British Journal of Pharmacology (1996) 117, 233–235

SPECIAL REPORT

Diadenosine tetraphosphate-induced inhibition of ATP-sensitive K^+ channels in patches excised from ventricular myocytes

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Diadenosine 5′,5″-P^1,P^4-tetraphosphate (Ap_4A) has been termed 'alarmone' due to its role in intracellular signaling during metabolic stress. It is not known whether Ap_4A could modulate ATP-sensitive K^+ (K_{ATP}) channels, a family of channels regulated by the metabolic status of a cell. We applied the single-channel patch-clamp technique to measure the effect of Ap_4A on K_{ATP} channels. When applied to the intracellular side of patches, excised from guinea-pig ventricular myocytes, Ap_4A inhibited K_{ATP} channel activity, in a reversible and concentration-dependent (half-maximal concentration ~ 17 μM) manner. We conclude that Ap_4A, a naturally occurring diadenosine polyphosphate, is actually an inhibitor of the myocardial K_{ATP} channel.

Keywords: ATP-sensitive K^+ channel; diadenosine 5′,5″-P^1,P^4-tetraphosphate; diadenosine polyphosphate; alarmone; channel gating; guinea-pig; heart; cardiomyocyte

Introduction Diadenosine 5′,5″-P^1,P^4 tetraphosphate (Ap_4A) has been termed putative 'alarmone' to denote that this dinucleotide polyphosphate is synthesized during metabolic challenges and could act homeostatically under stress conditions (Varshavsky, 1983). In several cell types a direct intracellular effect of Ap_4A has been demonstrated on enzymes with nucleotide-binding domains which are associated with cellular metabolism (Yakovenko & Formazyuk, 1993).

ATP-sensitive K^+ (K_{ATP}) channels are gated by intracellular ATP, and provide a link between cellular metabolism and membrane excitability (Ashcroft & Ashcroft, 1990; Davies *et al.*, 1991; Edwards & Weston, 1993). In the myocardium, modulation of K_{ATP} channel activity during metabolic stress has been related to intracellular mononucleotides, such as ATP (Nichols & Lederer, 1991; Findlay, 1994; Terzic *et al.*, 1994b). It is unknown whether a dinucleotide polyphosphate with putative 'alarmone' properties, such as Ap_4A, could also affect K_{ATP} channel activity. Therefore, we evaluated the effect of Ap_4A on cardiac K_{ATP} channels.

Methods Ventricular myocytes were isolated from guinea-pig hearts, and the inside-out configuration of the patch-clamp technique used to record channel activity (Terzic *et al.*, 1994a). Patch pipettes (3–5 MΩ) were filled with (in mM): KCl 140, $CaCl_2$ 1, $MgCl_2$ 1, HEPES-KOH 5 (pH 7.4), and the intracellular side of excised patches exposed to (in mM): KCl 140, $MgCl_2$ 1, EGTA-KOH 5, HEPES-KOH 5 (pH 7.3) in the absence and presence of ATP or AP_4A (Sigma). Single-channel recording was conducted at a holding potential of −60 mV (21–23°C) using a patch-clamp amplifier (Axopatch 1C). Data, stored on tape using a PCM converter system (Instrutech), and low-pass filtered at 1–1.5 kHz (−3 dB) by a Bessel filter (Frequency Devices), were sampled at 4 kHz, and analyzed with the 'BioQuest' software (developed by Dr A.E. Alekseev). Channel activity was expressed as NP_o (N = number of channels in the patch; P_o = open probability of each channel). Data are represented as mean ± s.e.mean. Statistical significance of differences between two means was determined with Student's t test, and $P<0.05$ considered significant.

Results Upon excision of a patch from a cardiomyocyte, vigorous openings of K_{ATP} channels appeared, and could be blocked by 200 μM ATP (Figure 1a). At equimolar concentrations of K^+ on the external and internal sides of a patch, these channels had a unitary conductance of $\sim$90 pS, as described for myocardial K_{ATP} channels (Findlay, 1994; Terzic *et al.*, 1994b). Addition of AP_4A (50 μM) to the intracellular side of a patch, did not affect the magnitude of the unitary current flowing through a K_{ATP} channel (5.7 ± 0.3 *vs.* 5.7 ± 0.3 pA at −60 mV in the absence and presence of AP_4A, respectively; $P>0.05$, $n=9$). Yet AP_4A (50 μM) induced immediate inhibition of K_{ATP} channels (Figure 1b(i)). The NP_o, was 4.37 ± 1.11 in the absence, and 0.60 ± 0.22 in the presence of 50 μM AP_4A ($P<0.01$, $n=9$; Figure 1b(ii)). The effect of AP_4A was partially reversible (Figure b(i), and the NP_o returned to 2.81 ± 0.80 following washout of AP_4A ($n=9$; Figure 1b(ii)). The inhibitory effect of AP_4A on K_{ATP} channels was concentration-dependent. The concentration-response relationship was fitted to a Hill equation with a half-maximal concentration estimated at 17 μM, and a slope factor of 1.2 (Figure 2).

Discussion This study demonstrates that Ap_4A, a naturally occurring dinucleotide polyphosphate, inhibits myocardial K_{ATP} channels. This represents a previously unrecognized property of Ap_4A, that could relate to the proposed intracellular potential of this molecule to regulate cellular metabolism.

The dinucleotide polyphosphate, Ap_4A, was effective when applied in micromolar concentrations to the intracellular side of excised patches. The effect of Ap_4A was concentration-dependent suggesting the involvement of a saturable binding site. The potency and efficacy of Ap_4A in blocking K_{ATP} channels was comparable to that described for the ATP-evoked K_{ATP} channel inhibition (Nichols & Lederer, 1991; Findlay, 1994; Terzic *et al.*, 1994b). Since Ap_4A inhibited K_{ATP} channels in the absence of intracellular GTP, it implies that a GTP-binding

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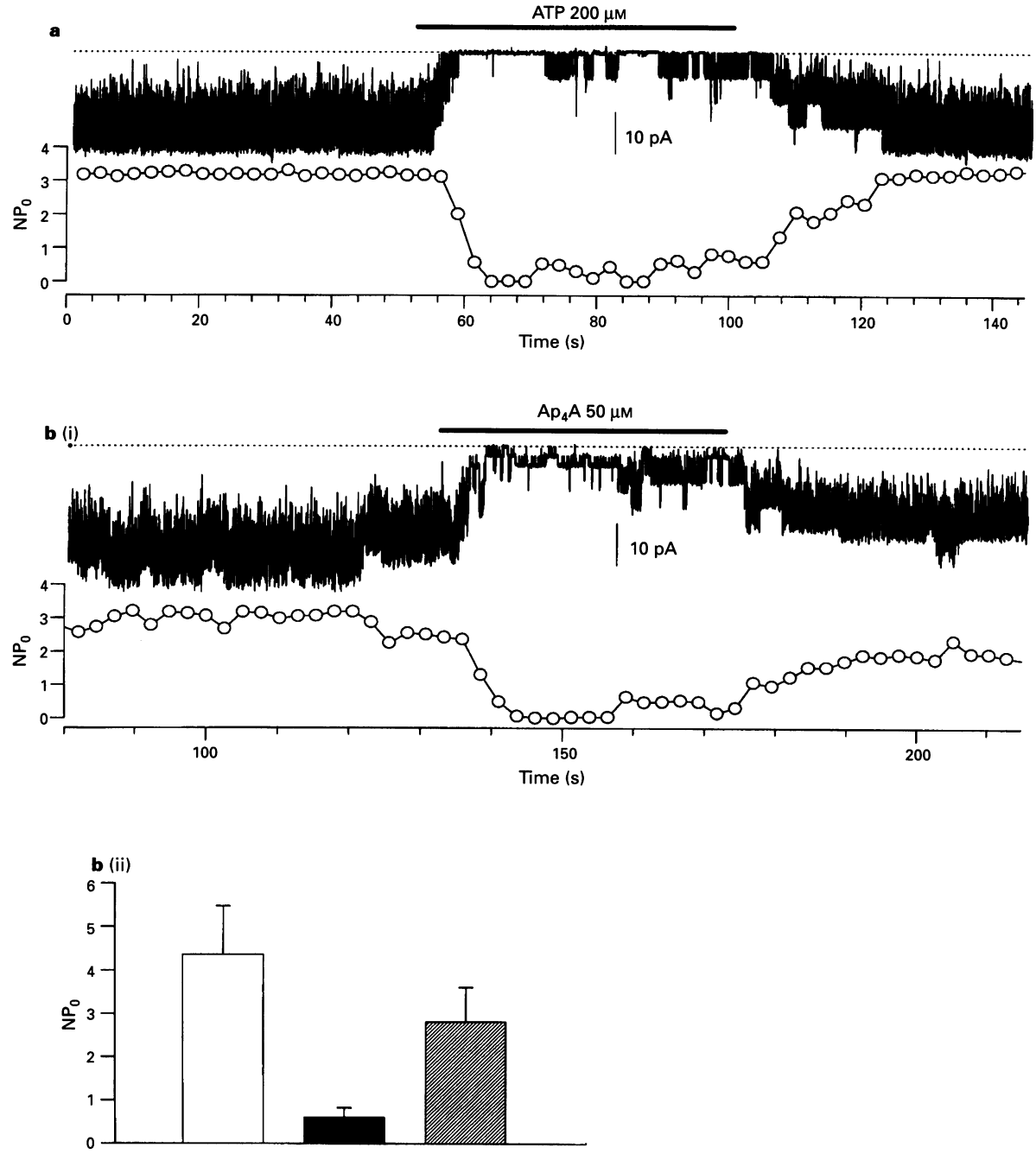

Figure 1 ATP-(a) and Ap_4A- (b) induced inhibition of K_{ATP} channels. Upper traces in (a) and (b(i)): channel records. Lower traces in (a) and (b(ii)): corresponding NP_o values calculated over 2.5-s long intervals. Dotted lines: zero current level. (b(ii)) Average NP_o prior to (open column), during (solid column), and after (hatched column) application of 50 μM Ap_4A to the intracellular side of patches.

protein is not required to transduce this effect. These findings probably exclude the possibility that the effect of Ap_4A on K_{ATP} channels was due to an extracellular action on purinoceptors (Baxi & Vishwanatha, 1995). Rather, Ap_4A could have acted directly on intracellular binding sites either on the K_{ATP} channel itself or associated proteins. Previously, interactions of Ap_4A with intracellular nucleotide-binding enzymes have been associated with the binding of Ap_4A to nucleotide-binding sites (Baxi & Vishwanatha, 1995). Thus, the site of action of Ap_4A could include the putative ATP-binding inhibitory or other nucleotide-binding site(s) of K_{ATP} channels (Edwards & Weston, 1993; Findlay, 1994; Terzic *et al.*, 1994a). Regardless of the site of action of Ap_4A, the present study suggests that cardiac K_{ATP} channels could be gated not only by ATP and related mononucleotides, but also by the dinucleotide polyphosphate, Ap_4A.

The physiological importance of the action of Ap_4A on K_{ATP} channels is, at present, unknown. Ap_4A is synthesized intracellularly, yet the concentrations of Ap_4A in cells at rest are in the pico- to nanomolar range (Yakovenko & Formazyuk, 1993). However, endogenous Ap_4A has a considerably longer intracellular half-life than ATP (Baxi & Vishwanatha, 1995), and under stress conditions, Ap_4A can rise to concentrations $>>1$ μM (Varshavsky, 1983). Provided that the affinity of K_{ATP} channels for Ap_4A in intact myocytes is similar to that measured in excised patches, it is under stress

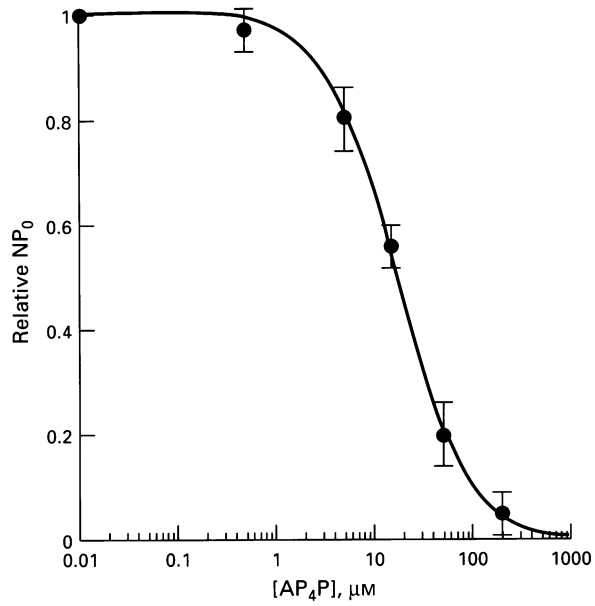

Figure 2 Concentration-dependent inhibition of K_{ATP} channels by Ap_4A. At different concentrations of Ap_4A, relative channel activity was obtained with reference to values recorded in the absence of Ap_4A. Data are from 6–9 patches for each point. Solid line was drawn according to the equation: $y = 1/\{1 + ([Ap_4A]/K_i)^{n_H}\}$; y = relative NP_o at each Ap_4A concentration ($[Ap_4A]$), $K_i = [Ap_4A]$ at half-maximal inhibition of channels = 17 μM; n_H = Hill coefficient = 1.2.

conditions that sufficient levels of intracellular Ap_4A could be synthesized to affect channel activity. Hence, the putative role of Ap_4A in the intracellular regulation of K_{ATP} channels awaits definition.

The authors acknowledge the expert contribution of Dr A.E. Alekseev with computer programming. A.T. is a recipient of the Ruth Salta Young Investigator Award from the American Health Assistance Foundation and of the Faculty Developmental Award from the Pharmaceutical Research and Manufacturers of America Foundation.

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(Received September 20, 1995
Accepted October 4, 1995)