Inhibition of a calcium-activated, non-selective cation channel, in a rat insulinoma cell line, by adenine derivatives

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The effects of adenosine and adenine nucleotides on a calcium-activated non-selective cation channel, present in the plasma membrane of an insulin-secreting cell line CRI-Gl were investigated. Single-channel currents were recorded from inside-out membrane patches and the adenine derivatives applied to the solution bathing the cytoplasmic aspect of the membrane surface. The activity of this channel is shown to be inhibited by all the derivatives tested. The potency sequence for inhibition was found to be AMP > ADP > ATP > adenosine.

(CRI-Gl cell) *Nucleotide effect* *Adenine derivative* *Cation channel*

1. INTRODUCTION

Non-selective cation channels, activated by calcium, with a conductance of between 20 and 25 pS are commonly found in the plasma membrane of mammalian excitable [1,2] and non-excitable [3,4] cells. We have shown previously that such a channel present in the plasma membrane of cells from a rat insulinoma cell line can be inhibited by the presence of 1 mM ATP, or its non-hydrolysable analogue AMPPNP, applied to the cytoplasmic side of the excised patch [5]. It is possible that ATP-sensitive channels may provide a link between substrate metabolism and stimulation of insulin secretion [6]. We now report that this non-selective cation channel is also sensitive to the other adenine nucleotides and their nucleoside.

2. MATERIALS AND METHODS

All experiments were performed on cells of the rat pancreatic islet cell line, CRI-G1, which were passaged at weekly intervals and maintained in Dulbecco's modified Eagles medium at 37°C in a humidified atmosphere of 95% air/5% CO_2 [7]. Cells were used between 2 and 6 days inclusive after plating. All experiments were performed on inside-out membrane patches excised from these cells using standard procedures [8]. Single-channel current recordings were made using an EPC-7 patch clamp amplifier and stored on magnetic tape for subsequent replay into a Gould 2200 chart recorder. All records shown were filtered by the chart recorder at 0.14 kHz. The open-state probability was determined off-line using a single-channel current analysis program (provided by J. Dempster, University of Strathclyde) run on a PDP 11/23 mini-computer. Stretches of data, 60 s in duration, were filtered at 600 Hz (8 pole Bessel) and sampled at a frequency of 3.3 kHz. The open-state probability was calculated by measuring the total time channels were in the open state and expressing this time as a proportion of the total time of the recording, taking into consideration the number of active channels observed. The ionic composition of the solution in the patch pipette was (mM): 135 NaCl, 5 KCl, 1 $MgCl_2$, 1 $CaCl_2$, 10 Hepes (pH 7.2); and in the bathing solution (mM):

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140 KCl, 1 $MgCl_2$, 1 $CaCl_2$, 10 Hepes (pH 7.2). The free Ca^{2+} and Mg^{2+} concentrations were maintained at 1 mM by increasing their total levels by the appropriate amount to compensate for chelation by ATP. These values of free calcium and magnesium were calculated using a computer program as described in [9] and the free calcium concentration checked directly with a calcium electrode.

The adenine derivatives were applied directly to the cytoplasmic face of the patch by superfusion of the bath. All experiments were performed at room temperature (23–25°C).

3. RESULTS

Under the conditions of these experiments whereby the membrane patch is exposed to an asymmetric cation distribution, a high calcium concentration (1 mM) at the cytoplasmic side and is clamped at a membrane potential of −45 to −50 mV the major active channel observed is the 25 pS non-selective cation channel [10]. Openings of the ATP-sensitive potassium (ATP-K^+) channel, which is also present in these cells [11], were infrequent and only appeared as small outward current steps (not shown).

The effects of adenosine and its nucleotide derivatives on the activity of the calcium-activated non-selective cation (Ca^{2+}-NS^+) channel are illustrated in fig.1. Each of the four sets of records has been obtained from a different inside-out patch and the control records show clearly the variability in the number of active channels observed (usually 1–5). Fig.1a illustrates the effect of ATP, a concentration of 100 μM producing 86% inhibition, and 1 mM complete abolition, of channel activity. We have observed this effect of ATP on a total of 11 patches. On testing the other adenine nucleotides we discovered that they too inhibited the activity of this channel and indeed were more potent than ATP. Application of 100 μM

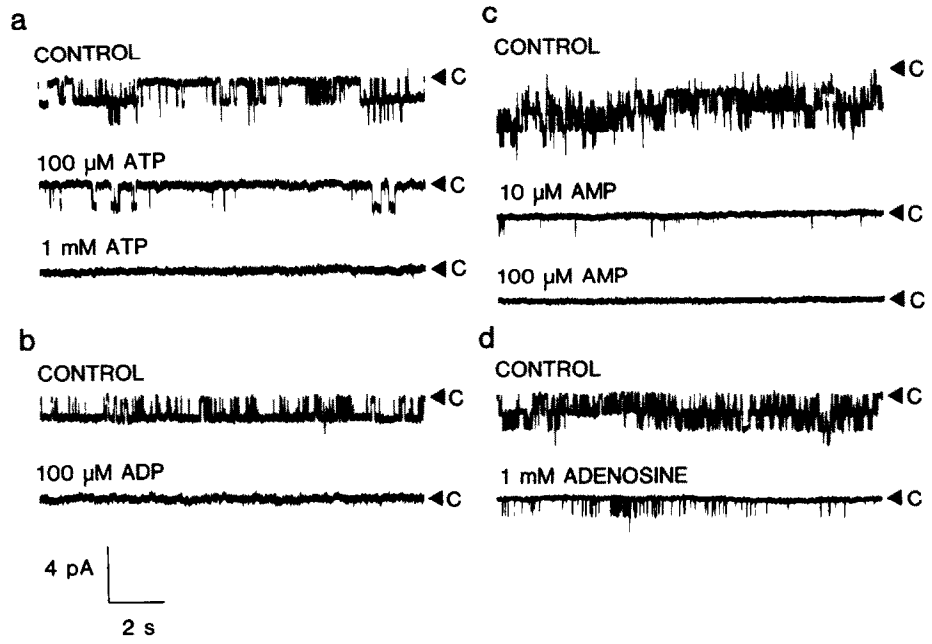

Fig.1. Single-channel current records from four individual inside-out membrane patches excised from CRI-G1 cells illustrating the actions of the adenine nucleotides and adenosine. Openings are shown as downward deflections (i.e. inward current) from the closed state (c) in all traces. The membrane potential was −45 mV for (a, c, d), and −50 mV for (b). The open-state probabilities for each of the traces are as follows: (a) control, 0.370; 100 μM ATP, 0.051; 1 mM ATP, 0; (b) control, 0.492; 100 μM ADP, 0; (c) control, 0.425; 10 μM AMP, 0.012; 100 μM AMP, 0; (d) control, 0.346; 1 mM adenosine, 0.03.

ADP was as effective as 1 mM ATP (fig.1b, n = 4). The monophosphate derivative, AMP, was found to be most potent (fig.1c), a concentration of 10 μM generally causing >90% inhibition and 100 μM complete abolition of channel activity (n = 4). In one patch where 10 μM AMP completely inhibited the channel, a concentration of 1 μM reduced channel activity by 94%. However, the nucleoside, adenosine, did not follow this potency sequence (fig.1d), 1 mM only causing 91% inhibition (n = 3).

Although not shown in this figure the effects of each of these adenine derivatives was immediate and completely reversible by washing, and could be reproduced many times with the same membrane patch. In addition the effect of these agents appeared not to be dependent upon the patch membrane potential. For example, 100 μM ADP had a similar inhibitory action at both negative and positive membrane potentials (e.g. −40 and 40 mV). The inhibitory action of these nucleotides was specific for the adenine base. GMP at concentrations of 100 μM and 0.5 mM did not reduce the open-state probability of this channel at both positive and negative membrane potentials (n = 3).

4. DISCUSSION

Recently there have been a number of reports describing the presence in plasma cell membranes of a potassium-selective channel that is susceptible to inhibition by ATP applied to the cytoplasmic side of isolated membrane patches. For example, such channels have been reported to exist in rabbit and guinea-pig cardiac muscle [12,13], frog skeletal muscle [14] and rat pancreatic β-cells [6]. This potassium channel appears to be sensitive primarily to the triphosphate of adenosine; ADP is approx. 10-times less effective, whilst AMP and adenosine are totally ineffective.

In contrast to the potassium channel, this study shows that the Ca^{2+}-NS^+ channel, although seemingly having a similar susceptibility to ATP, is much more sensitive to ADP and AMP. These potency sequences are, at first glance, somewhat similar to the two types of extracellular purinergic receptors, P_2 and P_1, respectively [15]. Indeed, the similarity between the ATP-K^+ channel sensitivity and the P_2 receptor has been previously reported [13]. However, they are not totally analogous because adenosine is much less effective at inhibiting the Ca^{2+}-NS^+ channel thus not conforming with the sequence for the P_1 purinoceptor.

Unfortunately, it is not clear what the function of the Ca^{2+}-NS^+ channel is in those cells in which it has been described, although it has been suggested [16] that it may contribute to the electrical activity of rhythmically firing cells. Whilst β-cells may exhibit rhythmic electrical activity during glucose stimulation [17] it also must be questioned whether the concentration of, or sensitivity to, calcium in vivo would be sufficient to activate their Ca^{2+}-NS^+ channel. Nevertheless the finding that substances closely involved in energy metabolism can act as antagonists to both K^+ selective and non-selective cation channels has important implications for the interactions between energy metabolism and cell excitability. It also raises a number of interesting questions amenable to further investigation. For example, are the inhibitory actions of adenine nucleotides on the Ca^{2+}-NS^+ channel a general phenomenon; is the channel physiologically active in β-cells and if so what role does it play in the complex electrical changes observed during β-cell stimulation? The observations which we report here suggest important and subtle interactions may occur between the availability of energy and cell membrane potential during cell activities such as secretion and contraction.

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