PS SA1

Protein–protein interaction and cation–chloride cotransporters

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The yeast-2-hybrid method was used to examine the interaction between intracellular domains of cation–chloride cotransporters (amino and carboxyl termini) and regulatory proteins. We have identified two Ste20-related kinases as putative interactors with cation–chloride cotransporters (Piechotta *et al.* 2002). The role of the kinases is analysed in terms of potential phosphorylation of cotransporters, as well as in terms of acting as an intermediate in the activation of MAPK pathways.

Piechotta K *et al.* (2002). *J Biol Chem* **227**, 50812–50819.

PS SA2

Physiological and pathophysiological consequences of targeted disruption of the murine Na^+–K^+–$2Cl^-$ isoform 1 (*Nkcc1*) gene

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The electroneutral Na^+–K^+–$2Cl^-$ cotransporters are expressed in a wide variety of epithelial and non-epithelial tissues. Two distinct isoforms (NKKC1 and NKCC2) have been identified to date. NKCC1 is located predominantly in the basolateral membrane of epithelia, where it is involved in fluid secretory processes. Since NKCC1 was first cloned from the shark rectal gland (Xu *et al.* 1994), Northern blot analysis has also localised this isoform in tissues as diverse as the kidney, stomach, lung, heart, skeletal muscle and neurones. However, it is only recently, since the development of the targeted gene disruption technique, that we have begun to fully understand the functional role of this important transport protein (Delpire & Mount, 2002). My presentation will discuss recent insights into the physiological role and pathophysiological consequences of disruption of the *Nkcc1* gene, with particular reference to the effects on inner ear function (Delpire *et al.* 1999), and salivary gland secretion (Evans *et al.* 2000).

Delpire E *et al.* (1999). *Nature Genetics*, 192–195.

Delpire E & Mount DB (2002). *Ann Rev Physiol* **64**, 803–843.

Evans RL *et al.* (2000). *J Biol Chem* **275**, 26720–26726.

Xu J-C *et al.* (1994). *Proc Natl Acad Sci USA* **91**, 2201–2205.

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PS SA3

Structure–function relationships in the Na^+-coupled Cl^- transporters

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PS SA4

Diuretics and renal sodium transporters

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In the kidney, thiazide and loop diuretics are secreted from proximal tubule via organic anion transporters and bind to apical sodium transporters, viz. the Na^+–Cl^- cotransporter in the distal convoluted tubule and the Na^+–K^+–$2Cl^-$ cotransporter in the thick ascending limb, respectively. Studies in animal models suggest that expression of the transporters may be affected by long-term diuretic administration at the target sites of renal tubule as well as at downstream segments. This information will be of help in understanding the adaptive responses to long-term diuretic use.

Abdallah JG *et al.* (2001). *J Am Soc Nephrol* **12**, 1335–1341.

Na KY *et al.* (2003). *Am J Physiol Renal Physiol*, F133–143.

PS SA5

Differential expression of cation–chloride cotransporters in rat pancreatic α-cells and β-cells

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An increase in plasma glucose concentration stimulates insulin secretion by causing a depolarisation of β-cell membrane potential. The closure of K_{ATP} channels is a major factor in the depolarisation, but the activation of anion channels may also contribute (Best *et al.* 1997). By contrast to β-cells, glucagon secretion from α-cells is inhibited by an increase in plasma glucose (Best & McLaughlin, 2003). We are currently investigating the hypothesis that the activation of volume-activated anion channels by glucose, causes a hyperpolarisation of the α-cell membrane potential and a depolarisation of the β-cell.

To test this hypothesis, the expression of cation–chloride transporters in cells of the pancreatic islets has been investigated. Islet cells were isolated from the pancreas of rats which had been killed by inhalation of an overdose of halothane . In β-cells there is functional and immunocytochemical evidence for the expression of NKCC1 (Majid *et al.* 2001). NKCC1 is not, however, expressed in α-cells. Thus, NKCC1 (a Cl^- accumulator) is expressed in cells in which Cl^- is above equilibrium, i.e. activation of anion channels is excitatory. RT-PCR has demonstrated the expression of mRNA for KCC3a, KCC3b and KCC4 in pancreatic islet cells. Cell volume studies suggest that these KCCs are expressed in α-cells, but not β-cells (Davies *et al.* 2002). This conclusion is supported by preliminary immunocytochemical data, which show that the KCC proteins are coexpressed with glucagon, but not insulin or somatostatin.

These data therefore indicate that KCCs (Cl^- extruders) are expressed in the α-cells, in which Cl^- must be below equilibrium, i.e. activation of Cl^- channels is inhibitory.

In conclusion, KCCs are expressed in α-cells and NKCC1 in β-cells. The data are therefore consistent with the different putative roles of anion channels in these cells.

Best L *et al.* (1997). *Exp Physiol*, 957–966.

Best L & McLaughlin J (2003). *Curr Op Genet* (in press).

Davies SL *et al.* (2002). *J Physiol* **544.P**, 109*P*.

Majid A *et al.* (2001). *Pflugers Arch* **442**, 570–576.

PS SA6

Physiological roles of KCC transporter isoforms revealed by knock-out mouse models

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Hamburg

PS SA7

A role for KCCs in human cervical carcinogenesis

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Human cervical carcinogenesis is accompanied by the over-expression of mRNA transcripts for KCC 1, KCC 3 and KCC 4. KCC 3 is the most abundant isoform in these cells, where its function may be in cell proliferation. Following expression in 3T3 cells, KCC 3 is not involved in RVD, and is not activated by hypotonic shock. There is differential expression of KCC 3 during the cell cycle. Inhibition of KCC 3 with DIOA or a dominant negative leads to inhibited cell growth and decreased tumour potential.

Shen MR *et al.* (2001). *J Physiol*, 347–362.

Shen MR *et al.* (2001). *Proc Natl Acad Sci U S A* **98**, 14714–14719.