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[\[Abstracts contents page\]](#) [\[Ion Channels abstracts\]](#)University of Leeds (2002) **J Physiol** **544P**, **S229**

Communications

***In vitro* recovery of ATP-sensitive potassium channels in β -cells from patients with hyperinsulinism in infancy; effects of low temperature and BPDZ 154**

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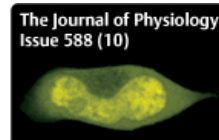
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Hyperinsulinism in infancy (HI) is the most common cause of recurrent or persistent hypoglycaemia in early childhood. The disease is principally caused by defects in the ATP-sensitive K channel (K_{ATP}) genes *ABCC8* (*SUR1*) and *KCNJ11* (*Kir6.2*), which can result in altered nucleotide regulation, channel gating, subunit assembly or subunit trafficking. Loss of K_{ATP} channels causes inappropriate Ca^{2+} channel activity and uncontrolled insulin release as a consequence. In this study we have investigated the cell surface expression of functional K_{ATP} channels in β -cells isolated from patients with HI by maintaining isolated cells under a variety of controlled conditions designed to modulate post-translational events associated with the trafficking of membrane proteins. Insulin-secreting cells were isolated from two patients with diffuse HI. Patients N79 and N94 were unrelated, and failed to respond adequately to diazoxide and/or Sandostatin or Octreotide *in vivo*. As a result both patients required a subtotal pancreatectomy to control hypoglycaemia.

Following surgery (with informed consent and Local Ethics Committee approval), a controlled collagenase digestion procedure was used to isolate intact islets of Langerhans, and single β -cells were liberated by mechanical agitation in a standard Ca-free extracellular solution. Single cells were subsequently maintained under standard tissue culture conditions at 37 °C or at either 37 or 25 °C in the presence or absence of: (1) 10 nM phorbol myristate acetate (PMA), 2 mM forskolin and 100 mM 3-isobutyl-1-methylxanthine (IBMX); (2) 2.5 mM 4-phenylbutyrate; or (3) the K_{ATP} channel agonist 10 mM BPDZ 154, for up to 40 h. The surface expression of functional K_{ATP} channels was assessed by patch-clamp methods using isolated patches of cell membrane. RNA was extracted from isolated tissue using standard protocols and RT-PCR performed to document the expression of K_{ATP} channel mRNAs using specific oligonucleotide primers directed against *SUR1* and *Kir6.2*. In N79 β -cells maintained at 37 °C, limited K_{ATP} channel activity was seen in only 38 % of cells ($n = 3/8$) as a consequence of defects in the C-terminal region of *SUR1* (oligonucleotide primers to *Kir6.2* and all three regions of *SUR1* generated PCR products in control cells, whilst in N79 only *Kir6.2* and the N-terminal region of *SUR1* were amplified). By contrast when N79 β -cells were maintained at 25 °C (either with or without exposure to 2.5 mM 4-phenylbutyrate), 73 % of cells ($n = 8/11$) expressed functional channels that responded to ADP (0.5 mM) and diazoxide (0.5 mM). Maintenance of N79 β -cells at 37 °C in the presence of 100 mM IBMX, 10 nM PMA and 2 mM forskolin did not enhance expression of functional K_{ATP} channels ($n = 3$). Under standard cell culture conditions at 37 °C, there were no operational K_{ATP} channels in N94 β -cells, $n = 6$. However, when cells were maintained at 37 °C in tissue culture media supplemented with either IBMX, PMA and forskolin or 10 mM BPDZ 154, this led to a recovery of K_{ATP} channel currents that were inhibited by ATP, $n = 4/10$ cells.

These data document that modulation of post-translational events can potentially lead to the recovery of



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endogenous K_{ATP} channel function in HI β -cells.

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