 48 kDa and a less pronounced at 90 kDa, are labeled by radioactive 8-Az-ATP (177). 2-MeSATP and ATP$\gamma S$ both partially and specifically inhibited labeling of the 48-kDa band. This was related to activation of the fast Ca$^{2+}$ transient by 2-MeSATP but not by ATP$\gamma S$ and of the Na$^+$/P$^-$ cotransport by ATP$\gamma S$ only. However, much caution should be taken during these binding studies, not only because it would be difficult to distinguish sites with relatively similar binding affinities but mostly because secured experimental conditions are difficult to settle. Optimal binding conditions to obtain reliable equilibrium parameters require a 15-min incubation, a time during which >75% of the radio-ligand must remain intact, a situation not easily reached in view of the high ecto-ATPase activities (42).

F. Ionotropic P2X Receptors

Several reviews directly aimed at P2X receptors have appeared (17, 59, 64, 112, 149, 178, 349–351, 403, 448, 459).

### Table 2. Ionotropic P2X purinoceptor: characteristics and presence in the heart

<table>
<thead>
<tr>
<th>Amino Acids</th>
<th>[ATP], $\mu$M</th>
<th>[α,β-Met-ATP], $\mu$M</th>
<th>TNT-ATP, nM</th>
<th>PPADS, $\mu$M</th>
<th>Cation</th>
<th>Desensitization</th>
<th>Heart</th>
<th>Reference Nos.</th>
</tr>
</thead>
<tbody>
<tr>
<td>P2X$_1$</td>
<td>300</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>Na$^+$/K$^+$/Ca$^{2+}$</td>
<td>Fast</td>
<td>+</td>
<td>47, 348</td>
</tr>
<tr>
<td>P2X$_{1,5}$</td>
<td>472</td>
<td>10</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>Na$^+$/K$^+$</td>
<td>Slow</td>
<td>+</td>
<td>348</td>
</tr>
<tr>
<td>hP2X$_1$</td>
<td>397</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>Na$^+$/K$^+$</td>
<td>Fast</td>
<td>0</td>
<td>47, 173</td>
</tr>
<tr>
<td>hP2X$_2$</td>
<td>397</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>Na$^+$/K$^+$</td>
<td>Fast</td>
<td>+</td>
<td>171, 348, 447</td>
</tr>
<tr>
<td>P2X$_2$</td>
<td>388</td>
<td>20</td>
<td>100</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>Slow</td>
<td>(+)</td>
<td>172</td>
</tr>
<tr>
<td>P2X$_3$</td>
<td>455</td>
<td>15</td>
<td>&gt;100</td>
<td>2</td>
<td>Na$^+$/K$^+$/weak</td>
<td>Slow</td>
<td>+</td>
<td>99</td>
</tr>
<tr>
<td>P2X$_4$</td>
<td>379</td>
<td>10</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>Na$^+$/K$^+$/Ca$^{2+}$</td>
<td>Slow</td>
<td>+</td>
<td>406, 5, 30</td>
</tr>
<tr>
<td>P2X$_{2,3}$</td>
<td>105</td>
<td>200</td>
<td>&gt;100</td>
<td>45</td>
<td>Pore</td>
<td>Slow</td>
<td></td>
<td>84, 286</td>
</tr>
<tr>
<td>P2X$_{1,5}$</td>
<td>5</td>
<td></td>
<td>1</td>
<td>1</td>
<td>Na$^+$/K$^+$/Ca$^{2+}$</td>
<td>Slow</td>
<td>+</td>
<td>477</td>
</tr>
<tr>
<td>P2X$_{2,3}$</td>
<td>6</td>
<td>12</td>
<td>&gt;100</td>
<td></td>
<td>Pore</td>
<td>Slow</td>
<td></td>
<td>280</td>
</tr>
</tbody>
</table>

* Splice variants P2X$_{2,3}$ (265 amino acids) (61) and P2X$_{2,3}$ (361 amino acids) (478) have been found. + The agonist response of mouse ortholog mP2X$_1$ is potentiated by P2X antagonists (478). (+), Found in fetal but not adult. α,β-Met-ATP, α,β-methylene adenosine 5′-triphosphate; TNT-ATP, 2′,3′-O-(2,4,6-trinitrophenyl)adenosine 5′-triphosphate.
dissociated smooth muscle cells of the rat tail artery proposes that UTP is an effective agonist but 100-fold less potent than ATP (318). Both suramin and pyridoxal-phosphate-6-azophenyl-2′,4′-disulfonic acid (PPADS), but not amiloride, block this current. The conductance, 19 pS at negative potential, shows anomalous inward rectification. These electrophysiological properties are close to those observed in smooth muscle (24). The cloned 399-amino acid-long protein is mostly extracellular and contains two transmembrane domains plus a pore-forming motif that bears a striking similarity with the P domain of the inward rectifying K⁺ channel, Kv 2.1 (205). A similar molecular architecture was reported for the mechanosensitive channels of Caenorhabditis elegans (211) and the related amiloride-sensitive epithelial sodium channel (77). However, there is no primary sequence homology between these channels and the P2X purinoceptor.

b) P2X₂ RECEPTOR. Essentially the same structure and properties were reported by Brake et al. (60) for a P2X₂ channel cloned from a complementary DNA library constructed from PC12 mRNA screened in oocytes. The homomeric P2X₂ channel is most readily characterized by its insensitivity to α,β-met-ATP and its lack of desensitization during ATP applications of up to 10 s. Its conductance is 21 pS at −100 mV in 150 mM NaCl. The behavior of this cloned P2X₂ receptor resembles native P2X receptors on PC12 cells but differs from those on vascular smooth muscle and vas deferens where α,β-met-ATP acts as a potent agonist. ATP, ATPγS, and 2-MeSATP were roughly equipotent agonists. CTP and 2′-deoxy-ATP (dATP) elicited small currents, whereas UTP, GTP, ADP, and AMP were inactive. As in neurons (96, 286), extracellular Zn²⁺ potentiates the ATP effect on the cloned receptor by shifting the EC₅₀ for ATP to 15 μM. The intracellular COOH terminus of P2X₂ receptor contains several consensus sequences for phosphorylation by protein kinase A (PKA) and by PKC. After its reexpression in HEK-293 cells, dialyzing with phorbol 12-myristate 13-acetate (PMA) fails to alter the ATP-induced cationic current. However, 8-bromo-cAMP or the purified catalytic subunit of PKA causes a reduction in the magnitude of the ATP-activated current without altering its kinetics of inactivation or its reversal potential (90).

Both groups (60, 487) noticed the similarity of the P2X₁ and P2X₂ receptors with a partial cDNA called RP-2 that was isolated from cells induced to die (358). This led them to suggest that extracellular ATP might initiate programmed cell death as is known from the application of ATP to various cells.

c) P2X₃ RECEPTOR. A single cDNA (P2X₃ receptor) was obtained from a rat dorsal root ganglion library (84, 285). Both ATP and α,β-met-ATP evoked fast-activating and rapidly desensitizing current. However, only the coexpression of P2X₃ and P2X₂ yielded ATP-activated currents that closely resembled the α,β-met-ATP-sensitive, non-
was, thus, suggested that Cx43 forms “half-gap junctions” in response to extracellular ATP (40). Second, both ATP and low-Ca solutions similarly evoked dye leakage from Novikoff hepatoma cells (293). The latter solution is known to induce hemigap-junction channels with single-channel conductance of 145 pS in catfish retina (124). Third, in HEK-293 cells overexpressing Cx43, as well as in ventricular cardiomyocytes, low-Ca solutions and metabolic inhibition open hemichannels (234). The latter case represents a physiopathological situation during which, not only cations, but also ATP could leak out of the cardiac cells and might induce autocrine stimulation.

P2X7 receptors are insensitive to UTP and inhibited by isoquinolene derivatives 1-(N,N-bis[5-isoquinolinesulphonyl]-N-methyl-L-tyrosyl)-4-phenylpiperazine (KN62), oxidized ATP (460), and calmidazolium (88, 224, 495). P2X7 receptors in macrophages and lymphocytes cause activation of phospholipase D (143) and modulate lipopolysaccharide signaling and inducible nitric oxide synthase expression in macrophages (220).

2. Regulation

As for K+ channels, several cloned P2X-receptor subunits are required to form an ion channel that enables the formation of homeric and heteromeric receptors that might provide distinct or novel characteristics. To what extent such in vitro constructs might reflect physiological conditions is illustrated below.

A) HOMO- AND HETEROmeric receptors. Like P2X2 and P2X3 that combine to form a unique heteromeric channel (285, 400), with specific properties (496, 497), others assemble. In cells expressing the heteromeric P2X2/3, αβ-met-ATP evoked biphasic currents with a pronounced nondesensitizing plateau phase (477). The heteromeric assembly was suggested by in situ hybridization studies (280) and is confirmed by coimmunoprecipitation (477). Similarly, P2X4 and P2X5 are major subunits with highly overlapping mRNA distribution in the mammalian central nervous system (99).

A systematic study of subunit P2X coassembly has been tested by protein-protein interactions using a coimmunoprecipitation assay (476). P2X4 and P2X2 do not form heteromers, whereas P2X6 needs to be associated in heteromers with P2X1,2,4,5 to form an active channel. It has been proposed that a novel structural motif of quaternary structure of P2X receptors together (as with voltage-dependent Na+, Ca2+, or K+ channels) with a bundle of α-helices contributed by the putative transmembrane segment M2 near the COOH terminal (405) might lead to a tetrameric (as with the nicotinic channel) or pentameric organization that forms a pore. In another elegant study, it is suggested that P2X1, P2X4, or P2X3 receptors form stable trimers (344). The dose-response curves for ATP activation of the 30-pS P2X2 channel showing a Hill coefficient of 2.3 also suggests the channel to be a trimer (131).

B) permeability. The substituted cysteine accessibility method was used to identify parts of the molecule that form the ion pore of the P2X2 receptor (405). L338 and D349 are on either side of the channel gate with D349 located near the middle of P2X2 channel. It is a negatively charged amino acid conserved among all the seven P2X receptors. It is thus possible that D349 is the site of permeant cation binding and be responsible for ionic selectivity (131). The asparagine residue Asn-333 was found to regulate the conductance such that the unitary conductance of 80 pS in 100 Na+ was roughly halved when it was replaced by isolateucine (N333I) (396).

The mechanism of inward rectification has been extensively studied in several types of channels. Small peptides that formed the channel might aggregate to lead to an intrinsic inward rectification (252). More often inward rectification results from open channel block by Mg2+ (310) and polyamines (296, 343). The ATP-receptor subunit P2X2 expressed in Xenopus oocytes and in HEK-293 cells shows profound inward rectification in all patch-clamp recording conditions. That suggests single-channel conductance may decrease when membrane potential becomes more positive, and furthermore, there is a substantial contribution of voltage-dependent gating to inward rectification of the steady-state current-voltage relation (537). P2X activation by external ATP (see sect. ivA1) was suggested.

Externally applied ATP activates a voltage-independent conductance on single smooth muscle cells dispersed from rabbit ear artery (31). This is also true in amphibian atrial cells (167). However, weak inward rectifying properties are shown for the ATP-induced current in rat ventricular, guinea pig atrial (204, 311, 424), and rabbit sinoatrial node (SAN) cells (436).

With the use of a combination of whole cell patch-clamp and fura 2 fluorescence measurements in HEK-293 cells transfected with hP2X4, it was determined that the recombinant channel allows a substantial amount of Ca2+ to permeate. The 8% current carried by Ca2+ is very close to the value previously reported for native ATP-induced currents in sympathetic neurons (411). Such a high Ca2+ permeation through hP2X4 receptors suggests that activation of these proteins may directly carry Ca2+ that will contribute to synaptic transmission (94).

The Ca2+ permeability of P2X4 receptors is greater than that of P2X2 receptors (Pc,Na/Pc,Ca = 3.9 and 2.2, respectively) despite no difference between the two receptors with respect to their permeability to a series of monovalent organic cations (148). In later work, advantage was taken of the clearly different properties of P2X2 and P2X3 (α,β-met-ATP sensitivity and desensitization) to compare Ca2+ permeability in homomorphic and heteromeric expressed receptors (496). P2X3 has a relatively low Ca2+
permeability ($P_{\text{Ca}}/P_{\text{Na}}$ 1.2–1.5) that dominates the heteromer P2X$_{2/3}$ Ca$^{2+}$ permeability while the heteromeric receptor is nearly as sensitive to external Ca$^{2+}$ inhibition as the P2X$_2$ receptor. These properties reinforced the view that the native $\alpha_2\beta$-met-ATP-sensitive receptor in nodose ganglion neurons is a P2X$_{2/3}$ heteromultimer (496). P2X$_{2/3}$ desensitization is much slower than that of P2X$_3$ subtype alone; thus the heterogeneous expressed P2X$_{2/3}$ acquires more effective Ca$^{2+}$ dynamics than P2X$_2$ or P2X$_3$ receptor alone (481).

c) Desensitization. P2X receptors can be divided into two broad groups according to whether they show fast desensitization within 100–300 ms or slowly if at all (Table 1). Desensitizing currents and the mechanisms of P2X-receptor desensitization have been recently reviewed (403). Briefly, rapidly desensitizing P2X receptors are activated by ATP, $\alpha_2\beta$-met-ATP, and 2-MeSATP. They include recombinant P2X$_1$ and P2X$_6$. The non-desensitizing $\alpha_2\beta$-met-ATP-insensitive P2X receptors are the expressed cloned P2X$_2$, P2X$_4$, P2X$_5$, P2X$_6$, and P2X$_7$ receptors. Several mechanisms have been proposed to account for desensitization. Desensitization implies the first or the second hydrophobic domain since substitution of these domains in chimeric P2X$_1$ or P2X$_2$ receptors confer the characteristic (516).

The rate of desensitization of the heterologously expressed P2X$_1$ receptor channel in HEK-293 cells has been reported to be 50–150 ms, while it is 5–10 s when recorded from Xenopus oocytes (147, 516). A slower rate of desensitization of the P2X$_1$ receptor from rat vas deferens stably expressed into HEK-293 cells was observed by the second day of culture. This effect was reversed by cytchalasins B and D (362). Mutations, M332I and T333S, identified in the porelike domain near or within the second putative transmembrane domain also prevented changes in kinetics.

Desensitization of P2X$_4$ receptors expressed in either Xenopus oocytes or HEK cells is markedly accelerated by increasing ATP concentrations (538). However, this concentration-dependent effect could in part be attributable to the concomitant acidification that occurs when high concentrations of ATP are dissolved in weakly buffered solutions (456). Desensitization of P2X$_2$ receptor was proposed to be controlled by alternative splicing (61). The splice isoform P2X$_{2\beta}$ or P2X$_{2\beta\beta}$, which lacks a stretch of COOH-terminal amino acids (Val370-Gln438), exhibits rapid and complete desensitization, whereas the wild-type channel desensitizes slowly (268, 442). The Pro373-Pro376 sequence of P2X$_{2\beta}$ represents a functional motif that is critical for the development of the slow desensitization profile (267).

The truncated P2X$_3$ clone lacking the NH$_2$-terminal intracellular region expresses functional channels that do not desensitize in oocytes (254). Desensitization of the ATP-gated cation channel P2X$_3$ is abolished by removal of external Ca$^{2+}$ or by cyclosporin pretreatment (254). The rate of desensitization is also decreased by injection of the autoinhibitory peptide CaNA457–481 in the oocyte. Thus it is thought that P2X$_3$ desensitizes through a Ca$^{2+}$-dependent calcineurin-mediated phosphorylation on NH$_2$-terminal residues (254).

3. P2X receptors in the heart

Most efforts in cloning of P2X receptors and their tissue distribution has been devoted to the brain. Much less is known for the heart and cardiomyocytes in particular. Immunoreactivity has been first reported with antisera against P2X$_1$ receptors on rat cardiac tissues that showed positive staining at the intercalated disk (501). One of the most remarkable characteristics of the rat P2X$_3$ receptor was its apparent tissue expression pattern with transcripts restricted to nociceptive neurons (84, 285). However, major species difference seems to exist, since P2X$_3$ was found in human heart (173) despite the fact that this gene is highly conserved from rat to human, coding for channel proteins that share 93% identity. The majority of sequence variations are located within the putative extracellular loop. P2X$_3$ is also found in human fetal heart where it appears to be the most abundant P2X-receptor subtype (47). Electrophysiological characterization showed however minor differences with an inhibitory potency of suramin five times lower in human than in rat (IC$_{50}$ 15 vs. 3 $\mu$M, respectively) (173). It is also remarkable that hP2X$_3$ had a high affinity for CTP (EC$_{50}$ ~20 $\mu$M). The authors could not rule out the possibility that the detection of human P2X$_3$ RNA in heart and spinal cord reflects neuronal transcripts located in primary sensory afferents densely innervating these structures.

In situ hybridization histochemistry was recently performed together with RT-PCR on the rat cardiovascular system (348). In heart sections, the authors noticed that mRNA transcripts for P2X$_1$, P2X$_2$, and P2X$_3$ all colocalized in smooth muscle cells of coronary vessels with no specific apparent positivity in myocardium. However, RT-PCR from microdissected tissues from various areas of the heart confirm the presence of P2X$_1$, P2X$_2$, and P2X$_4$ receptor mRNAs, with strong signals in the atria and only the typical band for P2X$_4$ in ventricles (348). Furthermore, two splice variants of the P2X$_2$ were identified. The discrepancy between the PCR and in situ hybridization data might result from a too low level of mRNA expression for detection or, as well, tiny blood vessels and nerve endings may also contribute.

Northern blot and RT-PCR analysis demonstrate P2X$_4$ transcripts in many tissues of the rat including blood vessels and heart (447). In a subsequent study, Garcia-Guzman et al. (171), characterization of recombinant human P2X$_4$ receptor reveals pharmacological differences in the two species. The homology to the rat P2X$_4$ receptor
shows 87% identity. Zn\(^{2+}\) increases the apparent gating efficiency at low concentration (5–10 \(\mu M\)) but inhibits the ATP-evoked current at 100 \(\mu M\) and higher. hP2X\(_4\) and rP2X\(_4\) receptors display similar agonist potency profiles, but the human receptor has a notably higher one.

The P2X\(_5\) purinoceptor was cloned in rat heart (172) simultaneously to its cloning in brain (99). Like in brain, functional expression of the recombinant rat P2X\(_5\) receptor shows a current that resembles mostly the P2X\(_3\) phenotype: slow desensitization, inhibition by PPADS and suramin, and no activation by \(\alpha,\beta\)-met-ATP. The EC\(_{50}\) for ATP is 8 \(\mu M\). The rank order of potency is ATP \(\geq\) 2-MeSATP > AMP ~ ADP > dATP ~ \(\beta,\gamma\)-met-ATP. No current is recorded after application of \(\alpha,\beta\)-met-ATP, CTP, GTP, UTP, or adenosine, each at 500 \(\mu M\) (172).

P2X\(_1\) receptor mRNA level is increased 2.7-fold in rats under congestive heart failure (216).

G. Metabotropic P2Y Receptors

To date a total of 13 P2Y receptor-like DNA sequences have been cloned (P2Y\(_{1,11}\), tp2Y, and fb1). However, the P2Y\(_7\) receptor is definitely a leukotriene B\(_4\) receptor, and there is no functional evidence that P2Y\(_6\), P2Y\(_9\), and P2Y\(_{10}\) receptors are nucleotide receptors. Therefore, there are currently five genuine human P2Y receptors (P2Y\(_{1,2,4,6,11}\)). It is most likely that the chicken P2Y\(_3\) receptor is the avian ortholog of the P2Y\(_6\) receptor, whereas Xenopus P2Y\(_2\) and tp2Y are probably orthologs of the P2Y\(_4\) receptor. Members of the P2Y-receptor family couple to heteromeric G proteins which, in turn, activate intracellular second messenger systems to modulate the physiological function of the cells. All have a wide tissue distribution (Table 3).

A number of specific reviews have appeared (18, 44, 59, 100, 191, 255, 403).

1. Characteristics

a) P2Y\(_1\) receptor. The first cloning of a P2Y receptor from chick brain was reported in 1993 (514). Subsequently, P2Y\(_1\) was isolated from a range of species including rat, mouse, bovine, and human with a wide tissue distribution including heart (12, 47, 199, 473, 511). Pharmacological characterization of the P2Y\(_1\) receptor indicates that only purines are active. UTP and derivatives are not active at this receptor subtype (443). More precisely, the P2Y\(_1\) receptor had been initially characterized by an agonist potency order of 2-MeSADP > 2-MeSATP > ADP > ATP (57, 58, 427, 514). However, this has been questioned (198, 283). Whereas ADP and 2-MeSADP are potent full agonists, purified ATP and 2-MeSATP rather act as weak antagonists on the heterologously expressed hP2Y\(_1\). This observed adenine nucleoside diphosphate specificity of the P2Y\(_1\) receptor is very similar to the pharmacological selectivity of the P\(_2T\) receptor in platelets (217). It is now well established that part of the ADP effect on platelets (calcium release, shape change) is mediated by the P2Y\(_1\) receptor. Other effects (cyclase inhibition) are mediated by a still uncharacterized distinct receptor, whereas aggregation requires both receptors (283). The apparent discrepancy with ATP being a weak but full agonist on P2Y\(_1\) expressed in HEK and an antagonist on the same receptor expressed in Jurkat T cells was analyzed in a recent study and attributed to differences in the degree of P2Y\(_1\)-receptor reserve (361). In another recent work, adenosine 2’-phosphate-5’-phosphate (A2P5P) and adenosine 3’-phosphate-5’-phosphate (A3P5P) inhibited ADP-induced platelet shape change and aggregation and

Table 3. Mammalian metabotropic P2Y purinoceptor: characteristics and presence in the heart

<table>
<thead>
<tr>
<th>P2Y</th>
<th>Ortholog</th>
<th>Amino Acids</th>
<th>Agonists</th>
<th>Antagonists†</th>
</tr>
</thead>
<tbody>
<tr>
<td>hP2Y(_1)</td>
<td>mP2Y(_1)</td>
<td>362</td>
<td>2-MeSADP &gt; ADP &gt; ATP(^*)</td>
<td>++</td>
</tr>
<tr>
<td>rP2Y(_1)</td>
<td>bP2Y(_1)</td>
<td>373</td>
<td>UTP = ATP &gt; Ap4A (2-MeSATP, ADP, UDP inactive)</td>
<td>++</td>
</tr>
<tr>
<td>rP2Y(_2)</td>
<td>mP2Y(_2)</td>
<td>365</td>
<td>UTP, ATP(_\gamma)S (\approx) ATP, ITP (2-MeSATP, UDP inactive)</td>
<td>+</td>
</tr>
<tr>
<td>rP2Y(_4)</td>
<td>361</td>
<td>ATP (\approx) UTP &gt; Ap4A &gt; ATP(_\gamma)S</td>
<td>0</td>
<td>Heart</td>
</tr>
<tr>
<td>hP2Y(_6)</td>
<td>rP2Y(_6)</td>
<td>379</td>
<td>UDP (\geq) UTP &gt; ATP &gt; ADP</td>
<td>-</td>
</tr>
<tr>
<td>hP2Y(_{10})</td>
<td>371</td>
<td>ATP &gt; 2-MeSADP (\geq) ADP (UTP, UDP inactive)</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

\(^*\) ATP was suggested to be an antagonist (197, 198, 283). † A2P5P (57), A2P5P, and A3P5P (197, 198) are specific antagonists at hP2Y\(_1\). +, 0, Degree of antagonistic effects; (−), not determined. 2-MeSATP, 2-methylthio-adenosine 5’-triphosphate; ATP\(_\gamma\)S, adenosine 5’-O-(3-thiotriphosphate).
likely the avian ortholog of the mammalian P2Y6 receptor. Ca2+
and ADP. ATP: A P2-PURINERGIC AGONIST IN THE MYOCARDIUM 777

b) P2Y2 RECEPTOR. Concurrently in 1993, a receptor was
isolated from mouse neuroblastoma cells and found to be
activated by UTP and by ATP with equal potency and
efficacy. ATPγS and ITP are less potent while 2-MeSATP
and α,β-met-ATP are weak partial agonists (299). Or-
thologs of P2U, now named P2Y3, have subsequently been
isolated from human and rat (365, 511). P2Y2 is expressed
in a wide variety of tissue including heart (81, 407, 511).
After expression in Xenopus oocytes, P2Y2 coupled to
both classes of G proteins to increase an endogenous
Ca2+-dependent Cl− channels and mediate a pertussis
toxin-sensitive increase in the inward-rectifier K+ chan-
nels of the Kir3.0 subfamily (330).

c) P2Y3 RECEPTOR. The P2Y3 was cloned from chick
brain and exhibits the following rank order of agonist
potency: UDP > UTP > ADP > ATP (512). It is most
likely the avian ortholog of the mammalian P2Y4 receptor.

d) P2Y4 RECEPTOR. Another clone originally thought to
be the pyrimidinoceptor, P2Y4, was isolated from human
genomic DNA libraries (104, 340). The hP2Y4 receptor is
highly selective for UTP, whereas ATP, ADP, and ITP
appear to be weak partial agonists (104, 340, 342). This
rules out P2Y4 as a true pyrimidinoceptor (440). It is not
agonized by suramin, nor by PPADS (but see for
rP2Y4), and is blocked by RB2. Pyrimidinoceptors were
mainly found in rat sympathetic neurons. Recently, a
rP2Y4 has been cloned (48, 513). It shares 83% overall
identity with the hP2Y4 and is less related to the rP2Y2
with 51% identity. Its agonist profile is close to the native
P2Y1 receptor: ATP ~ UTP > Ap4A > ATPγS > 2-MeSATP.
It is also strongly activated by ITP and UDP, although the
latter could be a partial agonist. These observa-
tions, including suramin efficiency, help differentiating
the rP2Y4 from the orthologs of P2Y2 (48, 154). ATP and
UTP act on the same receptor, since they show cross-
desensitization. Besides, the hP2Y4 is among the few G
protein-coupled receptors to show no N-glycosylation
consensus sequence (104), but the rat ortholog does
(513).

e) P2Y6 RECEPTOR. The P2Y6 was cloned from a rat
aortic smooth muscle library and from a human placenta
cDNA library; the two orthologs show 88% amino acid
identity (80, 103). As for P2Y2 receptors, the selectivity of
P2Y4 receptors for UDP had to be reexamined because of the
use of UDP preparations contaminated by UTP and the
degradation of UTP into UDP during incubation of the
cells (342). With these precautions the P2Y6 receptor is
activated most potently by UDP and weakly by UTP, ATP,
and ADP.

f) P2Y11 RECEPTOR. The newly cloned human P2Y re-
ceptor provisionally called hP2Y11 is characterized by
considerably larger second and third extracellular loops
(101). It exhibits only 33% homology with hP2Y1, its clos-
est homolog, and 28% homology with hP2Y2. It shows one
potential site for N-linked glycosylation and two potential
sites for phosphorylation by PKC or calmodulin-depend-
ent protein kinases. hP2Y11 couples positively to both
phosphoinositide and adenylyl cyclase, a unique feature
among the P2Y family. Following stable expression in
1321 N1 astrocytoma and CHO-K1 cells, the rank order of
agonist potency for the two pathways is ATPγS ~ BzATP
> dATP > ATP > adenosine 5’-O-(2-thiodiphosphate)
(ADPβS) > 2-MeSATP > ADP with UTP and UDP being
inactive, thus showing hP2Y11 is presently the most ade-
nine nucleotide selective P2Y receptor (106).

g) DETERMINANTS OF ATP BINDING ON P2Y PURINOCEPTORS.
Site-directed mutagenesis of P2Y2 led to the suggestion
that the charged amino acids His-262, Arg-265 in TM6 and
Arg-292 in TM7 interact with the phosphate groups of the
nucleotides (145). The amino acid sequence of hP2Y4
shows some similarities with the P2Y2 receptor at sites
directly involved in the binding of negatively charged
phosphate groups. Three residues are conserved: His-262,
Arg-265, and Arg-292, whereas Arg-265 is replaced by a
Lys-259 in P2Y1 as in the P2Y6 (105, 340). Moreover,
Lys-289 residue also plays a major role, since its substi-
tution by Arg shifts the preference of the P2Y2 receptor
for triphosphate nucleotides to diphosphate nucleosides
(145). Note that this Lys residue is conserved in both P2Y4
and in P2Y6; the latter which, however, has a clear pref-
ereence for UDP.

2. Coupling to G proteins and second messengers

Members of the P2Y family are quite diverse in se-
quence, more so than other known G protein-coupled
receptor families. The third intracellular loop and the
COOH-terminal tail, the two regions implicated in G pro-
tein specificity in the other G protein-coupled receptors,
varry greatly among the P2Y family. Nevertheless, P2Y
receptors have all been shown to be coupled through a
Gq/11 protein to the inositol 1,4,5-trisphosphate (IP3)
pathway.

Activation of the recombinant P2Y1 receptor medi-
ates IP3 formation and increases intracellular Ca2+
concentration ([Ca2+]i), but it does not change cAMP (443).
However, in a recent study BzATP, an antagonist of rat
and human P2Y1 expressed in Jurkat cells, prevents the
ADP-induced inhibition of the cAMP pathway (493). IP3
and [Ca2+]i increases can stimulate a variety of pathways
including PKC, phospholipase A2 (PLA2), and nitric oxide
synthase which subsequently can activate other pathways
such as phospholipase D (PLD) and mitogen-activated
protein kinase (MAPK).
Activation of P2Y$_4$ and P2Y$_6$ receptors also leads to the formation of inositol phosphates (103, 104) and a rise in [Ca$^{2+}$]$_i$ (80, 340). However, the two receptors seem to involve distinct G proteins. The P2Y$_4$ but not the P2Y$_6$ response was inhibited by pertussis toxin (80, 102, 409). hP2Y$_{11}$ has the unique property of simultaneously activating the adenyl cyclase with the same rank order of potency of agonists (101, 106). Surprisingly, the ATP derivative AR-C67085, a potent inhibitor of ADP-induced platelet aggregation, was the most potent agonist at the recombinant hP2Y$_{11}$ receptor (106).

3. P2Y receptors in the heart

The expression of P2Y$_{1,2,4,6}$ receptor transcripts was reported by RT-PCR in rat heart (511). All receptor sequences could be amplified from neonatal whole heart, with P2Y$_6$ appearing the most abundant transcript of the four. However, in neonatal cardiomyocytes, P2Y$_1$ is expressed at higher levels than the others. In adult myocytes, P2Y$_1$, P2Y$_2$, and P2Y$_6$ could be amplified while P2Y$_4$ could not be detected (511). Two major conclusions can be made from this work: 1) the need to specifically work on isolated cardiomyocytes, and 2) P2Y expression varies during development with the arrest of P2Y$_4$ expression in adult cardiomyocytes. P2Y$_2$, P2Y$_4$, and P2Y$_6$ receptors were recently cloned in fetal human heart using degenerated oligonucleotides (47). Other works reporting the presence of one or the other of the P2Y receptors are quoted in Table 2. P2Y$_{11}$ was not found in human heart using Northern blot analysis (101). In a recent study however, using a new quantifying RT-PCR protocol, P2-receptor mRNA expression was compared in control and rats under congestive heart failure. In the sham-operated rats, P2Y receptors are expressed at a higher level than P2X$_1$ receptors, with P2Y$_6$ being the most abundant. In failing hearts, a prominent change was seen: P2X$_1$ and P2Y$_2$ receptor mRNA levels were increased 2.7 and 4.7, respectively (216). Extending their study to adult human myocardium, the authors could detect P2X$_1$, P2Y$_1$, P2Y$_2$, P2Y$_6$, and P2Y$_{11}$ receptors in both right and left atria and ventricles, while no P2Y$_4$ receptor was seen.

H. Adenine Dinucleotide Receptors

The selectivity and activity of adenine dinucleotides for neurally derived recombinant P2 purinoceptors were studied using P2X$_2$ and P2Y$_1$ subtypes expressed in *Xenopus* oocytes (378). Ap$_{4A}$ is as active as ATP but less potent (EC$_{50}$ ~15 and 4 μM, respectively) on P2X$_2$ receptors. Other adenine dinucleotides are inactive. In a previous work it had been shown that Ap$_{5A}$ is a partial agonist at the human ortholog hP2X$_1$ (147). However, Ap$_{5A}$ potentiates the ATP responses but not the Ap$_{4A}$ response at the P2X$_2$ subtype with an EC$_{50}$ of ~3 nM, and Ap$_{5A}$ also enhances the efficacy of suramin (378). In a recent work, Ap$_{5A}$ also potentiated the ATP response in rat cerebellar astrocytes (233). Such an effect appears to be mediated by the metabotropic P2Y receptors. At the P2Y$_1$ subtype, Ap$_{4A}$ is equipotent and active as ATP. Ap$_{4A}$ is a weak partial agonist and other dinucleotides are inactive. In another study, Ap$_{4A}$ is reported to be a potent agonist at P2Y$_2$, but not P2Y$_1$, P2Y$_4$, and P2Y$_6$ expressed in 1321 N1 human astrocytoma cells (341). Thus some dinucleotides have the capacity to potentiate ATP responses at both P2X and P2Y receptors.

To date, in cardiac tissues, only the presence of Ap$_{4A}$ receptor has been reported (202, 203, 504), with at least 77% of the active receptors on the plasma membranes (502). Recent work demonstrates photocross-linking of an Ap$_{4A}$ derivative with a 50-kDa polypeptide, suggesting a homogeneous population of receptors (42). At the high-affinity binding site, the apparent K$_d$ values for Ap$_{4A}$ and ATPγS are 0.08 and 0.04 μM, respectively; there is also a low-affinity site for ATPγS with a K$_d$ at 1.0 μM. The P2X agonist α,β-met-ATP did not compete effectively with these two agonists and indicates that the Ap$_{4A}$ receptor is a P2Y receptor in cardiac plasma membranes. The same group had previously suggested Ap$_{4A}$ binds to a specific 30-kDa polypeptide dinucleotide receptor. This observation resulted from receptor proteolysis. Ap$_{4A}$ receptor was shown to undergo at least two proteolytic processing steps, one of which is carried out by a serine protease, and this serine protease is required for receptor activation (202, 503, 504). Specific dinucleotide receptors activated by Ap$_{4A}$, but not ATP or UTP, have been suggested on synaptic terminals in guinea pig diencephalon and cerebellum (379). This follows the identification of binding sites that are highly selective, or even specific, for Ap$_{4A}$ as opposed to mononucleotides. This possibly represents P2D receptors, P2Y$_{ApnA}$ receptors, or also P4 receptor that are distinct from those activated by ATP to induce an elevation in intrasynaptosomal Ca$_{2+}$

I. Pharmacological Concerns

Despite much effort there is a still strong need of potent and subtype-specific P2 receptor agonists and antagonists. Several substances that display selectivity for P2X or P2Y subtypes have been recently carefully reviewed (403) and are summarized in Tables 1 and 2. In the following I would just like to draw attention to side effects of most of the presently available compounds that make their use difficult even in isolated cells.

It had been already recognized that the ecto-nucleotidase activity accounts, in most part, for the difference in the concentration curves of P2X agonists in vascular
smooth muscle contraction and electrophysiological response, since ATP degradation products might act besides ATP itself (250). Inhibitors of the ecto-ATPases include NaN3 or AP2A as well as many of the so-called purinergic antagonists, suramin, PPADS, RB2, FLP 66301, and FLP 67156 (39, 66, 82, 110, 111, 223, 524, 540). All the nonhydrolyzable ATP analogs also do (83, 377). Consequently, such an inhibition of ecto-ATPases might, in part, account for the observed facilitation effects of ATP in tissues or in cell incubation (82). Suramin is also an active inhibitor of protein-tyrosine phosphatases (550), a side effect of potential importance knowing the involvement of tyrosine kinase pathways after ATP stimulation (394). PPADS also appears to inhibit UTP- and ATP-induced Ca2+ mobilization by a nonspecific mechanism independent of P2Y-receptor recognition that might involve the inhibition of intracellular IP3 channels (494). Moreover, there might be even species selectivity. Thus rat recombinant P2X4 and P2X6 receptors are not blocked by PPADS, but the human homolog of the P2X4 receptor is (171).

Recently, 2',3'-O-(2,4,6-trinitrophenyl)adenosine 5'-triphosphate (TNT-ATP) has been reported to inhibit specifically P2X1 and P2X3 and heteromeric P2X2/3 with an IC50 of 1 nM. However, its use in tissue might be of limited interest due to its fast breakdown (497).

BzATP, an antagonist of P2X7 receptor, is also a photoaffinity probe that binds covalently to the nucleoside sites of ATPases (20, 360); it prevents ATP binding to α-sarcoglycan, a skeletal muscle ecto-ATPase (38), and to P2Y1 receptors (493).

Quinidine was first used by Burnstock (69) as a P2-receptor inhibitor. It is interesting to note that quinidine antagonizes the ATP-induced nonselective cationic current in rat ventricular cells (91) and the late phase of positive inotropy in guinea pig atria (135) in line with the view that a part of contractile activity might result from P2X-mediated Ca entry (169).

DIDS is a commonly used anion transport inhibitor. It has also been reported to block a number of presumably purinoceptor-mediated responses. Indeed, DIDS causes a long-lasting blockade of P2X receptor activity in rat vas deferens (65, 112, 319). This effect is in line with the fact that DIDS decreases the binding of [32P]ATP in rat parotid acinar cells (319). DIDS is now known to be a P2-receptor antagonist (65, 147, 445).

Ion selectivity of the P2X-receptor channels resembles that of nicotinic ACh, glutamate-activated, or serotonin-activated channels. It might be of interest to test blockers of these channels (local anesthetics QX314, QX222, d-tubocurarine, dizocilpine, and phencyclidine). In fact, d-tubocurarine is a blocker of P2X2 receptors (60).

### III. PHYSIOLOGICAL AND PHYSIOPATHOLOGICAL EFFECTS OF EXTRACELLULAR ADENOSINE 5'-TRIPHOSPHATE

#### A. Contractile Effects

Most initial reports describe negative inotropic effects after applying ATP in mammalian hearts, particularly in the atrium (75, 137, 159, 207, 212). These effects should be attributable to A1-adenosine receptor activation after ATP breakdown in the tissue. Later studies on guinea pig and rat atria revealed positive inotropic effects of ATP that developed after a transient rapid decrease (135, 168, 169, 306). In these studies, ATP, ADP, AMP, adenosine, α,β-met-ATP, β,γ-met-ATP, as well as UTP were shown to have this dual effect. Desensibilization by long exposure to α,β-met-ATP (135), suramin, but not RB2 could antagonize the positive inotropic effect (168), whereas 8-phenyltheophylline (8-PT) or 8-cyclopentyl-1,3-dipropylxanthine (DPCPX) prevented the negative effects (168, 306). It is noteworthy that 2-MeSATP induces only a negative inotropic effect (168). A similar dual inotropic effect of ATP is observed in human atrial strips (J. Alvarez and G. Vassort, unpublished results). Pyridoxal 5'-phosphate, an active form of vitamin B6, shows antagonism toward ATP-induced positive inotropic effect in perfused rat heart, with both pyridoxal and phosphate moities being essential for the action (508).

Fifty years ago, Green and Stoner (185) reported that after a brief period of cardiac arrest, adenine nucleotides had a strong positive inotropic effect in isolated perfused mammalian hearts. They reported that increasing states of phosphorylation of the adenosine molecule correlated well with the increasing inotropic effects. They suggested that the adenine nucleotides exerted directly their effect on the myocardium rather than through vasodilatation. Several reports established ATP as a full positive inotropic agent in frog ventricles (11, 161, 183, 489 and see references therein). ATP also induces positive inotropic effects in the ventricle of axolotl (321) but has no effect in several species atrial strips (320, 322, 323). Positive inotropy in frog heart is also induced by UTP, ITP, CTP, GTP, and their derivatives (489, 490, 527). The fast initial positive phase is followed, after a transient decrease, by a secondary increase of variable amplitude according to the agonists. ATP and its derivatives exert clear positive inotropy in rat papillary muscle (282, 421) and enhance cell shortening in various mammalian ventricular cardiomyocytes (Fig. 1) (91, 116, 381, 421).

A beneficial effect of adenine nucleotides on contractility of postischemic papillary muscles is long known; a situation where ATP effect was tentatively attributed to its high-energy supplier properties such as it would con-
Contribute to high-energy phosphate replenishment (434). However, with the present knowledge, positive inotropy in control and diseased cells could be attributable to an increase in L-type long-lasting Ca$^{2+}$ current ($I_{CaL}$) (204, 421–423) that mediates an increase in Ca$^{2+}$ transient. Moreover, ATP could enhance SR Ca$^{2+}$ release, although this mechanism is unclear (41, 91, 204, 389). Other mechanisms have also been suggested. They include an interaction of ATP and UTP with P2X receptors that mediate Ca$^{2+}$ entry (169). A similar mechanism in cultured chick embryo ventricular myocytes could account for the observation that ATP roughly doubles cell shortening (381). ATP analogs mimic this effect with an efficacy and potency order of ATP$>$ADP$>$AMP$>\alpha\beta$met-ATP but not $\alpha\beta$-met-ATP has a strong positive effect while UTP is weak. In contrast, UTP, like ATP, triggers a strong phosphoinositide-stimulating hydrolysis while 2-MeSATP is a weak agonist, thus excluding a PLC-dependent effect (381). Positive inotropy can, in part, be attributed to the alkalinizing effect of ATP (391, 468) which sensitizes the contractile machinery to Ca$^{2+}$ (150). No direct effect on the contractile proteins was observed contrary to such observation after $\alpha_1$-adrenergic-mediated inotropy (388, 467).

In isolated ferret ventricular myocytes, extracellular ATP ($10^{-7}$ to $10^{-3}$ M) inhibits $I_{CaL}$ and decreases Ca$^{2+}$ transient and contraction. These effects were sustained (399). In this study, performed in a Na$^{+}$-free, K$^{+}$-free solution, there was also no evidence for ATP activating a nonselective cation current, $I_{ATP}$. In this case the Ca$^{2+}$ conductance via $I_{ATP}$ might be too small to measure.

### B. Chronotropic Effects

In their seminal work, Drury and Szent-Györgyi (137) reported a negative chronotropic effect of purines. Following studies suggested that ATP has dose-dependent...
effects; small doses producing tachycardia while relatively larger doses of ATP slow the heart and induce atrioventricular nodal conduction block (207, 455). ATP decreases the slow diastolic depolarization in Purkinje fibers but increases it in frog heart (269). However, these negative chronotropic effects might be the result of ATP degradation to adenosine (36) and the action of the latter on sinoatrial and atrioventricular nodes (372). This has been shown to be the case even during administration of ATP into the sinus node blood supply (i.e., intracoronary) in dogs (232), in which model the negative chronotropic action of ATP was attenuated by theophylline, a known antagonist of adenosine at the P1-receptor site (19, 371). The negative chronotropic action of ATP is independent of prostaglandins (375). These observations indicate that negative chronotropy is in part due to P1-purinergic activation by ATP or rather by its degradation product, adenosine (523). Thus ATP (Striadyne) was used to treat paroxysmal supraventricular tachycardia (331, 419). On the other hand, ATP caused cardiac acceleration in 40% of the rabbit hearts using the Langendorff-perfusion method (463). An excitatory effect of ATP in isolated cat atria was revealed by the previous addition of acetylcholine (4). These excitatory effects of ATP were not antagonized by either theophylline or pertussis toxin pretreatment, but were almost completely blocked by neomycin and indomethacin (463). On the basis of these data, it was concluded that the positive chronotropic effect of ATP was mediated by P2-purinoceptors coupled to prostaglandin synthesis via a pertussis toxin-insensitive pathway involving the stimulation of PLC (463).

It is difficult to anticipate the direct effect of ATP on sinus node rhythm. Extracellular ATP activates a time-independent, weakly inwardly rectifying current that is nonselective for monovalent cations (436). This current is not activated either by ADP, AMP, or adenosine, suggesting that the action of ATP is mediated by a P2 purinoceptor. In view of the critical role of \( I_{\text{Ca}} \) in the genesis of the action potential in SAN cells, it was proposed that extracellular ATP may play an important role in the regulation of heart rate (396). It is even more difficult to extrapolate data obtained in vitro (396, 463) to the human heart in vivo. Numerous studies in cats, dogs, and humans have indicated that extracellular ATP exerts a negative chronotropic action on cardiac pacemakers that is mediated in part by the vagus nerve in addition to the action of adenosine (30, 371, 374), an effect already reported in 1950 (185). The vagal effect is due to a cardio-cardiac depressor reflex elicited by the action of ATP on vagal afferent nerve terminals in the left ventricle (243), similar to its action on pulmonary vagal afferent terminals (374). In both organs (i.e., heart and lungs), the triggering of the vagal reflex by ATP is mediated by P2X receptors (243, 374).

In cultured adult guinea pig myocytes, chronotropic effects of ATP could be greatly enhanced in the presence of cardiac neurons that also possess P2 purinoreceptors (213). Thus ATP increases contractile rate in intrinsic cardiac neuron-myocyte cocultures by 40% under control conditions and much more (100%) after blockade of \( \beta \)-adrenergic receptors. In contrast, ATP induces much smaller effects (20%) in noninnervated myocyte cultures.

C. Pro- and Anti-Arrhythmic Effects of ATP

ATP was shown to produce transient tachycardia at low doses or to slow heart and to induce atrioventricular and His bundle blocks at higher doses (156, 207, 454). In fact, transient ectopic beats of atrioventricular junctional origin were consistently observed at the onset of the atrioventricular block produced by ATP. These premature beats were attributed to reentry phenomena, since positive chronotropic effects of ATP were never observed (484). Although these effects can be attributed to the fast formation of adenosine from ATP, in isolated rat ventricular myocytes, the sudden application of ATP at micromolar concentration induces cell depolarization and triggers automaticity (Fig. 2) (91, 424). A similar ATP-triggered automaticity in rat papillary muscles requires the uncaging of ATP by ultraviolet-light flash (Vassort, unpublished results) while the application of ATP by changing the bath solution was without significant effect. These effects could be attributable to activation of the fast transient nonselective cationic current \( I_{\text{ATP}} \) and cell depolarization.

In isolated ventricular myocytes of the guinea pig, ATP alone does not exert significant electrophysiological effect; however, when it is applied with drugs known to increase \( \left[ \text{Ca}^{2+} \right]_{\text{i}} \), ATP facilitates the induction of afterdepolarizations and triggered activity in ~60% of the cells (446). In the presence of isoproterenol, ATP increases the amplitude of the transient inward current \( I_{\text{n}} \), delayed afterdepolarizations (DADs), and \( I_{\text{CaL}} \). In the presence of either BAY K 8644 or quinidine, ATP further prolongs the action potential duration and also increases the amplitude of early afterdepolarizations (EADs) (446). These findings extend earlier observations regarding the interaction between the effects of catecholamines and ATP on \( I_{\text{CaL}} \) (125, 535) and support the hypothesis that the release of ATP into the extracellular space under pathophysiological conditions could be arrhythmogenic (276, 424).

In addition to these various effects at the cellular level, several other mechanisms triggered by extracellular ATP suggest that P2-purinergic stimulation can induce arrhythmia in the cardiac tissue. Both the transitory acidosis and the increase in intracellular \( \text{Ca}^{2+} \) decrease intercellular coupling (317). Tyrosine phosphorylation of protein Cx43 might reinforce this situation, since it closes the gap junction (277, 385). Complementary aspects are
the slowing of activation spread due to a decreased Na\textsuperscript{+} current (425) as well as the increase in tissue heterogeneity following the facilitation of \(I_{\text{K(ATP)}}\) and action potential shortening (13).

Is there a role for IP\textsubscript{3} in cardiomyocyte arrhythmia? The signaling role of IP\textsubscript{3} has been clearly established in many cell types in which it induces Ca\textsuperscript{2+} release from the endoplasmic reticulum (ER) (37, 94). IP\textsubscript{3} is generated following the action of many neurohormones that activate a G\textsubscript{q}-dependent PLC-\(b\) or, in the case of ATP or angiotensin II, the tyrosine kinase-dependent PLC-\(g\) (184, 395). In the heart, IP\textsubscript{3} has been involved in cardiac arrhythmias (138, 230, 520), heart failure (307), and graft rejection (152). In a recent work on neonatal rat cells, it is shown that uncaging IP\textsubscript{3} by ultraviolet light or by applying ATP (or PGF\textsubscript{2\alpha} that activates PLC-\(\beta\)) induces Ca\textsuperscript{2+} release around the nucleus from a ryanodine- and caffeine-insensitive compartment, probably the ER (231).

D. Hypertrophy

Hormones and mechanical stretch can induce cardiac growth. Extracellularly applied ATP constitutes a stimulus sufficient to induce changes in the pattern of

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**FIG. 2. Arrhythogenic effects of ATP.**

A: the extracellular application of 100 \(\mu\text{M}\) ATP on a rat ventricular cell induces membrane depolarization together with cell automaticity. \(V_m\), membrane potential. [From Christie et al. (91).]

B: ATP-induced delayed afterdepolarization and transient inward current in the presence of isoproterenol (10 nM ISO + 100 \(\mu\text{M}\) ATP) recorded on a guinea pig single ventricular cell. The action potential recorded at a basal stimulation frequency of 0.5 Hz (left) is followed by membrane potential recorded after 15-s overdrive stimulation applied at a frequency of 4 Hz (middle) and membrane current after a 10-s train of 300-ms depolarizing pulses from \(-80\) to \(+40\) mV at a frequency of 2 Hz (right). [From Song and Belardinelli (446).]

C: the addition of 10 \(\mu\text{M}\) ATP to the bathing solution induces oscillatory Ca\textsuperscript{2+} transients in a rat ventricular cell loaded with indo 1; however, this effect of ATP requires the presence of Mg\textsuperscript{2+}. [From Puce´at et al. (389).]

D: ATP at 20 \(\mu\text{M}\) stops the spontaneous activity of cultured neonatal rat ventricular myocytes loaded with fluo 3 that are beating at a high plating density. [From Jaconi et al. (231).]
expression of immediate-early genes such as c-fos and jun-B that is mediated by a Ca\textsuperscript{2+}-dependent pathway in neonatal rat ventricular myocytes (392, 534).

However, ATP does not induce cell hypertrophy. Studies on the intracellular signaling mechanisms responsible for myocardial cell growth have focused on MAPK, p\textsuperscript{42} and p\textsuperscript{44}MAPK (50, 382, 417, 470), or p\textsuperscript{38}MAPK (95, 529). These kinases are activated by both Ser-Thr kinases and tyrosine kinase and are involved during various neurohormonal stimulations and mechanical stress. Although ATP activates p\textsuperscript{42} and p\textsuperscript{44}MAPK (382) and p\textsuperscript{38}MAPK (5), it does not, like carbachol, transactivate cardiac-specific promoter/luciferase reporter genes, nor increases atrial natriuretic factor expression (382). These studies furthermore suggest that activation of c-fos, jun-B, PKC, and MAPK are not sufficient by themselves to stimulate hypertrophy. ATP might also activate an inhibitory pathway, as suggested by the fact that it prevents phenylephrine-induced hypertrophy (533). These effects are probably mediated by P2Y receptors (533). However, it has been reported that the autocrine release of ATP mediated by stress induces hypertrophy of cultured rat neonatal myocytes. This effect involves P2Y receptors and the activation of stretch-induced ERK (483).

E. Other General Effects of ATP in Cardiac Cells

1. Glucose transport inhibition

Extracellular ATP markedly inhibits glucose transport (157) by decreasing the amount of glucose transporters in the plasma membrane with a concomitant increase in intracellular microsomal membranes. P2X purinoceptors are not involved since a drastic reduction in extracellular Na\textsuperscript{+} or Ca\textsuperscript{2+} does not alter this effect of ATP. The rank order potency of P2 receptor agonists (ATP ≇ ATP\textsubscript{2}S ≇ 2-MeSATP > ADP > α,β-met-ATP) does not match that of any known P2Y purinoceptor subtype. Inhibition of transmembrane glucose transport was specific, since ATP did not inhibit the rate of glycolysis or the rate of pyruvate decarboxylation. The physiological significance of this inhibitory effect of ATP on glucose transport is unclear. It is noteworthy that glucose transport is reduced and is rate limiting in hypertrophied failing heart (462), a condition during which ATP release and extracellular content are expected to be enhanced.

2. Agonist-induced internalization of purinoceptors

In the face of persistent stimulation, the response of most receptors fades away. The process of receptor desensitization, one of the many forms of the G protein-coupled receptor regulation, has received much attention. Desensitization occurs generally via feedback regulation by the second messenger-stimulated kinases PKA or PKC that the G\textsubscript{q} and G\textsubscript{i} protein-coupled receptors activate, respectively. This was first reported for the β\textsubscript{2}-adrenergic receptor (55). Phosphorylation is followed by β-arrestin binding, a crucial step in internalization of most heptahedral receptors (153, 281).

Our knowledge of the turnover sequence activation/desensitization of the purinoceptor is rather limited. The agonist-induced desensitization and sequestration of the P2Y\textsubscript{2} receptor is attributable to its COOH terminus. Thus truncation of 18 or more amino acids does not alter UTP-stimulated increases in \([\text{Ca}^{2+}]_{\text{i}}\), while the concentration of UTP necessary to desensitize the receptor is increased 30-fold (174). The tagging of P2Y\textsubscript{2} receptor at its NH\textsubscript{2} terminus with a hemagglutinin-epitope sequence reveals that the receptor undergoes agonist-promoted movement to an intracellular compartment. However, this internalization does not establish any functional consequence and is not required for agonist-induced desensitization (450).

3. ATP and preconditioning

The mechanism of preconditioning, a brief ischemic treatment known to protect cardiac tissue from damage to subsequent ischemic episodes, is still a matter of debate. The possibility that p\textsuperscript{38}MAPK activation after ATP release intervenes in the process has been foreseen. p\textsuperscript{38}MAPK is activated by adenosine soon after ischemia (49, 316), a period during which ATP is released (276) and could be degraded. ATP itself activates p\textsuperscript{38}MAPK (5). ATP is also expected to be released during stretch by many cell types including cardiac cells (483), an experimental condition sharing conditioning mechanisms of protection with ischemic conditioning (189). Infarct size was reduced by various antagonists that share in common the prevention of an increase in K\textsuperscript{+} currents. They include blockers of adenosine receptors and PKC as well as gadolinium, Gd\textsuperscript{3+}, a blocker of stretch-activated channels including the ATP-activated K\textsuperscript{+} channel (5), and by glibenclamide, a blocker of the I\textsubscript{KATP} known to be enhanced by local intracellular ATP depletion following the activation of adenylyl cyclase by isoproterenol and ATP (13, 428).

4. Prostaglandin release

Biosynthesis and release of prostaglandins are increased by applying ATP to the perfused rabbit heart, an effect blocked by indomethacin (337). This effect was mimicked by ischemia, a condition which among other things increases extracellular ATP; ATP as well as UTP can induce prostaglandin release from cultured porcine endothelial cells and bovine aortic smooth muscle cells (121, 370). This P2Y-receptor effect involves mostly an increase in \([\text{Ca}^{2+}]_{\text{i}}\) (79, 298).

The large increase in prostaglandin synthesis corre-
lates with the acceleration of sinus pacemaker activity following the addition of ATP in the cardiac perfusate (463). However, ATP-induced prostaglandin release has not been characterized at the cardiomyocyte level. The involvement of prostaglandins to mediate the effects of ATP has been denied for both the increase in $I_{\text{CaL}}$ current (423) and cAMP synthesis (387).

F. Effects of Diadenosine Polyphosphates on Heart

In spontaneously beating guinea pig atria, diadenosine polyphosphates, Ap$_n$As, exert a negative chronotropic effect. They also reduce maximal contractile force and slow its development (218, 486). In guinea pig papillary or human ventricular muscles, Ap$_6$A alone was ineffective, but it attenuated isoprenaline-stimulated force (486). This is at odds with the primary observations that diadenosine penta- and hexaphosphate, Ap$_5$A and Ap$_6$A, have been shown to elicit phasic contraction in vas deferens and urinary bladder (453) and constrict rat aorta via an increase in $[\text{Ca}^{2+}]_i$ (429). These negative chronotropic and inotropic effects are mediated by A1-adenosine receptors. This has been confirmed in recent studies of Ap$_4$A, whose effects are abolished by DPCPX (338, 485). However, in human atrial and ventricular preparations, Ap$_4$A alone increases contractile force, an effect which is abolished by suramin (485). The positive inotropic effect was accompanied by prolonged contractile time parameters similarly to those observed during $\alpha_1$-adrenoceptor stimulation (469), although it was insensitive to prazozin. The mechanism is unknown; the authors tentatively suggest activation of a PLC.

The origin of bradycardia induced by Ap$_n$As in perfused heart and multicellular preparations was attributed to A$_1$-adenosine receptor activation (62). However, a detailed analysis at first using dipyridamole, an inhibitor of cellular adenosine uptake, indicates that in the tissue Ap$_n$As had no direct effect on A$_1$-adenosine receptor. Rather, the authors demonstrated that it is its degradation product adenosine that activates $I_{\text{K(ACh)}}$. In the same work, an activation of $I_{\text{K(ATP)}}$ is also observed. The latter is attributed to intracellular AMP increase following the hydrolysis of Ap$_n$As after its cellular influx (62) rather than to a direct effect of Ap$_n$As (237).

IV. MOLECULAR AND CELLULAR MECHANISMS

A. Modulation of Cardiac Transmembrane Ionic Currents

1. Purinergic-induced nonspecific cationic currents

The fast application to cardiac cells of ATP in the micromolar range elicits a rapid activating, fast desensitizing inward current $I_{\text{ATP}}$ (Table 4). Recovery occurs within a few minutes. Initially reported by Friel and Bean (167) in frog atrial cells, this observation was extended to mammalian atrial and ventricular cells (41, 91, 204, 363, 418, 420, 424, 430, 536). However, the presence of Ap$_n$As did not alter the recovery rate of $I_{\text{ATP}}$ in guinea pig atrial preparations (418, 420, 430, 536).

### Table 4. ATP-induced effects on cardiac ionic currents

<table>
<thead>
<tr>
<th>Current</th>
<th>Species</th>
<th>Pathway</th>
<th>Effects</th>
<th>Reference Nos.</th>
</tr>
</thead>
<tbody>
<tr>
<td>$I_{\text{ATP}}$</td>
<td>Frog atria</td>
<td>P2X</td>
<td>+</td>
<td>167</td>
</tr>
<tr>
<td></td>
<td>Mammalian</td>
<td>/</td>
<td>+</td>
<td>41, 91, 363, 424, 536</td>
</tr>
<tr>
<td>$I_{\text{ATP}},\text{SaS}$</td>
<td>Rabbit sinoatrial node</td>
<td>/</td>
<td>+</td>
<td>436</td>
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<tr>
<td>CI</td>
<td>Guinea pig atria</td>
<td>/</td>
<td>+</td>
<td>311</td>
</tr>
<tr>
<td></td>
<td>Rat, mouse ventricle</td>
<td>/</td>
<td>+</td>
<td>239</td>
</tr>
<tr>
<td></td>
<td>Guinea pig ventricle</td>
<td>P-Tyr on CFTR</td>
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<td>80, 438</td>
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<td></td>
<td>Guinea pig atria</td>
<td>/</td>
<td>0</td>
<td>363</td>
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<td>Na$^+$</td>
<td>Rat ventricle</td>
<td>Voltage shift</td>
<td>–</td>
<td>425</td>
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<tr>
<td></td>
<td>Frog atria</td>
<td>/</td>
<td>+</td>
<td>183, 527</td>
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<tr>
<td></td>
<td>Rat ventricle</td>
<td>/</td>
<td>+/-</td>
<td>7</td>
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<td>+</td>
<td>91, 116, 125, 126, 389, 435</td>
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<tr>
<td></td>
<td>Ferret ventricle</td>
<td>/</td>
<td>–</td>
<td>204</td>
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<tr>
<td></td>
<td>Hamster ventricle</td>
<td>/</td>
<td>–</td>
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<tr>
<td></td>
<td>Guinea pig atria</td>
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<tr>
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<td>170, 190, 312, 315</td>
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<tr>
<td></td>
<td>Rat atria</td>
<td>$I_{\text{K(Ca)}}$</td>
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<td>521</td>
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<td></td>
<td>Guinea pig atria</td>
<td>$I_{\text{K}}$</td>
<td>+</td>
<td>313, 315</td>
</tr>
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<td>Guinea pig ventricle</td>
<td>P-Tyr on $I_{\text{K}}$</td>
<td>+</td>
<td>309</td>
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<td></td>
<td>Rat ventricle</td>
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<tr>
<td></td>
<td>Guinea pig ventricle</td>
<td>cAMP synthesis</td>
<td>+</td>
<td></td>
</tr>
</tbody>
</table>

(\text{\textdagger}), Nondetermined pathway; (+), (–), increase or decrease in peak current amplitude; (0), no effect. CFTR, cystic fibrosis transmembrane conductance regulator. See text for definitions of currents.
It has also been seen in human cells (Alvarez and Vassort, unpublished results). The current has a reversal potential just below 0 mV. It could not be attributed to a Cl\(^-\) channel since neither internal nor external Cl\(^-\)ionic substitution altered the current. Rather, \(I_{\text{ATP}}\) is attributable to a nonselective cation channel allowing Na\(^+\), K\(^+\), and Ca\(^{2+}\), but not N-methyl-d-glucamine (NMDG) to flow through. \(I_{\text{ATP}}\) has linear (91, 167) or inwardly rectifying current-voltage relationships (204, 363, 424). Inward rectification of K\(^+\) currents has been attributed to intracellular Mg\(^{2+}\) and/or polyamines. Variations in the rectifying properties of the \(I_{\text{ATP}}\)-voltage relationships are of unknown origin; at first sight, they may be attributed to differences in the experimental conditions. Fluctuation analysis after ATP application yields a very low unitary conductance (363). Like ATP, 2-MeSATP is a very efficient agonist with \(EC_{50}\) 0.7–3 \(\mu M\) (363, 426) while ATP\(\gamma S\) and \(\alpha,\beta\)-met-ATP as well as GTP, ITP, UTP, ADP, AMP, and adenosine are not active (167, 424). However, \(\alpha,\beta\)-met-ATP at 100 \(\mu M\), and ATP\(\gamma S\) at 250 \(\mu M\), were reported to induce this current in atrial mammalian cells (91, 204). Ca\(^{2+}\) channel antagonists such as Ni\(^{2+}\), Co\(^{2+}\), or verapamil do not alter \(I_{\text{ATP}}\). However, increasing external Ca\(^{2+}\) reduces \(I_{\text{ATP}}\) (167). Opposite effects are seen when removing external Ca\(^{2+}\) (424), although \(I_{\text{ATP}}\) was unaffected by ryanodine treatment (204). Decreasing intracellular Ca\(^{2+}\) by BAPTA decreases and prolongs \(I_{\text{ATP}}\) (91, 421, 536). Mg\(^{2+}\) are required in the bathing solution for ATP to induce \(I_{\text{ATP}}\) (91, 424, 536). It is worth noting that generally divalent cations including Mg\(^{2+}\) reduce the affinity for ATP at P2X receptors (288).

\(I_{\text{ATP}}\) is blocked by quinidine (91). It is also inhibited by PPADS and cibacron blue (14, 426). However, these P2-receptor antagonists are known to interact with different ATP-binding sites apart from the specific purinoceptors.

The first thought, supported by the rapid activation and deactivation and by the nonspecificity of cation transfer, would be to relate \(I_{\text{ATP}}\) to ligand-operated channels. Such was the initial proposal in smooth muscle cells (31, 32) and sensory neurons (23). After eliminating “permeabilization” of the cells the way ATP does in mast cells (97), Friel and Bean (167) strengthened the comparison with nicotinic ACh receptor channels, although they could not rule out the involvement of a second messenger mechanism (probably Ca\(^{2+}\)).

The fast kinetic characteristics suggest to relate this current to either P2X\(_1\) or P2X\(_7\) receptors. However, on both receptor subtypes, \(\alpha,\beta\)-met-ATP is an active agonist while in cardiac cells this poorly hydrolyzable analog prevents the effects of ATP (167) without causing desensitization (426, 536). Also, in cardiac cells, fluctuation analysis of the ATP-induced current with Na\(^+\) as the main charge-carrying ion yields an apparent very small unitary conductance of 0.08 pS at a holding potential of −120 mV while 2-MeSATP evokes large whole cell currents, although the unitary conductance could not be detected using the same type of analysis (363). This situation is far from the single-channel unitary conductance (10–60 pS) reported for the expressed clones of P2X\(_1\) and P2X\(_7\) receptors (60, 487, 537).

With these observations, Scamps and Vassort (424) initially proposed a four-step cascade to account for the effects of rapid ATP application that implied the activation of the anion exchanger Cl\(^-\)/HCO\(_3^-\). Both \(I_{\text{ATP}}\) and acidosis requested Mg\(^{2+}\) and were not activated by UTP, CTP, ITP, GTP, and ADP (390). However, probenecid, an inhibitor of Cl\(^-\)/HCO\(_3^-\) exchanger, does not prevent \(I_{\text{ATP}}\) (M. Ugur and G. Vassort, unpublished results), and this hypothesis must be presently discarded. Led by the Mg\(^{2+}\) dependency and the observation that extracellular ATP induces phosphorylation of several membrane proteins, Christie et al. (91) proposed that the polyphosphate chain serves as a phosphate donor through action of a protein kinase intimately associated with the receptor. Furthermore, they suggested that either or both 32- and 47-kDa proteins could be involved in the formation of an aqueous pore that allows cation influx. Their view was supported by the weaker effect of ATP\(\gamma S\) and of \(\alpha,\beta\)-met-ATP in their hands. In conclusion, the present site of action of Mg\(^{2+}\) is unclear. Among several possibilities, one can suggest the following: Mg\(^{2+}\) interaction with P2X channels is compulsory for their opening; ATPMg\(^{2+}\) is the ligand of a given cardiac P2X subtype; or ATPMg\(^{2+}\) is the substrate of an ecto-kinase that modulates P2X channels. Presently, the protein support of this fast-activating, transient, nonselective cationic current, or its mechanism of induction by ATP on cardiac cells is unsettled.

More recently, another ATP-activated cationic current was reported in rabbit sinoatrial cells (436). This current increases in a dose-dependent manner over the concentration range of 0.01–1 mM ATP, which indeed was the only active agonist. This current could be carried by various cations including Tris and NMDG, whose apparent permeabilities are only five times less than K\(^+\), Na\(^+\), or Ca\(^{2+}\). It shows a reversal potential near 0 mV and is weakly inward rectifying. This current does not appreciably desensitize within 3 min of ATP application. A similar long-lasting ATP-induced current was also elicited in rat ventricular cells over a very broad range of ATP concentrations (Ugur and Vassort, unpublished results). The ATP effect does not require the presence of Mg\(^{2+}\). BzATP is a weaker agonist than ATP, whereas neither PPADS nor oxidized ATP prevents the sustained ATP-induced currents (Ugur and Vassort, unpublished results). These pharmacological characteristics make it difficult to clearly attribute this current to the activation of a P2X\(_7\) receptor.

Several mammalian sequences homologous to Dro sophila Trps have been identified that represent a new
family of Ca$^{2+}$-permeable cation channels with at least TRPC$_1$ present in heart (517). Their mechanisms of activation are still poorly known but appear quite variable. They include intracellular Ca$^{2+}$ increase as follows from angiotensin II activation of expressed TRPC$_3$ (543). Similar effects of ATP have to be investigated.

These two ATP-induced nonspecific cationic currents have two major cellular consequences. First they depolarize the cell membrane and so might initiate spontaneous automaticity that will lead to arrhythmia. Arrhythmia might also occur as a consequence of Ca$^{2+}$ entry and Ca$^{2+}$ overload through these channels. However, it has been reported that extracellular EGTA and Ca$^{2+}$ channel blockers (nifedipine, verapamil) reduce the ATP-induced Ca$^{2+}$ transient while BAY K 8644 increases it; that suggests a more direct relation with the L-type Ca$^{2+}$ channel (126). How much of the ATP-induced effects on intracellular Ca$^{2+}$ can be attributed to the nonspecific cationic current remains to be determined. Although such Ca$^{2+}$ entry should be expected in cardiac cells, the Ca$^{2+}$ transient elicited by ATP has been generally attributed to other mechanisms (see below).

2. Cl$^{-}$ current

Extracellular ATP (5–50 μM) activates an outwardly rectifying, time-dependent Cl$^{-}$ current (I$_{Cl}$) in single guinea pig atrial myocytes. ADP, AMP, and adenosine also activate I$_{Cl}$ (311). This is also true in single rat and mouse ventricular myocytes (222, 239, 284); however, there was no evidence for such a current in guinea pig ventricular cells (363). In mouse ventricular cells, this current is blocked by DIDS (which is now known to be a P2-receptor antagonist) and is not activated by either AMP or adenosine. ATPyS also elicits the Cl$^{-}$ current (284). The differential action of adenosine on I$_{Cl}$, activated by extracellular ATP in atrial and ventricular myocytes, could reflect different Cl$^{-}$ channels in these cells or species variability.

Neither the purinoreceptor subtype nor the signal transduction pathway mediating the action of ATP on I$_{Cl}$ is clearly known. Levesque and Hume (284) reported that activation of I$_{Cl}$ is not likely to be dependent on increased [Ca$^{2+}]_o$, since ATP activates the current in cells dialyzed with 10 mM EGTA or 20 mM BAPTA, a much faster Ca$^{2+}$ chelator. The ATP-induced Cl$^{-}$ current exhibits a weak outward rectifying conductance like the Cl$^{-}$ current activated by cAMP (357). However, a Cl$^{-}$ current was not activated by isoproterenol or forskolin application in mouse ventricular cells, although such occurs in other species (15, 193). Furthermore, adenosine, which is known to reverse the β-adrenergic-induced increase in cAMP in mouse ventricular cells (525) and I$_{Cl(cAMP)}$ in rabbit ventricle (194), does not prevent the effects of ATP. This supports the contention that cAMP is not involved in the activation of the ATP-induced Cl$^{-}$ conductance.

Attenuation of isoprenaline-induced I$_{Cl}$ in guinea pig ventricular myocytes by ATP has also been reported (404). In addition, it has been shown that genistein at 100 μM, but not daidzein, activates the cardiac chloride conductance (89, 438). This effect is antagonized by Na$_3$VO$_4$, an inhibitor of phosphotyrosine phosphatase. Comparison of I$_{Cl}$ activated by genistein and I$_{Cl}$ activated by forskolin led the authors to suggest that genistein activates the cAMP-dependent CFTR channel. Similarities in potency and efficacy of ATP for intracellular acidification (390) and in Cl$^{-}$ current activation were noted (239). It has thus been proposed that activation of a Cl$^{-}$ current near resting potential could lead to intracellular Cl$^{-}$ depletion and thus to the activation of the Cl$^{-}$/HCO$_3^-$ exchanger and subsequent intracellular acidification (239). The physiological importance of ATP enhancement of Cl$^{-}$ currents is not known. However, with a Cl$^{-}$ reversal potential around −45 mV in cardiac cells (122), these currents are potentially arrhythmogenic because they depolarize the membrane and shorten action potential plateau. The Cl$^{-}$ channels should be considered potential target sites for the development of antiarrhythmic agents.

3. Na$^{+}$ current

Extracellular ATP decreases the inward Na$^{+}$ current (I$_{Na}$) (425). Thus, in rat single cardiac ventricular myocytes, similar to its effect on I$_{Ca}$ (423), extracellular ATP in the micromolar range causes a leftward shift (5–8 mV) in both activation and availability characteristics of I$_{Na}$ so that the availability is reduced at resting potential (91, 425). At hyperpolarized potentials, I$_{Na}$ could be slightly increased due to the voltage shift in its activation kinetics (425). ATPyS and α,β-met-ATP exert similar effects but not UTP, β,γ-met-ATP, ADP, and adenosine. The voltage shift induced upon application of extracellular ATP is not affected by cholera toxin treatment, suggesting that a Gs protein and cAMP are not involved (425). The reduction in Na$^{+}$ current will slow the spread of activation and might contribute to the arrhythmogenic effects of ATP.

4. Ca$^{2+}$ current

More than two decades ago Goto et al. (183) using the double sucrose-gap technique reported that extracellular ATP and ADP enhance the calcium inward current (I$_{Ca}$) and the I$_{Ca}$-dependent phasic tension in muscle bundles isolated from the right atrium of the bullfrog. In a subsequent study, the same group determined that the action of ATP on I$_{Ca}$ and tension does not require the hydrolysis of ATP and is probably mediated by a receptor located at the outer surface of the affected cell membrane (527). This followed the primary observation that ATP is, like isoprenaline, able to restore electrical and mechani-
cal activities in Ca\textsuperscript{2+}-deficient solutions (11) or to induce slow action potentials in K\textsuperscript{+}-depolarized guinea pig hearts (430).

A) ACTIVATION. In single frog ventricle cells under whole cell patch clamp, ATP (1 \mu M) increases \( I_{\text{Cal}} \) by up to twofold; at higher ATP concentrations, the increase in \( I_{\text{Ca}} \) is smaller, and at 100 \mu M, ATP reduces this current (7). The ATP-induced increase in \( \text{Ca}^{2+} \) current is prevented by perturbations that block either signal transduction pathways involving the activation of PLC or its activity (7). These data were interpreted to suggest that the ATP-induced increase in \( \text{Ca}^{2+} \) current in frog ventricular myocytes is mediated by P2-purinoceptor and phosphoinositol turnover on the basis that neomycin specifically inhibits PLC, a probably misleading assumption (7).

Later detailed studies demonstrated that the extra- cellular application of micromolar ATP increases the L- type \( \text{Ca}^{2+} \) current, \( I_{\text{Cal}} \), in mammalian cells isolated from rat ventricular myocardium (91, 421, 423, 426, 536) and from guinea pig and rabbit atrial myocytes (204). ATP\gamma S exerts a similar effect, but adenosine is much less effective, and GTP, UTP, CTP, and ITP are without effect (421). The rank order of efficacy and potency of ATP analogs in increasing \( I_{\text{Cal}} \) amplitude is 2-MeSATP \textasciitilde ATP \textasciitilde ATP\gamma S, while \( \alpha,\beta\text{-met-ATP}, \beta,\gamma\text{-met-ATP}, \) and \( \beta,\gamma\text{-imido-ATP} \) have no effect (426). The single-channel conductance (~17 pS) is not changed by ATP\gamma S application on rat ventricular cells. However, both the probability of channel opening and availability of functional channels are enhanced after P2-purinergic stimulation (422). In contrast to the induction of the nonspecific cationic current by ATP, the enhancement of \( I_{\text{Cal}} \) does not require the presence of Mg\textsuperscript{2+} (424, 536). The activation of P2 purinoceptor leads to an increase of \( I_{\text{Cal}} \) via the activation of cholera toxin-sensitive \( G_{s} \) protein but is independent of cAMP or phosphoinositol turnover (423, 536) (Fig. 3). The ATP-induced increase in \( I_{\text{Cal}} \) does not involve a phosphorylation by PKA, since it occurs in the presence of 500 \mu M cAMP in the patch pipette (423) and is not prevented by the intracellular application of PKI (Alvarez and Vassort, unpublished results). ATP activates the tyrosine kinase pathway; however, the role of tyrosine phosphorylation on \( I_{\text{Cal}} \) in cardiomyocytes is, as yet, unknown. Src and FAK but not MAPK are involved in enhancing the \( \text{Ca}^{2+} \) current in smooth muscle cells, an effect that should be related to the association of Src to the \( \alpha_{1}\) subunit of the \( \text{Ca}^{2+} \) channel (219). Some other effects could also be involved in relation with the known interaction of tyrosine kinase with G proteins. Similarly, ATP increases the transient, low-threshold \( \text{Ca}^{2+} \) current (\( I_{\text{Cay}} \)) in frog atrial cells via a pathway that probably does not involve phosphorylation (9).

B) INHIBITION. In isolated ferret ventricular myocytes, extracellular ATP in the micromolar range inhibits \( I_{\text{Cal}} \) in a time- and concentration-dependent manner (398, 399).

Stable ATP analogs, 2-MeSATP, ATP\gamma S, and \( \alpha,\beta\text{-metATP} \), also cause inhibition, whereas suramin prevents it (398). The inhibitory effect of ATP cannot be blocked by either the muscarinic cholinergic antagonist atropine or the adenosine \( A_{1}\)-receptor antagonist DPCPX (399). The ATP-induced inhibitory effect is not dependent on \( \text{Ca}^{2+} \) influx or \( \text{Ca}^{2+} \) load, since it is observed with \( \text{Ba}^{2+} \) as charge-carrying ion or in the presence of BATPA (398), thus eliminating a mechanism related to the known \( \text{Ca}^{2+}\text{-dependent} \) \( \text{Ca}^{2+} \) inactivation of \( I_{\text{Cal}} \). ATP also inhibits \( I_{\text{Ca}} \) in guinea pig single SAN cells in a concentration-dependent manner (396, 446). The rank order of potency of ATP and related compounds inhibiting \( I_{\text{Ca}} \) in SAN cells is as follows: ATP \textasciitilde \( \alpha,\beta\text{-met-ATP} \textasciitilde 2\text{-MeSATP} \textasciitilde ATP\gamma S \textasciitilde UTP \textasciitilde ADP \textasciitilde AMP \textasciitilde \text{adenosine} (396). This potency order has not been reported with regard to previously identified P2-purinoceptor subtypes, suggesting the mediation of a novel purinergic receptor. Inhibition of \( I_{\text{Cal}} \) by ATP is also observed in the presence of guanosine 5’-O-(3-thiotriphosphate) (GTP\gamma S) (398, 399, 423) and was then poorly reversible (500). In guinea pig SAN cells, DPCPX and 8-PT as well as PPADS and suramin do not prevent the inhibitory effect of ATP of \( I_{\text{Ca}} \) which is, however, abolished by the PKC inhibitors staurosporine and calphostin. The authors proposed that in these cells the ATP-induced inhibition involves neither the P1 nor the P2 purinoceptors (396, 397).

Activation of the heterologously expressed P2Y\textsubscript{2} receptor induces inhibition of the N-type \( \text{Ca}^{2+} \) current in rat sympathetic neurons (154). ATP and UTP are equally active with an \( EC_{50} \textasciitilde \approx 0.5 \mu M \) similar to their \( EC_{50} \) for phosphoinositol signaling and their ability to stimulate phosphoinositol turnover. ATP also reduces L-type Ca\textsuperscript{2+} current and IP\textsubscript{3} production. Despite this, the authors suggested a direct action of a \( G_{i} \) protein inhibiting N-type channel gating (134). A \( G_{i} \) protein-mediated inhibition has been reported for the T-type current (8) and might account in part from the ATP-induced inhibition observed in frog heart cells at high ATP concentration.

![FIG. 3. Schematic regulation of the L-type \( \text{Ca}^{2+} \) current (\( I_{\text{CaL}} \)) following P2-purinergic stimulation. A \( G_{s} \) protein positively couples to the channel activity while the mechanisms of inhibition are unknown. PKG, protein kinase G.](http://physrev.physiology.org/)
Inhibition of $I_{\text{CaL}}$ is known to be mediated by activation of the cGMP-dependent protein kinase particularly when the cell has been previously stimulated by a β-adrenergic agonist (192, 201). An inhibition of basal $I_{\text{CaL}}$ by PKG was also reported (226, 466). Because purinergic stimulation increases cGMP in cardiac cells (423), such a pathway might well be involved in $I_{\text{CaL}}$ inhibition.

External ATP reduces binding of isradipine by 90% on cardiomcyocytes in 30 mM K$^+$ buffer in the presence or not of Ca$^{2+}$ and Mg$^{2+}$ (452). The reduction in binding sites might correspond in some ways to the inactivation of Ca$^{2+}$ channels leading to $I_{\text{CaL}}$ inhibition.

In rat ventricular cells, a transient inhibition of $I_{\text{CaL}}$ was observed when applying suddenly ATP that preceded the ATP-induced increase in $I_{\text{CaL}}$ (421). This inhibitory effect was attributed to the transient acidosis of the cells (389, 390), which is known to reduce $I_{\text{CaL}}$ (274). How much of the inhibitory effects described in other cells are attributable to ATP-induced acidosis has to be checked by the use of an inhibitor of the Cl$^-$/HCO$_3^-$ exchanger such as probenecid.

5. K$^+$ currents

A number of $K^+$ channels are present in cardiac myocytes that determine the shape of the action potential and frequency of beating. Several observations have indicated that most of these channels are regulated by extracellular ATP.

A) INWARD RECTIFYING $K^+$ CHANNELS, $I_{K(ACh)}$ AND $I_{K(ATP)}$.

Friel and Bean (167) first reported in bullfrog and bovine atrial cells that extracellular ATP activates an inwardly rectifying $K^+$ channel whose current-voltage relationship was similar to that activated by ACh. Further works on mammalian cells (170, 315, 521) demonstrated that P2 receptors, like the muscarinic cholinergic receptors and the $A_1$-adenosine receptors, are directly coupled to a $K^+$ channel via a pertussis toxin-sensitive G$_k$ protein. UTP also activates $I_{K(ACh)}$, an effect inhibited by pertussis toxin (521). These observations suggest the involvement of P2Y$_2$ or P2Y$_4$ receptors.

Activation of $I_{K(ACh)}$ by P2-purinergic agonists decreases monotonically in the mitotic presence of the agonist in the bath solution. As well, ATP application only transiently increases $I_{K(ACh)}$ activated by ACh before depressing the ACh effect (190, 313). This depressing effect of ATP is not mediated by a pertussis toxin-sensitive G protein and is not affected by PKC inhibitor. The potency order of ATP analogs in reducing $I_{K(ACh)}$ ATP ≥ 2-MeSATP ≥ $\alpha,\beta$-met-ATP indicates involvement of a P2Y receptor (312).

Recent studies have shown that extracellular ATP enhances the current flow through the intracellular ATP-sensitive $K^+$ channel $I_{K(ATP)}$ once it has been partially activated under conditions of metabolic stress (i.e., 100 μM of intracellular ATP) (13). The $K_{(ATP)}$ channel consists of a weak inward rectifier subunit Kir6.2 plus a member of the adenine nucleotide binding cassette (ABC) superfamily, SUR2 (228). $K_{(ATP)}$ channel activation during acute ischemia/hypoxia has been shown to exert a protective effect on the heart (186, 187). Studies in cardiac myocytes in vitro have suggested that the activation of $A_1$-adenosine receptors could result in the activation of $K_{(ATP)}$ channels (229, 258). However, at least in the hypoxic guinea pig heart in vivo, endogenous adenosine failed to activate $K_{(ATP)}$ channels (523). Several analogs of ATP, i.e., $\alpha,\beta$-met-ATP, 2-MeSATP, and ATP$\gamma$S, exert a similar effect to that of ATP in activating $I_{K(ATP)}$ in rat ventricular myocytes, whereas UTP and ADP have a relatively small effect and AMP and adenosine have no effect (14). The enhancement of $I_{K(ATP)}$ by extracellular ATP is inhibited by cholera toxin as well as by inhibition of adenylyl cyclase (Fig. 4) (13). Thus it has been suggested that the mechanism of this effect is the G$\kappa$-dependent activation of adenylyl cyclase which causes an increase in cAMP production and thereby reduces intracellular ATP level (13).

B) DELAYED OUTWARD RECTIFYING $K^+$ CHANNELS, $I_{Kr}$

ATP increases the delayed outward rectifier $K^+$ current ($I_{Kr}$) in guinea pig atrial cells (312, 314, 315). ADP also enhances $I_{Kr}$ while adenosine is without effect. PTX and theophylline as well as buffering intracellular Ca$^{2+}$ or PKC inhibition do not affect the activation by ATP (312, 314, 315). ATP was shown to selectively enhance the slow component of the delayed rectifier $K^+$ current ($I_{Kr}$), leaving the fast component $I_{Kr}$ unaffected since ATP increases similarly the current and its tail and since ATP effect is unaffected by E-4031, a known blocker of $I_{Kr}$ (313).

In various tissues, several $K^+$ currents can be modulated by tyrosine kinase, which is known to be activated by ATP. Thus the $\mu$-muscarnic ACh receptor potently inhibits a cloned delayed-rectifier $K^+$ channel, $K_{Ca1.2}$, through its direct phosphorylation via PYK2 (151, 221). The direct association of Src and tyrosine phosphoryla-

![FIG. 4. Proposed scheme accounting for the enhancement of the ATP-dependent $K^+$ current ($I_{K(ATP)}$) by P2-purinergic stimulation. The $G\alpha$ protein specifically couples to a given isoforn of adenylyl cyclase, ACV, whose activation induces a localized ATP depletion.](http://physrev.physiology.org/)
tion of hKv1.5 suppresses channel current (208). In guinea pig ventricular cells, the delayed rectifier K⁺ current \( I_\text{k} \) is concentration dependently increased by ATP with an EC\(_{50}\) of 1.86 μM. The ATP effect develops slowly; it is not affected by PKA and PKC inhibitors while genistein suppresses the response. That leads the authors to hypothesize that tyrosine phosphorylation is involved in P2Y-receptor stimulation of \( I_\text{k} \) (309).

In rat ventricular cells, ATP in the micromolar range also activates a delayed outward K⁺ current. The latter effect of ATP is mimicked by the application of arachidonic acid and blocked by AACOCF₃, a PLÀ₂ inhibitor as well as by inhibition of the cAMP pathway (5). These effects could be attributable to the activation of a two-pore domain K⁺ channel of the TWIK-1 family such as TRAAK (155).

c) HYPERPOLARIZATION-ACTIVATED CURRENT, \( I_f \). The hyperpolarization-activated current \( I_f \) is sensitive to neuro-modulation. \( \beta \)-Adrenergic stimulation facilitates \( I_f \) via a direct cAMP-induced leftward shift of its activation curve. These effects are antagonized by ACh (129) and would account for the modulation of SAN activity. As well, adenosine inhibits automaticity probably through the same mechanism (528). There is yet no report on the effects of purinergic agonist on \( I_f \). However, one might anticipate that ATP by increasing cAMP activates \( I_f \) and has a positive chronotropic effect.

B. Signal Transduction Pathways

The effects of extracellular ATP on second messengers such as cAMP, cGMP, and IP₃ have been investigated not only in whole heart tissues but also, in the most recent studies, in isolated cardiomyocytes.

1. cAMP and cGMP

A) cAMP. Whether ATP modulates intracellular cAMP in cardiac cells has long been controversial. An ATP-induced twofold increase in cAMP had long been reported in frog ventricle associated with the increase in contractile force (161). However, the authors noted that most of the increase in force was antagonized by indomethacin, implying an indirect effect via prostaglandin. Indomethacin was not used during cAMP assays. Zheng et al. (535) and Scamps et al. (423) observed that ATP does not significantly affect basal cAMP level in rat ventricular cardiomyocytes, but it facilitates the isoproterenol-induced increase in cAMP (535). In cardiomyocytes isolated from fetal mice, basal cAMP level is not changed by ATP, which, however, partially antagonizes the effect of isoproterenol (525). Reinvestigating the effects of purinergic agonists on rat neonatal and adult ventricular cardiomyocytes, we found that stimulation with ATP alone in the presence of the phosphodiesterase inhibitor IBMX increases the cAMP level by twofold; at the lower basal cAMP levels, a fourfold stimulation was observed (387). The effect of ATP on cAMP production is poorly potentiated by forskolin and is additive to that of submaximal concentrations of isoproterenol. The ATP-induced activation of the adenyl cyclase is mediated by a 45-kDa Gₐ protein, similar to that observed with isoproterenol stimulation. Both ATP and isoproterenol increase cAMP in HEK-293 cells expressing type V adenyl cyclase, whereas cAMP was only increased by \( \beta \)-adrenergic stimulation of HEK-293 expressing type IV and type VI adenyl cyclases. Thus, in rat cardiomyocytes, purinergic and \( \beta \)-adrenergic stimulations differentially activate cyclase isoforms; adenyl cyclase V appears the specific target of the purinergic stimulation (387). A paracrine effect involving PLÀ₂ activation and the formation of prostaglandins could be excluded, since this work was conducted on isolated cardiac cells. However, in intact tissues, an increase in cAMP following activation of prostaglandin synthesis (PG1₂) should be acknowledged, since ATP activates this pathway in endothelial cells (370).

The increase in cAMP has not yet been related to any particular purinoceptor subtype in cardiac cells (Fig. 5). A candidate would be the recently cloned P2Y₁₁ (101). In other cells such as Madin-Darby canine kidney cells, ATP increases cAMP preferentially through P2Y₂ relative to P2Y₁ and P2Y₁₁ (383, 384). Considering that ATP is rapidly degraded to adenosine, it should also be noted that ventricular cardiac cells possess both cAMP-decreasing \( \Lambda_1 \) and stimulating \( G_\text{s} \) isoforms; adenylyl cyclase V appears the specific target of the purinergic stimulation (387). A paracrine effect involving PLÀ₂ activation and the formation of prostaglandins could be excluded, since this work was conducted on isolated cardiac cells. However, in intact tissues, an increase in cAMP following activation of prostaglandin synthesis (PG1₂) should be acknowledged, since ATP activates this pathway in endothelial cells (370).

FIG. 5. Models for the control of cAMP level by P2Y receptors. In most cases P2Y receptors are reported to inhibit adenyl cyclase (AC). However, activation of the adenyl cyclase can occur \( J \) directly via a \( G_\text{s} \) protein coupled to a type V AC (387) and might follow stimulation of receptor (101; 2) via Ca⁺⁺ increase and calmodulin by which AC types I, III, and VIII are positively modulated (107, 438); Ca⁺⁺ also inhibits phosphodiesterase; and J via prostaglandin (PGE₂) release that activates an external \( G_\text{s} \) protein-coupled receptor R (383, 384). PDE, phosphodiesterase; PLC-β, phospholipase C-β; PLÀ₂, phospholipase A₂; IP₃, inositol 1,4,5-trisphosphate.
and cAMP-increasing A₂ adenosine receptors (27, 51, 451), although this has been disputed (200, 437).

b) cGMP. ATP, in the presence of IBMX, increases the cGMP content of isolated rat cardiomyocytes (423). A slowly developing increase in cGMP has also been reported in frog ventricles associated with the secondary decrease in contractile force (161).

c) UTP. Flitney and Singh (160) also reported that in the frog heart UTP, similarly to ATP, increases both cAMP and cGMP. Note, however, that in whole heart both effects could be secondary to activation of the prostanoid synthesis or to transphorylation by the NDPK. Indirect effects could be secondary to activation of the prostaglandin or cGMP. Note, however, that in whole heart both events known to be mediated by the kinase (534). These observations are of physiological relevance with regard to the likely specific role of PKC isoforms in cardiac function (392).

2. IP₃

Most P2Y receptors (i.e., P2Y₁, P2Y₂, P2Y₄, and P2Y₆) are coupled to PLC-β. In a recent study, ATP and UTP were both shown to activate Gₛ₁₁ and Gₐ₃ but not Gₑ, Gₛ₂, or Gₛ₃ protein in membranes from gastric and aortic smooth muscle as well as from heart (not isolated cardiomyocytes) (333). IP₃ formation induced by ATP and UTP was mediated concurrently by Gₛ₁₁-dependent activation of PLC-β₁ and Gₛ₂βγ-dependent activation of PLC-β₃. Phosphoinositide hydrolysis was thus partially inhibited by pertussis toxin. Moreover, specifically, in rat ventricles (282) and isolated fetal mouse cardiomyocytes (525), ATP accelerates phosphatidylinositol turnover and induces IP₃ formation. The ATP-induced IP₃ formation in rat ventricular myocytes should be attributed, in most part, to the activation of the PLC-γ by a tyrosine kinase pathway (395).

The role of IP₃ in cardiac cells is still unclear. In adult mammalian cardiomyocytes, IP₃ releases Ca²⁺ from the SR in mechanically skinned or chemically permeabilized cells (251, 352, 498, 499) and in voltage-clamped guinea pig cardiomyocytes (179). Despite that SR-Ca²⁺ release evoked by IP₃ is slower than that evoked by Ca²⁺, IP₃ enhances contractions (418, 499). In neonatal rat cells, caged IP₃ as well as purinergic stimulation triggers a spatially restricted intracellular Ca²⁺ release from a ryanodine- and caffeine-insensitive Ca²⁺ store enriched in IP₃ receptors. PLC-γ activation is required for the ATP effects. ATP, also in an IP₃-dependent manner, induces a depolarization of the mitochondrial membrane that is associated with a transient Ca²⁺ influx concomitant to the arrest in Ca²⁺ spiking of cardiomyocytes (231). These observations led the authors to propose that an integrated control of the filling state of Ca²⁺ pools accounts for the IP₃-dependent purinergic regulation of cardiac arrhythmic activity.

3. PKC

Direct evidence in favor of an ATP-induced increase in PKC activity had been obtained. ATP triggers redistribution from cytosol to the membrane of both ε- and δ-PKC, two Ca²⁺-insensitive PKC isoforms expressed in neonatal and adult cardiac cells. PKC also induces the phosphorylation of myristoylated alanine-rich C-kinase substrate (MARCKS) and the expression of c-fos and jun-B in neonatal cells, two events known to be mediated by the kinase (534). These observations are of physiological relevance with regard to the likely specific role of PKC isoforms in cardiac function (392).

4. Tyrosine kinase activation

Phosphorylation is a major modulating factor of protein activity. Specific kinases phosphorylate proteins on serine and threonine or on tyrosine residues. The first type of residue requires activation of PKA, PKC, and PKG as well as Ca²⁺-calmodulin kinase; the second implies either tyrosine kinases that display an intrinsic tyrosine kinase activity in their intracellular domain or the nonreceptor tyrosine kinases activated by G protein-coupled receptors (225). Various effects of tyrosine kinase have been reported on Ca²⁺ and K⁺ channels and on the gap junctions involved in cell-to-cell conduction (see Ref. 386 for review). A useful comparison could be made with the effects of angiotensin II whose role of tyrosine kinase in signal transduction in vascular smooth muscle has been recently reviewed (34). However, today, very little is known in the heart. Some comparison and arguments are taken from observations in other tissues.

Several nonreceptor tyrosine kinases are expressed in isolated cardiomyocytes: two members of the Src family pp60⁹⁵Src and p50⁹³Src, Src and Fyn (394, 416), and FAK (394). Several other nonreceptor tyrosine kinases are found in whole heart. PYK2 from the FAK family was shown in whole rat heart (130) but not in isolated neonatal or adult rat cells (394). Fes (78), Arg BP2 mostly located at the Z disks (506), and Tec (248) were also reported. Presently, there is no report about the presence of receptor tyrosine kinases in cardiomyocytes and heart.

ATP, like carbachol, phenylephrine, and endothelin, activates p₄² and p₄⁴MAPK isoforms in rat neonatal ventricular myocytes (382). It also activates them as well as p₃⁸MAPK in rat adult cardiomyocytes (5). Activation of the G protein-coupled P2Y₂ receptors by ATP and UTP stimulates p₄² and p₄⁴MAPK in endothelial cells (367) and in PC12 cells (444). In PC12 cells also, the activation of the Ca²⁺-dependent tyrosine kinase PYK₂ after ATP stimulation of P2X₂ receptors leads to MAPK activation (461).

The first evidence that ATP activates the tyrosine kinase pathways (Fig. 6) in adult rat cardiomyocytes was the labeling by an antiphosphotyrosine antibody of several proteins on Western blot, among them PLC-γ (395).
Both phosphorylation and translocation of PLC-γ depend on the presence of extracellular Ca²⁺. These events, as well as ATP-induced IP₃ formation, are prevented by genistein and herbimycin A, two potent tyrosine kinase inhibitors (395). The anionic exchanger AE₁ is also strongly phosphorylated on Tyr residues in ATP-stimulated cells (394). ATP stimulation of cardiomyocytes strongly increases both Src and Fyn activities. Fyn also associates to FAK, which is then phosphorylated on a Tyr residue, although its activity is unchanged (394). In addition, ATP stimulation of cardiomyocytes strongly phosphorylated on Tyr residues in ATP-stimulated cells (394). The activation of Fyn, a tyrosine kinase of the src family, leads to the phosphorylation of the Cl⁻/HCO₃⁻ exchanger and of the phospholipase C (PLC)γ to induce, respectively, acidosis and phosphatidyl-inositol bisphosphate hydrolysis to produce diacylglycerol (DAG) and inositol trisphosphate (IP₃). IP₃ might trigger Ca²⁺ release from intracellular stores, particularly the endoplasmic reticulum (ER). MgATP, rather than ATP, is required for the activation of the Cl⁻/HCO₃⁻ exchanger. Pip₂, phosphatidylinositol 4,5-bisphosphate.

Notwithstanding that ATP activates tyrosine kinase, its action is mediated by members of the Src family, whose action is mediated by members of the Src family, the src family; it negatively regulates both c-Src and Gs-
cyclase activity (16, 335). Overexpression of avian pp60src in fibroblasts results in an enhanced intracellular cAMP in response to β-adrenergic hormones that act through the stimulatory Gs protein (76, 301). More precisely, the Gα subunit can be phosphorylated by Src tyrosine kinase on a NH2-terminal and a COOH-terminal Tyr377 and Tyr377 residues in vitro (332), and the rate of binding of GTPγS to Gs protein is increased by pp60src (195). In this regard, it is interesting to note that caveolin, a major structural component of caveolae membranes, associates with H-Ras, c-Src, and other tyrosine kinases of Src family as well as with the α-subunit of G proteins including Gs; it negatively regulates both c-Src and Gs-ATPase activity (287, 289).

Notwithstanding that ATP activates tyrosine kinase, ATP (100 µM) and isoproterenol (1 µM) were just additive in increasing cAMP in neonatal cells (387). The use of supramaximal concentrations might have obscured synergistic effects; however, genistein did not alter ATP-induced cAMP increase (387). Such a modulating/controling effect of tyrosine kinase after ATP stimulation might occur on IP₃ formation. Gₛα and G₁₁α can be phosphorylated on Tyr-356. IP₃ formation is reduced after activating the m1-ACh receptor by carbachol in cells expressing a mutated G₁₁α (Y356F) protein. That suggests tyrosine phosphorylation events occur before activation of Gₛ/₁₁α and regulate its interaction with G protein-coupled receptors, thus accounting for the more active stimulation of PLC-β (294, 482).

The purinoreceptor subtype activating the tyrosine kinase cascade is unknown. However, it was previously shown (91, 390, 424) that the ATP-induced nonspecific cationic current and the ATP-induced acidosis requires the presence of Mg²⁺, a characteristic that is not generally recognized with the presently available cloned purinoceptors.

5. PLA₂ and PLD

Besides the activation of PLC and the production of IP₃, second messenger signaling of ATP cascades also include activation of PLA₂ (86, 291, 522). In the latter report in MDCK-D₁ cells (522), the ATP and UTP increased PLA₂ activity and arachidonic acid release involving either PKC-α or other PKC isoforms acting through MAPK activation. Previous reports indicate that ATP activates arachidonic acid metabolism in both whole heart and isolated cardiac myocytes (113, 463). Arachidonic acid is thought to mediate sinus rhythm acceleration via a cyclooxygenase metabolite, i.e., prostaglandins (463), or to enhance contractile force and intracellular Ca²⁺ transient after Ito inhibition and action potential prolongation (113). Arachidonic acid increase might also mediate the ATP-induced activation of a two-pore domain K⁺ current in rat cardiac cells (5). Another consequence could be an ATP-induced increase in cGMP content, since arachidonic acid has been reported to activate soluble guanylyl cyclase.

ATP effects also include activation of PLD mediated by PKC-ζ (376) or both a G protein, probably Rho, and
tyrosine kinases (139, 275). Thus it was observed ATP markedly enhances PLD stimulation by GTPγS; this effect is mimicked by vanadate, an inhibitor of protein tyrosine phosphatases and inhibited by tyrosine kinase inhibitors. The rank order of efficacy for stimulation of PLD activity in the presence of GTPγS is β,γ-met-ATP > ATPγS = ATP = ADP > 2-MeSATP > α,β-met-ATP = UTP and is independent from PLC activation in rat liver plasma membranes (303).

6. Intracellular Ca\(^{2+}\)

In quiescent and stimulated cells, the extracellular application of ATP in the micromolar range causes an about threefold increase in \([\text{Ca}^{2+}]_i\) (91, 116, 125, 126, 204, 389, 435). This increase results, in most part, from a large \(\text{Ca}^{2+}\) release by the SR, since caffeine and ryanodine markedly reduce it, although they do not prevent it all (41, 125, 204) even in the absence of external \(\text{Ca}^{2+}\) (389). In the frog heart, despite the limited SR, a role of this \(\text{Ca}^{2+}\) store in the facilitating effect of ATP had also been reported (345, 346). However, in most of these studies, a requirement for external \(\text{Ca}^{2+}\) was reported. This led some groups to suggest a relation with the increase in \(I_{\text{CaL}}\) associated with extracellular ATP (116, 125, 126, 204). Furthermore, ATP directly gates a nonslective cationic channel, \(I_{\text{(ATP)}}\) (41, 167, 536), through which a significant \(\text{Ca}^{2+}\) influx could also occur (see sect. VI). This is in line with the observation that both \(I_{\text{CaL}}\) and the ATP-induced increase in \(\text{Ca}^{2+}\) are \(\text{Mg}^{2+}\) dependent (91, 389, 424) and with the ATP-mediated influx of \(\text{Mn}^{2+}\) that quenched the indo 1 fluorescence signal (389). Other suggested mechanisms include phosphorylation of an extracellular protein leading to activation of a novel ion channel (91), as well as ATP-induced acidosis (389), leading to both \(I_{\text{ATP}}\) and the increase in \([\text{Ca}^{2+}]_i\) (424).

Several ATP analogs, ATPγS, α,β-met-ATP, ATPγS, and 2-MeSATP, also induce an increase in \([\text{Ca}^{2+}]_i\), while β,γ-met-ATP, β,γ-imido-ATP, AMP, and ADP are inactive (91, 116, 204, 389). In their study Christie et al. (91) noted that the ATP effect is biphasic with a large transient (1 min) peak followed by a weaker sustained \(\text{Ca}^{2+}\) increase. This was not reproduced by the other agonists, which furthermore could desensitize the ATP effect as they do for ATP (426). Thus, to summarize, ATP first activates \(I_{\text{ATP}}\) carried by various cations including \(\text{Ca}^{2+}\); \(I_{\text{ATP}}\) depolarizes the cell and triggers \(I_{\text{CaL}}\) followed by \(I_{\text{NaL}}\), so that tetrodotoxin could suppress the effect of ATP on \([\text{Ca}^{2+}]_i\) (41).

Increase in \(\text{Ca}^{2+}\) transient and contractile force might be also indirect, secondary to action potential prolongation following \(\text{K}^-\) current inhibition, or secondary to activation of the reverse mode of the \(\text{Na}^+/-\text{Ca}^{2+}\) exchanger following \(\text{Na}^+\) load. Thus arachidonic acid that is produced after ATP application inhibits \(I_{\text{CaL}}\); during the sub-

\[\text{FIG. 7. Regulation of intracellular pH (pH}_i\) by ATP. A: the sudden application of 10 μM ATP on a Snarf-1-loaded ventricular myocyte triggers first cell acidosis followed by alkalinosis. These effects are attributable in most part to the successive activation of the \(\text{Cl}^-/\text{HCO}_3^-\) exchanger and of the Na\(^+/-\text{H}^+\) antiport. [From Puce´ at et al. (390).] B: after blockade of the Na\(^+/-\text{H}^+\) antiport with ethylisopropylamiloride (EIPA), 10 μM ATP still accelerates the recovery from acidosis on a rat ventricular cell submitted to a NH\(_4\) pulse (20 mM for 1.5 min). This effect is attributed to the activation of the Na\(^+/-\text{Ca}^{2+}\) cotransport (467, 468). [From Tercic et al. (468). Copyright 1992 Springer-Verlag.]\]
The most remarkable effect on intracellular pH of a sudden application of micromolar extracellular ATP is a large (0.4 pH unit) and transient (1 min) acidosis that requires Cl\(^-\) in the extracellular milieu that has been attributed to the activation of the anionic Cl\(^-\)/HCO\(_3\)- exchanger (390). The activation of the exchanger is associated with a band 3-like protein phosphorylation on a tyrosine site (393, 394). More recent work has shown that isolated cardiomyocytes express both AE\(_1\) and AE\(_3\) isoforms. ATP induces tyrosine phosphorylation of AE\(_1\) (Fig. 6) while acidosis still occurs in cell in which AE\(_3\) expression was blocked. More precisely, ATP activates the tyrosine kinase Fyn and the association of both Fyn and FAK with AE\(_1\). Tyrosine kinase inhibitors and microinjection of either anti-Cst.1 antibody or recombinant COOH-terminal Src kinase (CSK), both of which prevent activation of Src kinases, significantly depress the ATP-induced activation of the anion exchanger. As well microinjection of an anti-FAK antibody directed against the domain of Fyn binding or overexpression of a dominant negative FAK (FAK\(_{397}\)) significantly blocks ATP-induced activation of the exchanger (394).

In conclusion, it is proposed that ATP by activating not-yet-identified receptors implicate both the alkalinizing ion exchangers, Na\(^+\)/H\(^+\) antiport and Na\(^+\)/HCO\(_3\)- co-transport, and the acidifying AE\(_1\) and thus provides the cell with a complementary functional buffering system bringing a beneficial protective way to face pH disturbances.

V. CONCLUDING REMARKS

The cloning of 7 P2X and 13 P2Y receptors during the last 5 years has considerably advanced our basic knowledge. However, the precise functions of those of these proteins which are expressed in cardiac myocytes are not clear. Not only is this due to the probable simultaneous expression of multiple receptors in each cardiomyocyte but also to the fact that P2X receptors can form complex homo- or heterotrimeric receptors whose activity differs with each oligomer (99, 131, 344). A similar complexity may also apply to P2Y receptors following the recent demonstration that G protein-coupled GAB\(_A\) receptors form heterodimers with specific and different properties compared with the individual homomeric receptors (246, 273). Analysis should also take into account that P2Y receptors might connect with only specific parts of an otherwise general pathway such as occurs with the coupling to isoform V of adenyl cyclase (387). Furthermore, as for other proteins, receptor phosphorylation can modulate channel activity that is shown by P2X\(_2\) receptors (90). In addition, some peculiar effects of ATP such as the activation of the tyrosine kinase pathways (387, 394) have yet to be associated with any particular purinergic receptor subtype. Therefore, there is still a long way to go to understand the behavior of integrated cellular P2 receptors from the expression of single cloned proteins. However, cloning and expression of these proteins are primary and necessary steps to develop efficient pharmacological tools, an urgent need in the face of the present paucity.

Several P2X mRNAs have been found in heart tissues; in as far as they are translated, the role of these proteins remains to be determined. As mentioned earlier, there is no clear P2X-related effect of ATP on cardiomyocytes despite \(I_{\text{ATP}}\) showing some broad similarities (rapid inactivation, nonselectivity, and cation permeability) with P2X-purinoceptor activation after reexpression. However, the three studies (91, 424, 536) in which the effects of Mg\(^{2+}\) were investigated report their need to elicit \(I_{\text{ATP}}\). That led these authors to suggest the involvement of ectoenzymes. This is at odds with the general belief that ATP\(^4\) is the preferred agonist of P2 purinoceptors. Mg\(^{2+}\) might also control receptor affinity; however, divalent and trivalent cations reduce ATP-activated currents in native and endogenous P2X receptors (91, 403, 424, 536), and Mg\(^{2+}\), in particular, have been shown to reduce P2X\(_2\) affinity to ATP (288). Mg\(^{2+}\) are also requested for the activation of the tyrosine kinase pathway and the Cl\(^-\)/HCO\(_3\)- exchanger. Moreover, in cardiomyocytes, it is noteworthy that ATP and its analogs modified on the purine base that are known good substrates for ecto-ATPases induce \(I_{\text{ATP}}\). The same compounds were also reported good agonist on P2Y\(_1\) purinoceptors. These effects were suppressed under conditions in which care was taken to purify the agonist solutions from contaminating ADP by treatment with a creatine phosphate/creatine phosphate kinase regenerative system. Similar control experiments need to be performed to determine the exact origin of \(I_{\text{ATP}}\).

ATP is released into the interstitial milieu from most cell types. UTP, by transphosphorylation and diadenosine polyphosphates, after degradation is another potential source of extracellular ATP. At the same time ATP is degraded to adenine nucleotides and nucleosides so that the response of the cardiomyocyte should be the integral of the various effects of these multiple compounds generated by the different surrounding tissues. Furthermore, under pathological conditions, this process could be accelerated by the macrophages and lymphocytes that have high ecto-ATPase activities and which invade the interstitial space. Also, upregulation of the enzymatic chain hydrolyzing extracellular ATP after ischemia could be expected in cardiac tissues as was observed in the forebrain after transient occlusion of brain arteries (63). Cardiomyocytes also exhibit both autocrine and paracrine purinergic activities. In this context, it has been reported that ATP might mediate the cell-to-cell spread of the Ca\(^{2+}\) signal in the absence of gap-junctional coupling such as occurs in mast cells or astrocytes (188, 356). Cardiac muscle is considered to be a syncytium. However, the
spread of electrical activation should be reduced in the presence of ATP both by $I_{Na}$ depression and cell uncoupling after intracellular Ca\(^{2+}\) release and acidosis. In these circumstances, anomalous activity might occur and propagate thanks to ATP diffusion and P2X-receptor activation that leads to cell depolarization and contraction, stretching of the neighboring cells, and further ATP release.

At this point it should be clarified that ATP is an agonist with both physiological and pathological effects. The first include positive or negative inotropy and chronotropy. These effects seem to be mostly mediated by P2Y receptors. However, when applied abruptly, ATP induces acidosis, cell depolarization, and arrhythmia mostly by acting through P2X receptors. It is noteworthy that most extracellular effects of ATP occur in the micromolar range, at a concentration at least 1,000-fold less than for its intracellular metabolic effects. Such a variation in stimulation profiles is not generally recognized for agonists acting within the same concentration range on a tissue with the same physiological status. Other possible roles of ATP concern various physiopathological aspects such as hypertrophy, preconditioning, and apoptosis. The latter was early mentioned by Brake et al. (60) who reported the extensive sequence similarity between P2X2 and the partial cDNA called RP-2 and isolated in immature thymocytes undergoing programmed cell death. More recently, apoptosis of dendritic cells was shown to depend on P2X\(_7\)/P2X receptor by a pathway, in most part, independent of known caspases (109). A common feature to these receptors is the increase in [Ca\(^{2+}\)]\(_i\) following their activation. Apoptosis is a process well established in cardiac cells under stress but for which an involvement of ATP remains to be elucidated. Few studies have investigated the presence of P2X receptors on isolated cardiomyocytes rather than on membranes obtained from the whole heart, but it is precisely this which is required to extend our knowledge of the change in P2-receptor subtype expression with age (47, 48, 511) or pathological conditions (216). These studies used PCR protocols. Very few antibodies are currently available. Further research will benefit from the recent developments in genome-based methods: expressed sequence tags, DNA microarrays, mRNA differential displays, or the serial analysis of gene expression. The acquired information about developmental and compensatory mechanisms are important to design possible pharmacological treatments.

Finally, Ap\(_\alpha\)As are a new class of signaling molecules that act both intra- and extracellularly to alter both cardiac chronotropy and inotropy. The specificity of diadenosine phosphate receptors and their signal transduction pathways are still poorly understood. Furthermore, Ap\(_\alpha\)As slowly formed nucleotides and nucleosides that further multiply the difficulties of analysis. Cloning of these receptors and detailed studies after reexpression are prerequisite to better understand the roles of Ap\(_\alpha\)As in cardiac tissues.

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