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Diadenosine-hexaphosphate is an inhibitory ligand of myocardial ATP-sensitive K^+ channels

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Abstract

P^1P^6-di(adenosine-5')hexaphosphate (Ap_6A) has been recently demonstrated in mammalian cells, yet its function remains unknown. Using single-channel current recordings, we studied the effect of Ap_6A on guinea-pig cardiac ATP-sensitive K^+ (K_{ATP}) channels. When applied to the intracellular side of excised membrane patches, Ap_6A produced a concentration-dependent inhibition (half-maximal inhibitory concentration: 14 μM; Hill coefficient: 1.1) of K_{ATP} channel activity. We conclude that Ap_6A is a potent inhibitory ligand of K_{ATP} channels.

Keywords: Diadenosine hexaphosphate; ATP-sensitive K^+ channel; Heart

Recently, the existence of dinucleotide polyphosphates, such as diadenosine tetra-, penta-, and hexaphosphate, has been demonstrated in the cytosol of mammalian cells (Baxi and Vishwanatha, 1995). While the extracellular effects of dinucleotide polyphosphates, in particular the regulation of vascular tone and platelet aggregation, has been clearly shown (Schluter et al., 1994), the intracellular function of these ATP-related nucleotides remains rather obscure (Yakovenko and Formazyuk, 1993). Tentatively, the intracellular actions of diadenosine tetraphosphate and pentaphosphate have been related to the regulation of cell cycle, and the inhibition of adenylate kinase (Yakovenko and Formazyuk, 1993; Baxi and Vishwanatha, 1995). Yet, there is no detailed information as to the intracellular action of Ap_6A. Due to the structural similarity with ATP, and other mononucleotides (Yakovenko and Formazyuk, 1993), it is conceivable that Ap_6A might regulate a nucleotide-dependent protein, such as the ATP-sensitive K^+ (K_{ATP}) channel. The cardiac K_{ATP} channel is a prototype of an intracellular nucleotide-gated ion channel (Noma, 1983). Therefore, the aim of the present study has been to define whether Ap_6A can affect K_{ATP} channel activity.

Ventricular myocytes were isolated by enzymatic dissociation from adult guinea-pig hearts (Terzic et al., 1994a). The inside-out configuration of the patch-clamp technique (Hamill et al., 1981) was used to record current flowing through K_{ATP} channels. Patch pipettes had a resistance of 3–5 MΩ, and were filled with (in mM): KCl 140, $CaCl_2$ 1, $MgCl_2$ 1, Hepes-KOH 5 (pH 7.4). Once excised, the intracellular side of membrane patches was exposed to (in mM): KCl 140, $MgCl_2$ 1, EGTA-KOH 5, Hepes-KOH 5 (pH 7.3) in the absence and presence of Ap_6A (Sigma). Single-channel recording was conducted at room temperature (21–23°C), using a patch-clamp amplifier (Axopatch 1C). The holding potential was −60 mV. Data were stored on tape using a PCM converter system (Instrutech), reproduced, low pass filtered at 1–1.5 kHz (−3 dB) by a Bessel filter (Frequency Devices 902), sampled at 4 kHz, and analyzed off-line with the BioQuest software analysis program (developed by Dr. A.E. Alekseev). Channel activity was expressed as NP_O, where N is the number of channels in the patch and P_O the open probability of each channel. Data are represented as mean ± S.E.M. where appropriate. Statistical significance of differences between two means was determined with the Student's t-test, and a value of $P < 0.05$ was considered to be statistically significant.

Following excision of a membrane patch from a ventricular myocyte, characteristic openings of K_{ATP}

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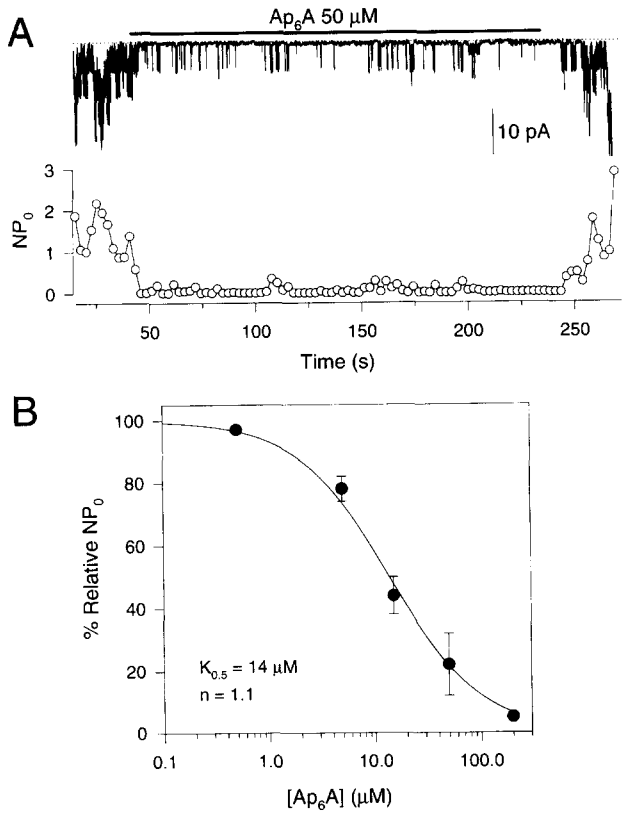

Fig. 1. Diadenosine-hexaphosphate inhibits myocardial ATP-sensitive K^+ channels. (A) Upper trace: continuous channel record. Lower trace: NP_O values, corresponding to channel record, calculated over 2.5-s long intervals. Dotted line corresponds to the zero current level. Holding potential was −60 mV. (B) Concentration-dependent effect of Ap_6A. At different concentrations of Ap_6A, the relative channel activity expressed as NP_O was obtained with reference to values recorded in the absence of Ap_6A. Data are from 4 membrane patches for each data point. Solid line was drawn according to the equation, $y = 1/\{1+([Ap_6A]/K_i)^n\}$, in which y = relative NP_O at each Ap_6A concentration, $[Ap_6A]$ = concentration of Ap_6A, K_i = the concentration of Ap_6A at half-maximal inhibition of the channel, and n = Hill coefficient.

channels appeared (see Noma, 1983; Findlay, 1994; Terzic et al., 1994b). Addition of Ap_6A (50 μM) to the intracellular side of a patch did not affect the magnitude of the unitary current flowing through a K_{ATP} channel (5.4 ± 0.4 vs. 5.4 ± 0.4 pA at a holding potential of −60 mV in the absence and presence of Ap_6A, respectively; $n = 4$). But, Ap_6A (50 μM) induced immediate inhibition of K_{ATP} channel activity (Fig. 1A). The average NP_O value was 3.49 ± 1.00 in the absence, and 0.66 ± 0.30 in the presence of 50 μM AP_6A ($P < 0.01$; $n = 4$). The effect of Ap_6A on K_{ATP} channel activity was concentration-dependent. The half-maximal inhibitory concentration was estimated to be 14 μM and the Hill coefficient was 1.1 (Fig. 1B).

This study demonstrates that Ap_6A, a naturally occurring dinucleotide polyphosphate, antagonized myocardial K_{ATP} channels. The potency of Ap_6A to block K_{ATP} channel activity was moderately higher than that described for the ATP-evoked K_{ATP} channel inhibition since the half-maximal concentration of ATP to inhibit myocardial K_{ATP} channels is usually in the range of 30 μM (see Noma, 1983; Findlay, 1994; Terzic et al., 1994b). The effect of Ap_6A appeared to be mediated through an intracellular action as the membrane non-permeable Ap_6A was effective when applied to the intracellular side of excised patches.

These findings probably exclude the possibility that the inhibitory effect of Ap_6A on K_{ATP} channels is due to its known extracellular action on purinoceptors (see Baxi and Vishwanatha, 1995). Rather, it is likely that Ap_6A could act on intracellular nucleotide binding sites of K_{ATP} channels or associated proteins. The present study would, then, indicate that the cardiac K_{ATP} channel could be affected not only by ATP and related mononucleotides, but also by the dinucleotide polyphosphate, Ap_6A.

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References

Baxi, M.D. and J.K. Vishwanatha, 1995, Diadenosine polyphosphates: their biological and pharmacological significance, J. Pharmacol. Methods 33, 121.

Findlay, I., 1994, Interactive regulation of the ATP-sensitive potassium channel of cardiac muscle, J. Cardiovasc. Pharmacol. 24 (Suppl. 4), S6.

Hamill, O.P., A. Marty, E. Neher, B. Sakmann and F.J. Sigworth, 1981, Improved patch-clamp techniques for high-resolution current recordings from cells and cell-free membrane patches, Pflüg. Arch. 391, 85.

Noma, A., 1983, ATP-regulated K^+ channels in cardiac muscle, Nature 311, 147.

Schluter, H., E. Offers, G. Bruggemann, M. Van der Giet, M. Tepel, E. Nordhoff, M. Karas, C. Spieker, H. Witzel and W. Zidek, 1994, Diadenosine phosphates and the physiological control of blood pressure, Nature 367, 186.

Terzic, A., I. Findlay, Y. Hosoya and Y. Kurachi, 1994a, Dualistic behavior of ATP-dependent K^+ channel towards intracellular nucleoside diphosphates, Neuron 12, 1049.

Terzic, A., R.T. Tung and Y. Kurachi, 1994b, Nucleotide regulation of ATP-sensitive K^+ channels, Cardiovasc. Res. 28, 746.

Yakovenko, I.N. and V.E. Formazyuk, 1993, Diadenosine oligophosphates: metabolic pathways and role in regulation of the functional activity of cells, Biokhimiya 58, 3.