Biochemical Pharmacology, Vol. 54, pp. 219–225, 1997.

ISSN 0006-2952/97/$17.00 + 0.00
PII S0006-2952(97)00262-1

COMMENTARY

Intracellular Diadenosine Polyphosphates

A NOVEL FAMILY OF INHIBITORY LIGANDS OF THE ATP-SENSITIVE K^+ CHANNEL

*Aleksandar Jovanovic, Alexey E. Alekseev and Andre Terzic**

DIVISION OF CARDIOVASCULAR DISEASES AND CLINICAL PHARMACOLOGY UNIT, DEPARTMENTS OF MEDICINE AND PHARMACOLOGY, MAYO CLINIC AND FOUNDATION, ROCHESTER, MN 55905, U.S.A.

ABSTRACT. Intracellular diadenosine polyphosphates (Ap_nA) are signal molecules that alert the cell under stress conditions. Herein, we review evidence that has recently identified a novel target for Ap_nA, namely the ATP-sensitive K^+ (K_{ATP}) channel. These channels are abundant in pancreatic β-cells and cardiac myocytes where they are essential in coupling the cellular metabolic state with membrane excitability. The potency and efficacy of Ap_nA to inhibit K_{ATP} channel activity were first described in cardiac K_{ATP} channels, and appear similar to those of intracellular ATP, the primary ligand of K_{ATP} channels. Also, the inhibitory ligand action of Ap_nA is dependent upon the operative condition of K_{ATP} channels and the presence of nucleotide diphosphates. In addition to a direct antagonism of channel opening, an indirect effect of Ap_nA on K_{ATP} channel activity has been associated with inhibition of adenylate kinase, a catalytic system believed essential for the regulation of channel opening. At present, the precise role for Ap_nA-induced K_{ATP} channel inhibition remains to be established. Yet, it is known that, under glucose challenge of pancreatic β-cells, intracellular concentrations of Ap_nA do increase to micromolar levels necessary to block K_{ATP} channels, leading to insulin secretion. Thus, the Ap_nA-mediated K_{ATP} channel gating represents a previously unrecognized pathway of channel regulation. BIOCHEM PHARMACOL 54;2:219–225, 1997. © 1997 Elsevier Science Inc.

KEYWORDS. adenylate kinase; alarmone; dinucleotide polyphosphates; glucose; K_{ATP} channels; stress

Intracellular Ap_nA† (where n = 2–6; Fig. 1) have emerged as important intracellular signal molecules in cells under stress [1, 2]. As Ap_nA are synthesized during metabolic challenge and can act homeostatically under stress conditions, these molecules, particularly Ap_4A, have been termed putative "alarmones" [3]. Specifically, during oxidative and heat shock stress, cytosolic Ap_4A rapidly rises in concentration [4, 5] and binds to several heat shock and oxidative stress proteins to inhibit their activities [6]. In several cell types, a direct intracellular effect of Ap_nA has been demonstrated on enzymes with nucleotide-binding domains, which are associated with cellular metabolism [1, 2].

Recently, a novel target for Ap_nA has been uncovered—the K_{ATP} channel [7]. Thus far, an inhibitory effect of Ap_nA has been determined on cardiac [8, 9] and pancreatic K_{ATP} channels [10], where these signaling molecules may serve a novel role in the regulation of K_{ATP} channel behavior.

It is well established that K_{ATP} channels are present at high density in cardiac myocytes and pancreatic β-cells where they link membrane excitability with the cellular bioenergetic state [11–15]. A defining attribute of K_{ATP} channels is their regulation by intracellular mononucleotide polyphosphates, primarily ATP [13, 16, 17]. In pancreatic β-cells, K_{ATP} channels mediate glucose-induced insulin secretion. When the glucose concentration in serum is high, intracellular ATP inhibits the opening of K_{ATP} channels, which in turn depolarizes the cellular membrane, and promotes Ca^{2+} influx, triggering insulin release [18, 19]. In the heart, K_{ATP} channels have been implicated in the shortening of the action potential duration and in cellular loss of K^+ that occurs during metabolic stress and hypoxia [20, 21]. During early ischemia, opening of K_{ATP} channels may promote cardiac arrhythmias [22, 23], although opening of K_{ATP} channels has also been implicated in the cardioprotective mechanism of ischemic preconditioning [24, 25].

This synopsis summarizes the actions of Ap_nA on K_{ATP} channels, as these intracellular molecules now emerge as a novel regulator family of channel gating.

AP_NA: DIRECT INHIBITORS OF K_{ATP} CHANNEL OPENING

Ligand Effect of Ap_nA on K_{ATP} Channels in Excised Membrane Patches

That Ap_nA act as inhibitory ligands of K_{ATP} channels was first described for Ap_6A in membrane patches excised from cardiac cells [7] (Fig. 2). Further investigation, using the

* Correspondence. Tel. (507) 284-2747; FAX (507) 284-9111; E-mail: terzic.andre@mayo.edu

† *Abbreviations:* Ap_nA, diadenosine polyphosphates; Ap_4A, diadenosine tetraphosphate; Ap_6A, diadenosine hexaphosphate; Ap_3A, diadenosine triphosphate; Ap_5A, diadenosine pentaphosphate; and K_{ATP}, ATP-sensitive K^+.

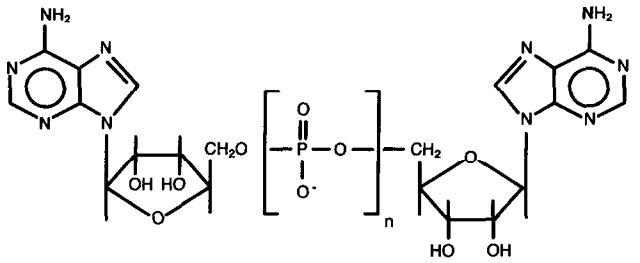

FIG. 1. General structure of Ap_nA, which are dinucleotides composed of two adenosine moieties linked through ribose 5′-carbons to the phosphate group chain. The number of phosphate groups (n) commonly varies from 2 to 6.

TABLE 1. Summary of the IC_{50} and Hill coefficient values defining the concentration-dependent inhibition of cardiac and pancreatic K_{ATP} channels by Ap_nA*

	Cardiac		Pancreatic	
	IC_{50} (μM)	Hill coefficient	IC_{50} (μM)	Hill coefficient
Ap_3A	ND†	ND	74	ND
Ap_4A	17	1.2	17	1.2
Ap_5A	16	1.6	ND	ND
Ap_6A	14	1.1	ND	ND

*Data from Refs. 7–10.
†ND = not determined.

inside-out configuration of the patch-clamp technique, indicated that, in addition to Ap_6A, micromolar concentrations of other Ap_nA, such as Ap_4A, as well as Ap_3A and Ap_5A inhibit K_{ATP} channel opening in a reversible manner [8–10, 26] (Fig. 2 and Table 1).

The idea that led to the discovery of Ap_nA-mediated inhibition of K_{ATP} channels was based on the previous observation that these molecules inhibit the activity of certain cytosolic nucleotide-binding enzymes through interaction with the intracellular ATP and/or AMP binding sites of targeted proteins [2, 27]. As the K_{ATP} channel structure also possesses nucleotide-binding domains [28–32], it was conceivable that Ap_nA could affect the behavior of K_{ATP} channels. Indeed, application of Ap_nA to the intracellular side of myocardial and pancreatic β-cell membrane patches decreased the probability of K_{ATP} channel opening [7–10, 26].

The site of action of Ap_nA is most likely intracellular, as these molecules are poorly membrane-permeable [2]. In addition, the effect of Ap_nA occurs in the absence of GTP [7–10], eliminating the possibility of the involvement of a GTP-binding protein in the transduction of the inhibitory effect of Ap_nA on K_{ATP} channels. The concentration-dependence and saturable nature of the action of Ap_nA on K_{ATP} channel opening (Fig. 3) suggest the existence of specific binding sites for Ap_nA within K_{ATP} channel subunits or a closely associated protein.

Regardless of the length of the phosphate chain, the potency of various Ap_nA, e.g. Ap_4A, Ap_5A, and Ap_6A, to inhibit K_{ATP} channels is within a close micromolar range (Table 1). This is in contrast with a variable affinity displayed by members of the Ap_nA family in binding to other nucleotide-binding proteins, such as adenosine kinase [33], adenylate kinase [27, 34, 35], terminal deoxynucleotidyl transferase and carbamyl phosphate synthase [36]. For

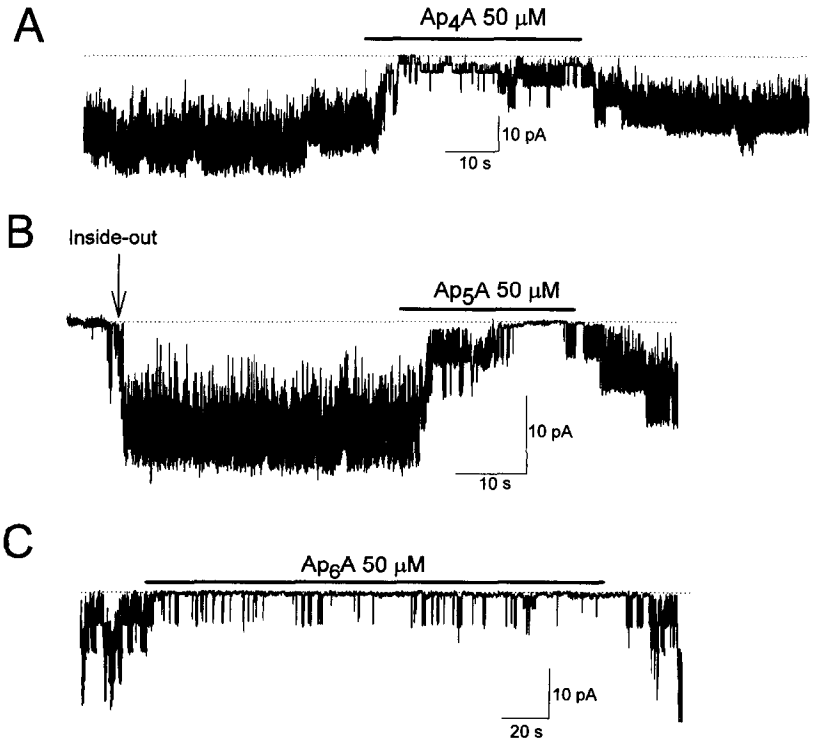

FIG. 2. Diadenosine tetra- (A), penta- (B), and hexaphosphate (C) (Ap_nA, n = 4–6)-induced inhibition of myocardial K_{ATP} channels. The patch-clamp technique was employed in the inside-out configuration. Holding potential: −60 mV. Modified from Refs. 7–9.

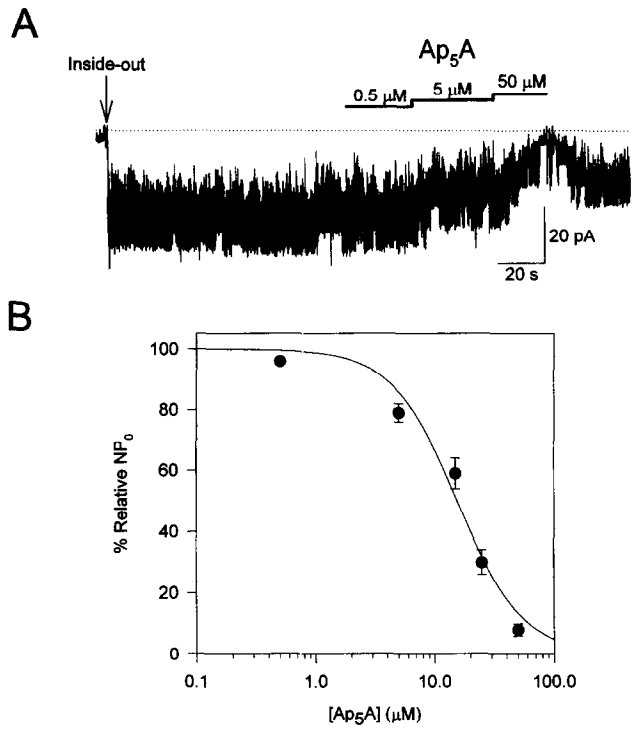

FIG. 3. Concentration-dependent effect of Ap_5A-induced inhibition of myocardial K_{ATP} channels. The patch-clamp technique was employed in the inside-out configuration. Holding potential: −60 mV. (A) Original record. (B) Concentration–response curve fitted by the Hill equation. Channel activity is expressed as NP_o, where N represents the number of channels and P_o the probability for channels to be open. Values, expressed as means ± SEM, are from 3–6 membrane patches for each data point. Slightly modified from Ref. 9. Reprinted with permission from *Naunyn-Schmiedebergs Arch Pharmacol* 353: 241–244, 1996. Copyright (1996) Springer-Verlag GmbH & Co. KG.

example, Ap_4A inhibits the activity of adenosine kinase [33], a property not shared by other members of the Ap_nA family. Despite their different selectivity toward other nucleotide-binding proteins and their different affinities for the ATP-binding site located on these proteins, it appears that the property of Ap_nA to inhibit K_{ATP} channels is common to all Ap_nA so tested.

Operative Condition-Dependent Regulation of K_{ATP} Channels by Ap_nA

K_{ATP} channels can function under two operative conditions: operative condition 1, when K_{ATP} channel activity is sustained spontaneously and not enhanced by nucleotide diphosphates, versus operative condition 2, when after the decline of spontaneous channel activity ("run-down") nucleotide diphosphates restore channel activity [15, 17, 37]. The effect of Ap_4A and Ap_5A on K_{ATP} channels under different operative conditions was tested in membrane patches excised from ventricular cardiac cells [26]. Under operative condition 1, application of nucleotide diphosphates prevented the Ap_4A- and Ap_5A-induced inhibition of spontaneous K_{ATP} channel activity (Fig. 4). In contrast, under operative condition 2, the nucleotide diphosphate-induced K_{ATP} channel openings were inhibited by Ap_4A (Fig. 4) or Ap_5A. Thus, the outcome of the interaction between an Ap_nA and nucleotide diphosphates on K_{ATP} channel opening is not constant but changes depending on the operative condition of the channel. The mechanism responsible for the switch in the responsive behavior of K_{ATP} channels toward a ligand is unknown. Phosphorylation of the channel protein has been suggested as a possible candidate [26, 37], as it is for the mechanism underlying the switch between operative conditions 1 and 2 [15, 16]. The K_{ATP} channel protein complex possesses several nucleotide binding and phosphorylation sites [29, 30] that could be involved in regulating the interaction of Ap_nA with K_{ATP} channels.

Comparison of the Effects of ATP and Ap_nA on K_{ATP} Channels

ATP and Ap_nA inhibit the opening of K_{ATP} channels with a similar micromolar potency (Table 2). The relationship between the concentration of ATP or Ap_nA on channel activity could be fitted by a Hill equation with similar values for the apparent Hill coefficient (Table 2). However, the precise number of molecules required to close the channel remains to be determined. Neither ATP nor Ap_nA affect the amplitude of the single channel current, but they decrease the probability

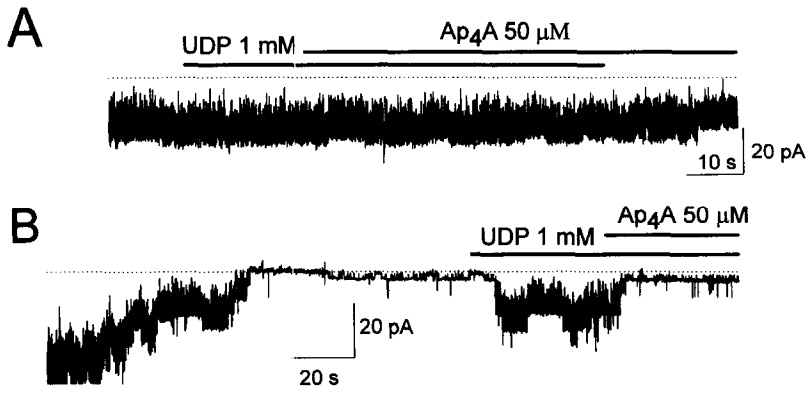

FIG. 4. Operative condition-dependent responsiveness of myocardial K_{ATP} channels to Ap_4A. The patch-clamp technique was employed in the inside-out configuration. Holding potential: −60 mV. (A) Operative condition 1 (spontaneous channel opening). (B) Operative condition 2 (nucleotide diphosphate-UDP-induced channel opening after "run-down"). Under operative condition 1, Ap_4A did not inhibit K_{ATP} channel activity in the presence of UDP. Under operative condition 2, Ap_4A did inhibit K_{ATP} channel activity in the presence of UDP. Modified from Ref. 26.

TABLE 2. Comparison of the effects of ATP and Ap_nA (n = 4–6) on myocardial K_{ATP} channels*

	Channel inhibition			Channel maintenance
	IC_{50} (μM)	Hill coefficient	Operative condition-dependent	
ATP	24–30	1.7–1.8	Yes (with UDP)	Yes (with Mg^{2+})
Ap_nA	14–17	1.1–1.6	Yes (with UDP)	No

*Data from Refs. 7–9, 26, and 37–40.

of channel opening, most likely by apparently reducing the time the channel spends in a burst [8–10, 13].

As described above for Ap_nA, the outcome of interaction between ATP and K_{ATP} channels is governed by the operative condition of the channel and the presence of nucleotide diphosphates [15, 37]. Thus, the interaction of K_{ATP} channels with both Ap_nA and ATP provides further evidence that the basal state of the effector system governs the outcome of a stimulus [37, 41, 42].

In contrast to ATP, which serves not only as a ligand to close the K_{ATP} channel but also maintains channel activity [12–16], Ap_nA did not promote sustained channel activity. The maintenance of channel activity by ATP can be observed after removal of this nucleotide, and is the exclusive property of the Mg^{2+} bound form of ATP (MgATP), and is not shared by non-hydrolyzable analogs of ATP. Since both Mg^{2+} and ATP hydrolysis are necessary for kinase activity, maintenance of K_{ATP} channel activity may relate to an enzyme-dependent action of ATP, such as phosphorylation. When K_{ATP} channels are in "run-down," treatment of membrane patches with MgATP reactivates spontaneous channel activity [13, 15, 16]. In contrast, Ap_nA do not reverse channel activity after "run-down" [8–10]. Thus, Ap_nA possess a "pure" inhibitory property on K_{ATP} channel opening, without having the ability to maintain channel opening. In this regard, the action of Ap_nA on K_{ATP} channels mostly resembles that observed with non-hydrolyzable analogs of ATP, such as ATPγS.

AP_NA: INDIRECT REGULATORS OF K_{ATP} CHANNEL BEHAVIOR?

The possibility that certain Ap_nA could regulate K_{ATP} channel activity through an indirect mechanism has been indicated recently in cardiomyocytes [43]. As has been already mentioned, the defining property of K_{ATP} channels is their inhibition by ATP, as is the effect of ADP to reverse the ATP-inhibited state [12, 13, 15–17]. However, the mechanism by which opening of K_{ATP} channels is governed *in situ* remains undetermined, and the conventional assumption that a fall in the cytosolic concentration of ATP determines channel opening has been contested [20]. The cytosolic concentration of ATP remains, even under metabolic stress, over 100-fold higher than the value required for K_{ATP} channel closure. Therefore, additional mechanisms must allow K_{ATP} channels to open in the presence of millimolar concentrations of cytosolic ATP. Recent studies suggest that the microenvironment surrounding K_{ATP} channels may regulate the ATP-dependent gating of these channels [38, 44]. Furthermore, evidence has been presented that transition from the closed (ATP-liganded) to the open (ADP-liganded) state of K_{ATP} channels is possible through catalytic transformation of ATP into ADP within the microenvironment surrounding K_{ATP} channels [43]. Such transition could be mediated through adenylate kinase activity, known to catalyze the transformation of ATP into ADP [43, 45–47]. Thus, adenylate kinase emerges as an important determinant of the composition of adenine nucleotide species at the K_{ATP} channel site. Of importance, Ap_nA, in particular Ap_5A, are potent blockers of adenylate kinase activity [27]. Indeed, Ap_5A inhibited the adenylate kinase-mediated transition of K_{ATP} channels from the closed to the open state [43] (Fig. 5). Such an effect of Ap_5A was observed at concentrations that were not sufficient to directly inhibit K_{ATP} channel activity, in accord with the high affinity of adenylate kinase for Ap_5A [27, 43]. Therefore, it is apparent that besides a direct action on K_{ATP} channels, Ap_5A may modulate the opening behavior of these channels through an indirect mechanism, i.e. through inhibition of adenylate kinase-dependent channel gating. At present, such indirect regulation of K_{ATP} channel behavior has been reported only for Ap_5A.

ROLE FOR AP_NA IN REGULATING K_{ATP} CHANNEL BEHAVIOR

Under resting conditions, the intracellular concentration of Ap_nA (<1 μM) is at least 10,000-fold lower than the concentration of ATP [1, 2]. However, it is established that after cell exposure to various oxidants or metabolic stress, cytosolic concentrations of Ap_nA could rise to micromolar levels [1, 2]. Furthermore, as described above, Ap_nA may act as alarmones to alert the cell to the onset of metabolic stress [3]. In pancreatic β-cells, evidence has been obtained that the Ap_nA-mediated regulation of K_{ATP} channels may be of importance in glucose-triggered insulin secretion [10]. It was shown that a glucose challenge of a pancreatic β-cell induces an important increase of the cytosolic concentra-

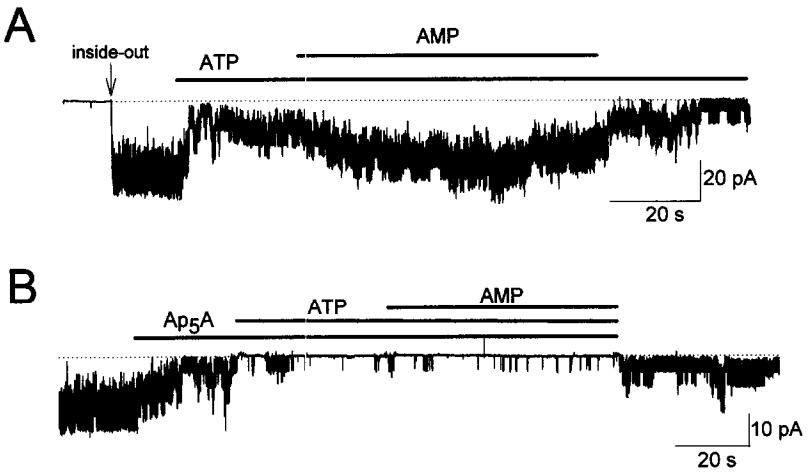

FIG. 5. Prevention by Ap_5A of the opening of myocardial K_{ATP} channels induced by substrates (AMP and ATP) of adenylate kinase. The patch-clamp technique was employed in the inside-out configuration. Holding potential: −60 mV. (A) Adenylate kinase-mediated activation of ATP-inhibited channels by AMP. (B) Prevention by Ap_5A of the opening of the K_{ATP} channel by the two substrates of adenylate kinase (ATP and AMP). Modified from Ref. 40.

tion of Ap_nA to micromolar concentrations sufficient to inhibit K_{ATP} channel opening [10]. By analogy to pancreatic β-cells, it is conceivable that under stress conditions the concentration of Ap_nA may also rise in other tissues sufficient to gate K_{ATP} channel opening. This would be of importance due to the critical cellular functions with which K_{ATP} channels have been associated, including vasodilation, skeletal muscle contraction, and neuroprotection [12–17, 48–51]. In particular, a role for Ap_nA in the regulation of K_{ATP} channels in the heart may be significant since the myocardium is under constant metabolic stress. Activation of K_{ATP} channels does appear to be protective against myocardial infarction [24, 25, 52], while it has also been reported that opening of K_{ATP} channels is associated with cellular loss of K^+ during ischemia and hypoxia with possible proarrhythmic consequences [20, 23].

An important question to be resolved is why the K_{ATP} channel system would rely on two inhibitory signaling pathways, the conventional ATP-dependent and the now described Ap_nA-dependent inhibition. It is tempting to speculate that under resting conditions in cardiac cells, for example, K_{ATP} channels would be closed by high cytosolic levels of ATP, while the concentration of Ap_nA would be too low to affect channel activity. Under metabolic stress with the decrease in the cytosolic concentration of ATP, as well as an associated adenylate kinase-mediated opening of K_{ATP} channels [43], intracellular levels of Ap_nA may rise to directly impede channel opening and/or block adenylate kinase-mediated channel opening. Thus, the role of Ap_nA may lie in counteracting the consequence of decreased intracellular ATP concentration under metabolic stress.

CONCLUDING REMARKS

Intracellular Ap_nA are signal molecules proposed to act homeostatically under stress conditions. Herein, we present evidence identifying Ap_nA as inhibitors of K_{ATP} channels, an important ion conductance that couples the metabolic state of a cell with membrane excitability.

The overall inhibitory profile of Ap_nA appears similar to that of intracellular ATP, the primary ligand of K_{ATP} channels. However, the dual ability of ATP to both block as well as maintain K_{ATP} channel activity is not shared by Ap_nA. Thus, the inhibitory activity of Ap_nA mostly resembles that reported for non-hydrolyzable analogs of ATP.

At present, the mechanism through which Ap_nA inhibit K_{ATP} channels is unknown. Two possible mechanisms for Ap_nA-induced channel inhibition have been proposed: (1) a direct ligand inhibitory effect, probably mediated through binding to a specific binding site on a K_{ATP} channel subunit or on an associated protein, and/or (2) an indirect effect mediated through inhibition of adenylate kinase-mediated channel regulation. A particular feature of the direct inhibitory action of Ap_nA is its dependence upon the operative condition of K_{ATP} channels and the presence of nucleotide diphosphates. The indirect effect through inhibition of adenylate kinase may occur at a slightly lower concentration of Ap_nA than the direct ligand inhibitory effect.

The precise role related to the Ap_nA-induced K_{ATP} channel inhibition remains to be determined. It is known, however, that the intracellular concentration of Ap_nA does increase to levels sufficient to gate K_{ATP} channels under cellular challenges. Of particular importance is the ability of glucose to induce a rise in the cytosolic levels of Ap_nA within pancreatic β-cells, which in turn may play an important role in the K_{ATP} channel-dependent regulation of insulin secretion. Other roles for the Ap_nA-dependent K_{ATP} channel gating await to be identified.

In summary, this synopsis reviews the evidence for a previously unrecognized property of Ap_nA, as well as the discovery of a novel family of endogenous ligands gating K_{ATP} channel behavior.

The work performed in the laboratory of the authors was supported through grants from the American Heart Association and the Miami Heart Research Institute. A. J. is the recipient of a Merck Sharp & Dohme International Fellowship in Clinical Pharmacology.

References

1. Baxi MD and Vishwanatha JK, Diadenosine polyphosphates: Their biological and pharmacological significance. *J Pharmacol Methods* **33:** 121–128, 1995.
2. Yakovenko IN and Formazuyk VE, Diadenosine oligophosphates: Metabolic pathways and role in regulation of the functional activity of cells. *Biokhimiia* **58:** 3–24, 1993.
3. Varshavsky A, Diadenosine 5′-5″-P^1, P^4-tetraphosphate: A pleiotropically acting alarmone? *Cell* **34:** 711–712, 1983.
4. Baker JC and Jacobson MK, Alteration of adenyl dinucleotide metabolism by environmental stress. *Proc Natl Acad Sci USA* **83:** 2350–2352, 1986.
5. Lee PC, Bochner BR and Ames BN, AppppA, heat shock stress, and cell oxidation. *Proc Natl Acad Sci USA* **80:** 7496–7500, 1983.
6. Johnstone DB and Farr SB, AppppA binds to several proteins in *Escherichia coli*, including the heat shock and oxidative stress proteins DnaK, GroEL, E89, C45 and C40. *EMBO J* **10:** 3897–3904, 1991.
7. Jovanović A and Terzic A, Diadenosine-hexaphosphate is an inhibitory ligand of myocardial ATP-sensitive K^+ channels. *Eur J Pharmacol* **286:** R1–R2, 1995.
8. Jovanovic A and Terzic A, Diadenosine tetraphosphate-induced inhibition of ATP-sensitive K^+ channels in patches excised from ventricular myocytes. *Br J Pharmacol* **117:** 233–235, 1996.
9. Jovanovic A, Alekseev AE and Terzic A, Cardiac ATP-sensitive K^+ channel: A target for diadenosine 5′,5″-P^1,P^5-pentaphosphate. *Naunyn Schmiedebergs Arch Pharmacol* **353:** 241–244, 1996.
10. Ripoll C, Martin F, Rovira JM, Pintor J, Miras-Portugal MT and Soria B, Diadenosine polyphosphates. A novel class of glucose-induced intracellular messengers in the pancreatic β-cell. *Diabetes* **45:** 1431–1434, 1996.
11. Noma A, ATP-regulated K^+ channels in cardiac muscle. *Nature* **305:** 147–148, 1983.
12. Ashcroft SJH and Ashcroft FM, Properties and functions of ATP-sensitive K^+-channels. *Cell Signal* **2:** 197–214, 1990.
13. Nichols CG and Lederer WJ, Adenosine triphosphate-sensitive potassium channels in the cardiovascular system. *Am J Physiol* **261:** H1675–H1686, 1991.
14. Lazdunski M, ATP-sensitive potassium channels: An overview. *J Cardiovasc Pharmacol* **24:** S1–S5, 1994.
15. Terzic A, Jahangir A and Kurachi Y, Cardiac ATP-sensitive K^+ channels: Regulation by intracellular nucleotides and K^+ channel-opening drugs. *Am J Physiol* **269:** C525–C545, 1995.
16. Findlay I, Interactive regulation of the ATP-sensitive potassium channel of cardiac muscle. *J Cardiovasc Pharmacol* **24:** S6–S11, 1994.
17. Terzic A, Tung RT and Kurachi Y, Nucleotide regulation of ATP sensitive potassium channels. *Cardiovasc Res* **28:** 746–753, 1994.
18. Ashcroft FM, Harrison D and Ashcroft SJH, Glucose induces closure of single potassium channels in isolated rat pancreatic β-cells. *Nature* **312:** 446–448, 1984.
19. Cook DL and Hales CN, Intracellular ATP directly blocks K^+ channels in pancreatic B-cells. *Nature* **311:** 271–273, 1984.
20. Weiss JN and Venketesh N, Metabolic regulation of cardiac ATP-sensitive K^+ channels. *Cardiovasc Drugs Ther* **7:** 499–505, 1993.
21. Findlay I, The ATP-sensitive K^+ channel of cardiac muscle and action potential shortening during metabolic stress. *Cardiovasc Res* **28:** 760–761, 1994.
22. Gasser RNA and Vaughan-Jones RD, Mechanism of potassium efflux and action potential shortening during ischemia in isolated mammalian cardiac muscle. *J Physiol (Lond)* **431:** 713–741, 1990.
23. Wilde AAM and Janse MJ, Electrophysiological effects of ATP-sensitive potassium channel modulation: Implications for arrhythmogenesis. *Cardiovasc Res* **28:** 16–24, 1994.
24. Gross GJ, ATP-sensitive potassium channels and myocardial preconditioning. *Basic Res Cardiol* **90:** 85–88, 1995.
25. Grover GJ, Protective effects of ATP sensitive potassium channel openers in models of myocardial ischaemia. *Cardiovasc Res* **28:** 778–782, 1994.
26. Jovanovic A, Zhang S, Alekseev AE and Terzic A, Diadenosine polyphosphate-induced inhibition of cardiac K_{ATP} channels: Operative state-dependent regulation by a nucleoside diphosphate. *Pflugers Arch* **431:** 800–802, 1996.
27. Lienhard GE and Secemski II, P^1,P^5-Di(adenosine-5′)pentaphosphate, a potent multisubstrate inhibitor of adenylate kinase. *J Biol Chem* **248:** 1121–1123, 1973.
28. Aguilar-Bryan L, Nichols CG, Wechsler SW, Clement JP IV, Boyd AE III, Gonzáles G, Herrera-Sosa H, Nguy K, Bryan J and Nelson DA, Cloning of the β cell high-affinity sulfonylurea receptor: A regulator of insulin secretion. *Science* **268:** 423–426, 1995.
29. Inagaki N, Gonoi T, Clement JP IV, Namba N, Inazawa J, Gonzales G, Aguilar-Bryan L, Seino S and Bryan J, Reconstitution of I_{KATP}: An inward rectifier subunit plus the sulfonylurea receptor. *Science* **270:** 1166–1170, 1995.
30. Inagaki N, Gonoi T, Clement JP, Wang CZ, Aguilar-Bryan L, Bryan J and Seino S, A family of sulfonylurea receptors determines the pharmacological properties of ATP-sensitive K^+ channels. *Neuron* **16:** 1011–1017, 1996.
31. Nichols CG, Shyng S-L, Nestorowicz A, Glaser B, Clement JP IV, Gonzales G, Aguilar-Bryan L, Permutt MA and Bryan J, Adenosine diphosphate as an intracellular regulator of insulin secretion. *Science* **272:** 1785–1787, 1996.
32. Isomoto S, Kondo C, Yamada M, Matsumoto S, Higashiguchi O, Horio Y, Matsuzawa Y and Kurachi Y, A novel sulfonylurea receptor forms with BIR (Kir6.2) a smooth muscle type ATP-sensitive K^+ channel. *J Biol Chem* **271:** 24321–24324, 1996.
33. Bone R, Cheng YC and Wolfenden R, Inhibition of adenosine and thymidylate kinases by bisubstrate analogues. *J Biol Chem* **261:** 16410–16413, 1986.
34. Bachmaier A, Just G and Holler E, *In vitro* competition between adenosine(5′)tetraphospho(5′)adenosine and deoxyribonucleic acid in the reaction with diamminedichloroplatinum(II). *Eur J Biochem* **161:** 621–627, 1986.
35. Rodriguez del Castillo A, Torres M, Delicado EG and Miras-Portugal MT, Subcellular distribution studies of diadenosine polyphosphates—Ap_4A and Ap_5A—in bovine adrenal medulla: Presence in chromaffin granules. *J Neurochem* **51:** 1696–1703, 1988.
36. Powers SG, Griffith OW and Meister A, Inhibition of carbamyl phosphate synthetase by P^1,P^5-di(adenosine 5′)-pentaphosphate: Evidence for two ATP binding sites. *J Biol Chem* **252:** 3558–3560, 1977.
37. Terzic A, Findlay I, Hosoya Y and Kurachi Y, Dualistic behavior of ATP-sensitive K^+ channels toward intracellular nucleoside diphosphates. *Neuron* **12:** 1049–1058, 1994.
38. Terzic A and Kurachi Y, Actin microfilament disrupters enhance K_{ATP} channel opening in patches from guinea-pig cardiomyocytes. *J Physiol (Lond)* **492:** 395–404, 1996.
39. Terzic A, Jahangir A and Kurachi Y, HOE-234, a second generation K^+ channel opener, antagonizes the ATP-dependent gating of cardiac ATP-sensitive K^+ channels. *J Pharmacol Exp Ther* **268:** 818–825, 1994.
40. Terzic A, Tung RT, Inanobe A, Katada T and Kurachi Y, G proteins activate ATP-sensitive K^+ channels by antagonizing ATP-dependent gating. *Neuron* **12:** 885–893, 1994.
41. Brady PA, Zhang S, López JR, Jovanovic A, Alekseev AE and Terzic A, Dual effect of glyburide, an antagonist of K_{ATP}

channels, on metabolic inhibition-induced Ca^{2+} loading in cardiomyocytes. *Eur J Pharmacol* **308:** 343–349, 1996.
42. Jovanovic A, Lopez JR and Terzic A, Cytosolic Ca^{2+} domain-dependent protective action of adenosine in cardiomyocytes. *Eur J Pharmacol* **298:** 63–69, 1996.
43. Elvir-Mairena JR, Jovanovic A, Gomez LA, Alekseev AE and Terzic A, Reversal of the ATP-liganded state of ATP-sensitive K^+ channels by adenylate kinase activity. *J Biol Chem* **271:** 31903–31908, 1996.
44. Brady PA, Alekseev AE, Aleksandrova LA, Gomez LA and Terzic A, A disrupter of actin microfilaments impairs sulfonylurea-inhibitory gating of cardiac K_{ATP} channels. *Am J Physiol* **271:** H2710–H2716, 1996.
45. Zeleznikar RJ, Dzeja PP and Goldberg ND, Adenylate kinase-catalyzed phosphoryl transfer couples ATP utilization with its generation by glycolysis in intact muscle. *J Biol Chem* **270:** 7311–7319, 1995.
46. Dzeja PP, Zeleznikar RJ and Goldberg ND, Suppression of creatine kinase-catalyzed phosphotransfer results in increased phosphoryl transfer by adenylate kinase in intact skeletal muscle. *J Biol Chem* **271:** 12847–12851, 1996.
47. Olson LK, Schroeder W, Robertson RP, Goldberg ND and Walseth TF, Suppression of adenylate kinase catalyzed phosphotransfer precedes and is associated with glucose-induced insulin secretion in intact HIT-T15 cells. *J Biol Chem* **271:** 16544–16552, 1996.
48. Edwards G and Weston AH, The role of potassium channels in excitable cells. *Diabetes Res Clin Pract* **28:** S57–S66, 1995.
49. Jovanovic A, Gojkovic LJ, Kazic T, Grbovic L and Tulic I, Relaxation of human uterine artery in response to pinacidil: Predominant role for ATP-dependent potassium channels. *Arch Int Pharmacodyn Ther* **327:** 344–354, 1994.
50. Nelson MT and Quayle JM, Physiological roles and properties of potassium channels in arterial smooth muscle. *Am J Physiol* **268:** C799–C822, 1995.
51. Ohno-Shosaku T and Yamamoto C, Identification of an ATP-sensitive K^+ channel in rat cultured cortical neurons. *Pfluegers Arch* **422:** 260–266, 1992.
52. Escande D and Cavero I, K^+ channel openers and "natural" cardioprotection. *Trends Pharmacol Sci* **13:** 269–272, 1992.