Create Account Contact Us Member Area My Physoc Profile Physiology Jobs

Member ID
Password
>> Login
>> Forgot Password

Search : >

You are here : Scientific Meetings

The Journal of Physiology Issue 588 (10)

Annual General Meeting
University of Manchester
Thursday 1 July
1.15pm

Public Lecture
Tracking down killers in the brain

International Grants
For physiology teachers & researchers of all levels
Apply by 30 June

Media Centre

Do you read Physiology News?
Take our online survey for a chance to win a fantastic netbook.

Proceedings of The Physiological Society

[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

Karen E. Cosgrove*, Ana-Maria Gonzalez*, Anne T. Lee*, Philippa Barnes*, Khalid Hussain†, Al Aynsley-Green†, Keith J. Lindley†, Bernard Pirotte‡, Philippe Lebrunñ and Mark J. Dunne*

*Biomedical Science, Sheffield University, Sheffield, UK, †Institute of Child Health, London, UK, ‡Department of Medicinal Chemistry, University of LiÅge, LiÅge, Belgium and ñLaboratory of Pharmacology, Free University of Brussels, Brussels, Belgium

Medline articles by:
Aynsley-Green, A
Gonzalez, A-M
Lee, AT
Pirotte, B
Hussain, K
Cosgrove, KE
Lindley, KJ
Dunne, MJ
Barnes, P
Lebrun, P

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-phenylbutryate; 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

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

All procedures accord with current local guidelines.

Where applicable, experiments conform with Society ethical requirements

Home | Data Protection & Privacy | Disclaimer | Sitemap | Powered by VCGenius
The Physiological Society. Peer House, Verulam Street, London WC1X 8LZ, UK. Registered Charity No. 211585