

An adenylate kinase is involved in K_{ATP} channel regulation of mouse pancreatic beta cells

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Abstract

Aims/hypothesis In a previous study, we demonstrated that a creatine kinase (CK) modulates K_{ATP} channel activity in pancreatic beta cells. To explore phosphotransfer signalling pathways in more detail, we examined whether K_{ATP} channel regulation in beta cells is determined by a metabolic interaction between adenylate kinase (AK) and CK.

Methods Single channel activity was measured with the patch-clamp technique in the inside-out (i/o) and open-cell attached (oca) configuration.

Results The ATP sensitivity of K_{ATP} channels was higher in i/o patches than in permeabilised beta cells (oca). One reason for this observation could be that the local ATP:ADP ratio in the proximity of the channels is determined by factors not active in i/o patches. AMP (0.1 mmol/l) clearly increased open channel probability in the presence of ATP (0.125 mmol/l) in permeabilised cells but not in excised patches. This suggests that AK-catalysed ADP production in the vicinity of the channels is involved in K_{ATP} channel regulation. The observation that the stimulatory effect of AMP on K_{ATP} channels was prevented by the AK inhibitor P^1, P^5 -di(adenosine-5')pentaphosphate (Ap_5A ; 20 μ mol/l) and abolished in the presence of the non-metabolisable ATP

analogue adenosine 5'-(β, γ -imido)triphosphate tetralithium salt (AMP-PNP; 0.12 mmol/l) strengthens this idea. In beta cells from AK1 knockout mice, the effect of AMP was less pronounced, though not completely suppressed. The increase in K_{ATP} channel activity induced by AMP in the presence of ATP was outweighed by phosphocreatine (1 mmol/l). We suggest that this is due to an elevation of the ATP concentration by CK.

Conclusions/interpretation We propose that phosphotransfer events mediated by AK and CK play an important role in determining the effective concentrations of ATP and ADP in the microenvironment of pancreatic beta cell K_{ATP} channels. Thus, these enzymes determine the open probability of K_{ATP} channels and eventually the actual rate of insulin secretion.

Keywords Adenine nucleotides · Adenylate kinase · AK1 knockout mice · Beta cells · Creatine kinase · K_{ATP} channel · Phosphotransfer

Abbreviations

| | |
|---------|--|
| AK1-KO | adenylate kinase 1 knockout |
| AK | adenylate kinase |
| AMPK | AMP-activated protein kinase |
| AMP-PNP | adenosine 5'-(β, γ -imido)triphosphate tetralithium salt |
| Ap_5A | P^1, P^5 -di(adenosine-5')pentaphosphate |
| CK | creatine kinase |
| i/o | inside-out |
| P_o | open probability |
| oca | open-cell attached |
| PCr | phosphocreatine |
| SUR1 | sulfonylurea receptor 1 |

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Introduction

The K_{ATP} channel of pancreatic beta cells plays a predominant role in insulin secretion because it links beta cell metabolism to electrical activity that triggers Ca^{2+} influx and finally exocytosis. K_{ATP} channel activity is regulated by ATP derived from beta cell metabolism. The bulk ATP concentration in beta cells ranges between 3 and 5 mmol/l at high glucose concentration [1–3]. In excised patches half-maximal inhibition of beta cell K_{ATP} channels can be achieved in the micromolar range [4, 5]. In intact cells, ATP concentrations required for channel inhibition are somewhat higher but still below the physiological cytosolic ATP concentration [6, 7]. Therefore, ATP-sensitive K^+ channels should be completely inhibited under physiological conditions; however, they are evidently active.

To reconcile the experimental observations with the physiological situation, it has been postulated that there must be additional regulatory mechanisms that determine the activity of K_{ATP} channels in beta cells. It is well accepted that ADP counteracts the inhibitory action of ATP [8–10]. While ATP exerts its inhibiting effect by binding at the inwardly rectifying potassium channel 6.2 (Kir6.2) subunit of the K_{ATP} channel, ADP interacts with the nucleotide-binding domains of the sulfonylurea receptor 1 (SUR1) subunit, thereby promoting channel opening. Therefore, ADP is at least as important as ATP in K_{ATP} channel regulation [11, 12]. However, the problem remains that bulk ATP and ADP concentrations would have to undergo large and rapid fuel-dependent changes to explain satisfactorily how the signal from glucose metabolism is transferred to the K_{ATP} channels. In particular, beta cell membrane potential oscillations, which include rhythmic changes in the open probability (P_o) of K_{ATP} channels [13–15], cannot be explained by global concentration changes of ATP in the millimolar range. Thus, it seems much more likely that alterations in the microenvironment of the channels determine P_o for the K_{ATP} channel. In the past decade, several suggestions have been put forward, with long-chain acyl-coenzyme A esters [16, 17], C16:0 sulfatide [18] and membrane phosphatidylinositol phosphates (especially phosphatidylinositol 4,5-bisphosphate (PIP2)) [19, 20] having been shown to increase the P_o of pancreatic K_{ATP} channels by reducing their ATP sensitivity. These regulatory mechanisms are more likely to be of significance in receptor-dependent signalling pathways [19–21] or under conditions of pathophysiological stress [16, 17, 22, 23]. Recently, it has been proposed that the ATP sensitivity of pancreatic K_{ATP} channels is primarily determined by interaction of Mg-nucleotides with the nucleotide-binding domains of the SUR1 subunit [3].

We have demonstrated previously that a membrane-associated creatine kinase (CK) is involved in K_{ATP}

channel regulation in native beta cells [24] and proposed that phosphocreatine (PCr) acts as a shuttle, transferring energy-rich phosphate to the plasma membrane where ADP is phosphorylated by a CK in close vicinity to the channels. It is tempting to speculate that several phosphotransfer enzymes together constitute a network that functions as a metabolic barrier. This enzyme network would on the one hand determine the actual ATP and ADP concentrations that the channels sense, and on the other hand shield beta cell K_{ATP} channels against bulk ATP and ADP concentrations. One good candidate for such an arrangement is an adenylate kinase (AK), which forms ADP using ATP and AMP as substrates. This enzyme would counterbalance ATP production by the CK. For cardiomyocytes it has been shown that macromolecular complexes consisting of CK, AK and K_{ATP} channels exist and that both enzymes are involved in the regulation of K_{ATP} channels [25, 26]. In the present paper we investigated whether AK activity could be involved in the regulation of K_{ATP} channels of pancreatic beta cells. We demonstrate here that the enzyme increases the P_o of the channels and suggest that it is part of a metabolic barrier shielding the K_{ATP} channels from bulk ATP and ADP concentrations.

Methods

Islet and cell preparation Experiments were performed on single pancreatic beta cells of fed male C57Bl/6N mice (20–30 g; Charles River, Sulzfeld, Germany) and AK1 knockout (AK1-KO) mice [27] (Bé Wieringa, Nijmegen, The Netherlands) that had been killed by CO_2 . Islets were isolated by collagenase digestion of the pancreas. Cells were dispersed in Ca^{2+} -free medium and cultured up to 4 days in RPMI 1640 medium supplemented with 10% fetal calf serum, 100 U/ml penicillin and 100 μ g/ml streptomycin [28].

Electrophysiology Open-cell attached (oca) and inside-out (i/o) measurements were performed using an EPC-9 amplifier and the Pulse software package (both from Heka, Lambrecht, Germany). Data were stored on videotape for later analysis with Igor software (Wavemetrics, Lake Oswego, OR, USA). Channel activity in single channel recordings is given as NP_o , the product of P_o calculated by point-by-point analysis and the number of active channels in the patch (N). Each experiment was started in the cell-attached configuration with a bath solution containing (mmol/l): 140 NaCl, 5 KCl, 1.2 $MgCl_2$, 2.5 $CaCl_2$, 0.5 glucose, 10 HEPES, pH 7.4 adjusted with NaOH (solution 1). For oca and i/o measurements the bath solution was changed to (mmol/l): 130 KCl, 5 EGTA, 0.5 glucose, 10

HEPES, 1.5 MgCl₂, pH 7.2 adjusted with KOH (solution 2). When ATP was added, this solution was adjusted to 3 mmol/l MgCl₂. The pipette solution consisted of (mmol/l): 130 KCl, 4 MgCl₂, 2 CaCl₂, 10 EGTA, 0.65 Na₂ATP, 20 HEPES, pH 7.15 adjusted with KOH.

The oca configuration was obtained as follows. After formation of a cell-attached patch, the bath solution was changed to solution 2 and the holding potential was set to -50 mV. A second pipette containing solution 2 with 0.05% saponin (*w/v*) was then brought to the other side of the cell. Positive pressure was applied to eject saponin-containing solution on to the surface of the cell. Permeabilisation was assessed by the development of a slight swelling of the cell and the increase in NP_o of K_{ATP} channels due to wash-out of cellular ATP [29, 30]. The saponin-containing pipette was then withdrawn. Single K_{ATP} channel currents were recorded at 25° to 28°C.

Pipettes were pulled with a DMZ Puller (Zeitz Instruments, Munich, Germany) using borosilicate glass (Clark, Pangbourne, UK). They had resistances of 7–12 MΩ when filled with pipette solution.

Chemicals HEPES was obtained from Roth (Karlsruhe, Germany), collagenase from Serva (Heidelberg, Germany) or Roche Diagnostics (Mannheim, Germany) and adenosine 5'-(β,γ-imido)triphosphate tetralithium salt (AMP-PNP) from Fluka (Seelze, Germany). RPMI 1640, fetal calf serum and penicillin–streptomycin were provided by Invitrogen (Karlsruhe, Germany). All other chemicals were purchased from Sigma-Aldrich (Seelze, Germany) and Merck (Darmstadt, Germany) in the purest form available.

Presentation of results Electrophysiological experiments are illustrated by recordings that are representative for the indicated number of experiments performed with different cells. Beta cells of at least two different cell preparations were used for each series of experiments. Where possible, values in the text are given as means±SEM for the indicated number of experiments. The statistical significance of differences between means was assessed by a Student's *t* test for paired values when two samples were compared; multiple comparisons were made by ANOVA followed by Student–Newman–Keuls test. A value of $p \leq 0.05$ was considered to represent a statistically significant difference.

ATP concentration–response curves were fitted with the Hill equation: $y = 100 / [1 + (x/IC_{50})^h]$, where y equals NP_o of the K_{ATP} channel in the test solution (taken as percentage of NP_o in the solution without ATP), x represents the ATP concentration used and IC_{50} is the ATP concentration that causes half-maximal block, h is the Hill coefficient. IC_{50} is given ±SD.

Results

Different ATP sensitivity of K_{ATP} channels in permeabilised beta cells vs cell-free patches In Fig. 1, K_{ATP} channel inhibition in dependence of the ATP concentration is compared in beta cells permeabilised by saponin (=oca) with patches in the *i/o* configuration. The pores formed by permeabilisation in the oca configuration allowed us to establish a range of intracellular nucleotide concentrations by extracellular application of ATP, AMP etc. [29, 30]. ATP concentrations of 3, 10, 30, 100 and 300 μmol/l were applied. NP_o under control conditions (without ATP) was taken as 100%. In each experiment the inhibitory effectiveness of a certain ATP concentration was determined first in the oca configuration and afterwards in the *i/o* configuration. The data reveal a clear right-shift of the curve obtained in the oca configuration vs that measured in the *i/o* configuration. The values for half-maximal inhibition amounted to 6.6 ± 0.7 μmol/l (*i/o*) and 27.5 ± 14.8 μmol/l (oca) ($n=4-8$ for different ATP concentrations).

Regulation of the K_{ATP} channel by an AK To test whether an AK is involved in the regulation of K_{ATP} channels of pancreatic beta cells, we added AMP to cells in the presence of ATP after formation of the oca configuration. AK-mediated production of ADP should increase NP_o of K_{ATP} channels. Figure 2a shows that 0.1 mmol/l AMP added in the presence of 0.125 mmol/l ATP clearly increased channel activity. To quantify the effect, NP_o under control conditions (ATP 0.125 mmol/l) in each experiment was taken as 1 and changes were referred to this value. AMP increased normalised NP_o from 1 to 5.95 ± 1.07 ($n=5$, $p < 0.001$ vs control). Removal of AMP reversed the effect. Normalised NP_o amounted to 0.92 ± 0.41 ($n=4$, $p < 0.01$ vs value with AMP) after wash-out of AMP.

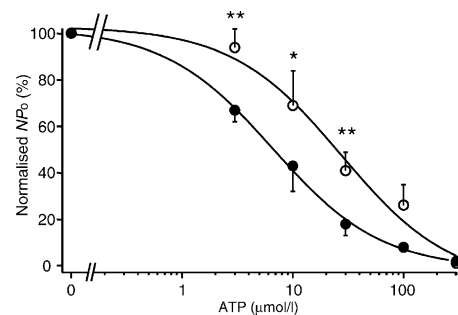


Fig. 1 K_{ATP} channel sensitivity to ATP inhibition is different in permeabilised cells and excised patches. The figure shows the relation between the ATP concentration and NP_o measured in the oca configuration (open circles) and in *i/o* patches (closed circles). Control values before addition of ATP were taken as 100%. $n=4-8$ for different ATP concentrations. * $p < 0.05$, ** $p < 0.01$

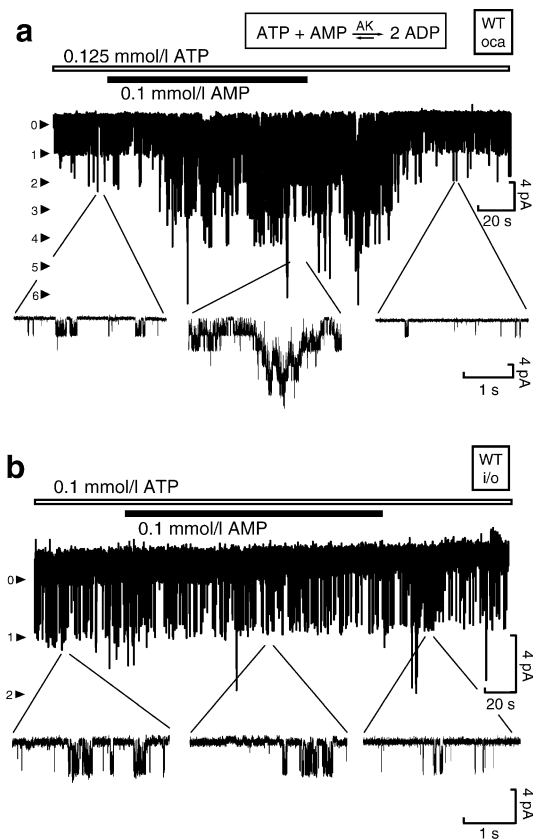


Fig. 2 K_{ATP} channels are activated by AMP in (a) oca patches but not in (b) i/o patches. **a** Representative experiment showing that NP_o of beta cell K_{ATP} channels markedly increased when 0.1 mmol/l AMP was applied in the presence of 0.125 mmol/l ATP. The number of open channels is indicated by the arrowheads on the left-hand side. The lower traces show parts of the experiment before and during application of AMP and after wash-out at extended time scales. The box above indicates the AK-catalysed reaction responsible for the channel opening. **b** Representative experiment showing that in cell-free patches AMP (0.1 mmol/l) did not increase K_{ATP} channel activity in the presence of ATP (0.1 mmol/l). The number of open channels is indicated by the arrowheads on the left-hand side

Lack of AK-mediated K_{ATP} channel regulation in excised patches To evaluate whether the discrepancy in ATP sensitivity of the K_{ATP} channel between i/o and oca was linked to AK activity, we repeated the experiments shown in Fig. 2a in i/o experiments. Addition of 0.1 mmol/l AMP in the presence of 0.1 mmol/l ATP did not alter channel activity under these conditions (Fig. 2b). Moreover, these data rule out the possibility that the stimulatory effect of AMP on pancreatic K_{ATP} channels in the oca configuration is a direct effect of addition of this metabolite. The slight reduction of NP_o in the presence of AMP (to 0.72 ± 0.26 ; $n=5$, NS vs control) is likely to be channel rundown because it persisted after wash-out (0.34 ± 0.06 , $n=4$, $p < 0.05$ vs control). In control experiments under the same experimental conditions as above, 0.2 mmol/l ADP was still able to open K_{ATP} channels in the presence of ATP 0.1 mmol/l

(67.68 ± 33.03 vs 1 under ATP alone, $n=5$) (data not shown). Thus, ADP remains capable of increasing the channel activity under conditions where AMP fails to do so. This indicates that the failing effect of AMP in i/o experiments is due to the lack of AK activity.

Inhibition of the AK by P^1, P^5 -di(adenosine-5')pentaphosphate If an AK is responsible for the increase of NP_o in oca experiments after addition of AMP (0.1 mmol/l), the effect should be prevented by the AK inhibitor P^1, P^5 -di(adenosine-5')pentaphosphate (Ap₅A) [31, 32]. Indeed, Fig. 3 shows that addition of 20 μmol/l Ap₅A completely suppressed channel activation by AMP in the oca configuration. Channel NP_o , taken as 1 under control conditions (ATP 0.125 mmol/l + Ap₅A 20 μmol/l), was even decreased with AMP to 0.54 ± 0.17 ($n=6$, $p < 0.01$ vs control), falling subsequently to 0.31 ± 0.11 after wash-out ($n=5$, $p < 0.01$ vs control). The further decline after wash-out is probably due to channel rundown. Diadenosine polyphosphates have been found to directly inhibit K_{ATP} channel activity in cardiac cells and pancreatic beta cells [33]. Therefore, in experiments with Ap₅A, AMP was added when a constant channel activity was reached in the presence of ATP and Ap₅A. Furthermore, we ruled out the possibility that Ap₅A prevents channel activation by ADP. To do this, we applied ADP (0.2 or 0.5 mmol/l) in oca patches in the presence of 0.125 mmol/l ATP and 20 μmol/l Ap₅A (Fig. 4). Again, channel NP_o in the presence of ATP was taken as 1. In the presence of Ap₅A, it amounted to 1.80 ± 0.37 in the series with 0.2 mmol/l ADP and 0.93 ± 0.24 in the series with 0.5 mmol/l ADP, increasing to 25.39 ± 13.10 and 20.83 ± 7.63 , respectively after addition of ADP ($n=4$ and $n=5$). This shows that ADP is able to activate K_{ATP} channels in the presence of the inhibitor.

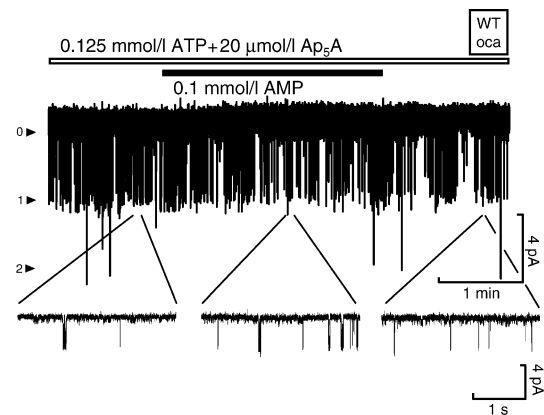


Fig. 3 The effect of AMP can be blocked by the AK inhibitor Ap₅A. In an oca experiment AMP (0.1 mmol/l) was not able to increase K_{ATP} channel activity when the AK was inhibited by 20 μmol/l Ap₅A (with 0.125 mmol/l ATP). The number of open channels is indicated by the arrowheads on the left-hand side

K_{ATP} channels of AK1-KO murine beta cells still exhibit regulation by AMP AK1-KO mice (first described by Janssen et al. [27]) lack the cytosolic AK1 isoform, which could be responsible for the AK activity that regulates the beta cell K_{ATP} channel as shown above. Therefore, we performed oca experiments as in Fig. 2 to explore whether the regulation by AMP is abolished in AK1-KO mice. Contrary to our expectation, this was not the case and AMP still increased NP_o in the AK1-KO cells (Fig. 5). However, the increase was smaller than in wild-type beta cells (AMP increased the normalised NP_o from 1 to 2.65 ± 0.33 in AK1-KO cells, $n=5$, $p<0.001$ vs control, $p<0.05$ vs AMP in wild-type beta cells). These data suggest that AK1 is not the only AK isoform responsible for K_{ATP} channel regulation in pancreatic beta cells.

AMP does not activate K_{ATP} channels in the presence of a non-metabolisable ATP analogue (AMP-PNP) If activity of an AK is responsible for the opening of the K_{ATP} channels, as suggested from Fig. 2, the reaction would not be expected to take place in the presence of a non-metabolisable ATP analogue. According to this expectation, addition of 0.1 mmol/l AMP did not increase NP_o when the non-metabolisable ATP analogue AMP-PNP (0.12 mmol/l) was used instead of ATP in oca experiments (Fig. 6). Channel NP_o was taken as 1 in the presence of AMP-PNP and decreased to 0.41 ± 0.14 with AMP ($n=4$, $p<0.01$ vs control; wash-out 0.36 ± 0.10 , $n=4$, $p<0.01$ vs control). In three of these experiments, AMP (0.1 mmol/l) was subsequently tested for an effect in the presence of ATP (0.05–0.125 mmol/l) and indeed elicited a five- to eightfold increase in channel activity (data not shown). This strengthens the argument that the presence of a metabolisable

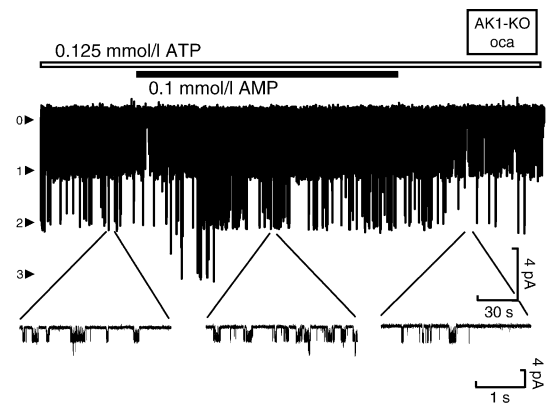


Fig. 5 K_{ATP} channels in AK1-KO mice are still activated by AMP in oca experiments. In the presence of 0.125 mmol/l ATP, 0.1 mmol/l AMP is still able to activate K_{ATP} channels in AK1-KO mice, but to a lesser extent than in wild-type beta cells. The number of open channels is indicated by the arrowheads on the left-hand side

nucleotide is needed as the substrate for AK-mediated phosphotransfer to produce the channel openings.

Effect of PCr Assuming an interaction between AK and CK in pancreatic beta cells, one would expect PCr to reduce the AMP-induced channel activity because CK forms ATP by transferring phosphate from PCr to ADP. We observed that PCr (1 mmol/l) completely reversed the increase in NP_o that was evoked by the activity of the AK (Fig. 7). As in the experiments presented in Fig. 2, normalised NP_o was increased about sixfold after addition of AMP (6.31 ± 1.16 , $n=3$, $p<0.01$ vs control). Subsequent application of PCr decreased the NP_o to levels equal to those in the presence of ATP alone (1.02 ± 0.21 , $n=3$, $p<0.01$ vs value with AMP). The effect was reversible upon removal of PCr.

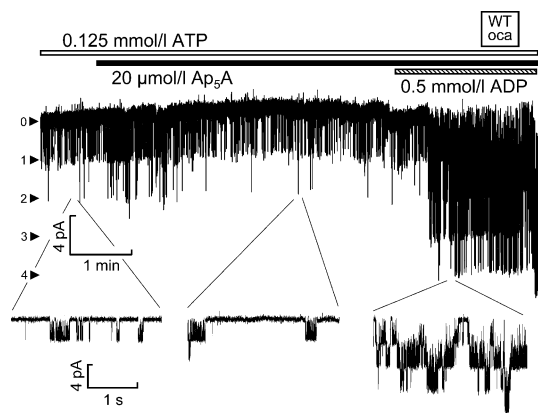


Fig. 4 Ap_5A does not prevent channel activation by ADP. Ap_5A (20 μ mol/l) in the presence of 0.125 mmol/l ATP does not prevent 0.5 mmol/l ADP from augmenting the channel activity in oca experiments. The number of open channels is indicated by the arrowheads on the left-hand side

Discussion

For native beta cells it has been widely accepted that glucose enhances the intracellular ATP concentration and the ATP:ADP ratio. However, this concept does not explain how glucose metabolism and global intracellular ATP and ADP concentrations are interlinked with K_{ATP} channel activity regulation. Experimental data regarding this regulatory network are inconsistent [34–38]; moreover, the high intracellular ATP concentration as such questions the simple assumption that bulk ATP and ADP concentrations regulate beta cell K_{ATP} channel activity [3].

One important finding of our work presented here is the right-shift of the concentration–response curve for K_{ATP} channel inhibition by ATP in the oca configuration vs excised patches. This supports the idea that the P_o is

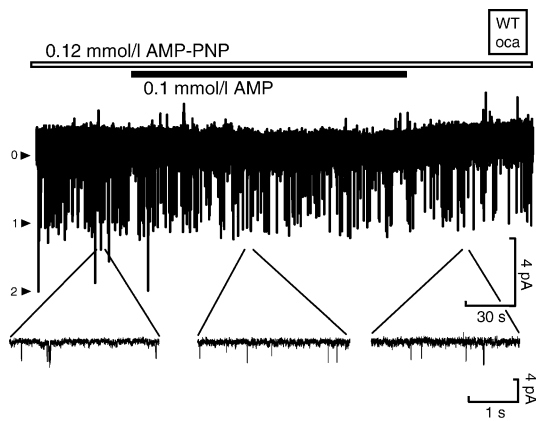


Fig. 6 Lack of activation of K_{ATP} channels by AMP in the presence of AMP-PNP. AMP (0.1 mmol/l) fails to increase the NP_o of K_{ATP} channels in oca experiments where a non-metabolisable ATP analogue (AMP-PNP, 0.12 mmol/l) is used instead of ATP. The number of open channels is indicated by the arrowheads on the left-hand side

regulated by the ATP and ADP concentration in the microenvironment of the channels rather than by the total cellular ATP and ADP concentration. Interestingly, a similar result has been reported for cardiomyocytes [30] and very recently for pancreatic beta cells [3]. The different ATP sensitivity of K_{ATP} channels in oca and excised patches of heart cells was explained by augmented ATP consumption by ATPases and the presence of glycolytic enzymes associated with K_{ATP} channels in the oca configuration [30]. In beta cells the difference in the ATP effectiveness between the diverse patch configurations was explained by altered Mg-nucleotide stimulation in oca vs i/o patches at the nucleotide-binding domains of SUR1 [3]. In addition to the above, our data strongly suggest that high-energy phosphoryl exchange between adenosine phosphonucleotides is involved in the regulation of the K_{ATP} channel activity in pancreatic beta cells.

Theoretically, nucleotides could also affect beta cell function by stimulating purinergic receptors or changing the activity of the AMP-activated protein kinase (AMPK). Activation of purinergic receptors by binding nucleotides at the extracellular site is reported to increase insulin secretion [39]. However, potentiation of insulin secretion is not reconcilable with the AMP-induced opening of K_{ATP} channels and thus we rule out the possibility that the observed effects were due to interaction of the nucleotides with purinergic receptors. AMP also activates the general fuel-sensor AMPK, which has been shown to inhibit glucose oxidation and ATP synthesis in beta cells [40]. However, our results with a non-metabolisable ATP analogue, where AMP failed to increase K_{ATP} channel activity in oca measurements (Fig. 6), argue against the possibility that AMPK is involved in the regulation observed in Fig. 2.

AKs reversibly catalyse phosphotransfer between AMP, ADP and ATP. Several isoforms have been identified: these are localised in mitochondria and cytosol or are plasma membrane-bound (for review see [41]) and contribute to the regulation of adenine nucleotide concentration(s) in microdomains of the cell [41].

According to Smink and co-workers [42], different isoforms of the mRNA for AK are found in mouse pancreatic islets. Moderate expression of mRNA for AK1, AK5 and AK7 as well as an enriched expression of mRNA for AK2 and AK3 have been observed so far. For human primary beta cells a moderate expression of mRNA for AK1 has been described whereas the expression of mRNA for AK2 is enriched and that of AK3L1 is only low. No data are available yet for the expression of other isoforms.

Our assumption that AK activity determines local ATP concentration in pancreatic beta cells is based on the following observations: (1) that NP_o of K_{ATP} channels is increased by AMP in the presence of ATP; (2) that the effect is only observed in permeabilised cells with intact enzyme activity, but not in small excised patches (see below), where ADP is still able to open the channels; (3) that the effect is suppressed by the AK inhibitor Ap₅A; and (4) that AMP only activates K_{ATP} channels in the presence of ATP, but not in the presence of the non-metabolisable analogue AMP-PNP. Moreover, we demonstrated that PCr counteracted AK-induced K_{ATP} channel activation, suggesting the involvement of CK activity. This confirms our previous observation of the existence of CK-mediated phosphotransfer in pancreatic beta cells [24].

The lack of effect of AMP in i/o patches points to a role for cytosolic and/or mitochondria-associated AK isoforms. However, Larsson et al. [43] observed an increase in

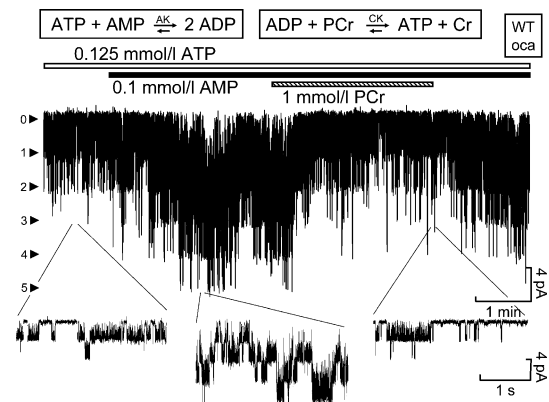
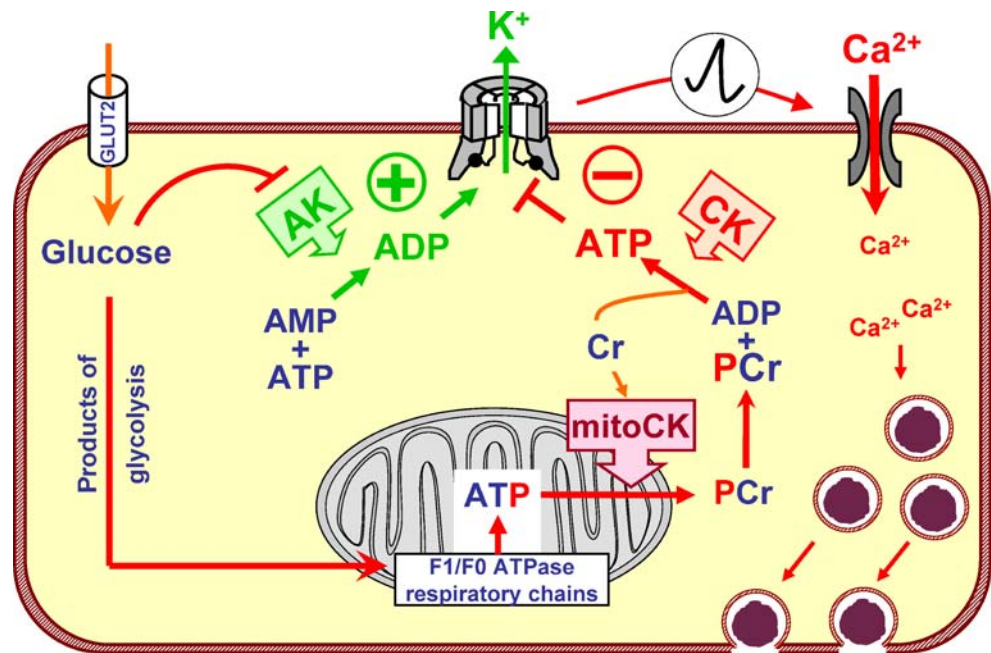


Fig. 7 PCr reverses the activation of K_{ATP} channels by AMP in oca patches. PCr (1 mmol/l) counteracts the effect of AMP (0.1 mmol/l). The boxes above show the reactions catalysed by AK and CK. The number of open channels is indicated by the arrowheads on the left-hand side

Fig. 8 Model illustrating the involvement of AK and CK activities in phosphotransfer networking and the regulation of beta cell K_{ATP} channels. *Cr* creatine, *mitoCK* mitochondrial CK



channel activity with AMP in cell-free macro-patches with extremely low pipette resistances (2–3 vs 8–11 M Ω in our experiments). Macro patches may facilitate the accumulation of nucleotides generated by AK activity or could result in the excision of cytosolic components. According to this concept, we also observed an increase of channel openings by AMP in i/o patches using pipettes with resistances lower than 4 M Ω (data not shown).

Compared with beta cells, the contribution of phosphotransfer mediated by AK and other enzymes to the regulation of K_{ATP} channels in heart cells is much better understood [44, 45]. Carrasco and co-workers [26] showed in cardiomyocytes that AMP activates K_{ATP} channels in the presence of ATP in the oca configuration. This effect was reversed by PCr and abolished in cells from AK1-KO mice. Thus, in heart cells the AK1 isoform seems to be involved in K_{ATP} channel regulation together with a cytosolic CK isoform. Carrasco et al. [26] have further demonstrated that the AK1 is physically associated with a K_{ATP} channels in heart cells, an observation described before for a CK [25].

In beta cells from AK1-KO mice, by contrast, an AK-dependent regulation of the K_{ATP} channel is preserved. However, this regulation is less pronounced than in wild-type beta cells. Possibly, therefore, another AK activity than AK1 must be involved in the regulation of beta cell K_{ATP} channels. Alternatively, AK1 is the only relevant K_{ATP} channel-regulating isoform in wild-type beta cells and other AK isoforms can take over this function in AK1-KO cells to compensate for its loss.

Olson and co-workers [46] have shown that increasing the glucose concentration from substimulatory to stimula-

tory values inhibits the AK-catalysed phosphorylation of AMP by ATP in HIT-T15 cells. This would result in reduced formation of ADP and thus enhanced closure of K_{ATP} channels by ATP, a situation that actually occurs after glucose stimulation in beta cells. Very importantly, the authors were able to demonstrate that the glucose-induced decrease in AK-catalysed phosphotransfer occurs prior to or at the time of K_{ATP} channel closure, and not as a consequence of events provoked by K_{ATP} channel inhibition, including membrane depolarisation, Ca^{2+} influx and insulin secretion. The authors reported [46] that glucose-induced changes of total intracellular ATP and ADP are not detectable or are marginal in HIT-T15 cells. However, overall ATP metabolic flux is increased due to the increased energy demand of glucose-stimulated secretion, resulting in augmentation of ATP hydrolytic consumption and ATP regeneration. Taken together, these data [46] strongly support the view that not bulk ATP and ADP, but the concentration of the nucleotides in the microenvironments of K_{ATP} channels determine the P_o of the channels.

Interestingly, Ap_nAs were reported to be synthesised in beta cells and to increase with rising glucose concentrations [47]. Thus, beta cell endogenous AK inhibitors may form a link between rising glucose concentrations and inhibition of AK.

For pancreatic beta cells we propose a model (Fig. 8) in which AK- and CK-mediated exchange of high-energy phosphoryl groups functions as a metabolic barrier shielding beta cell K_{ATP} channels against total cellular ATP and ADP concentrations. According to this model, ATP produced in the glycolytic-mitochondrial pathway is transferred

to the K_{ATP} channels via a PCr shuttle system and re-equilibration by coupled AK helps to determine the local ATP and ADP concentrations that govern channel behaviour. Future work should help to reveal the role of partitioning of phosphotransfer enzymes and mitochondrial distribution in cell cortical areas underneath sites of K_{ATP} in the cell membrane.

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Duality of interest The authors declare that there is no duality of interest associated with this manuscript.

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