Glucagon activates calcium and chloride conductance in rat hepatocytes

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Glucagon is a 29-amino acid peptide hormone which, together with insulin, is the key regulator of glucose homeostasis. The main target for glucagon is the liver where it activates glucose synthesis and release and inhibits synthesis of glycogen. While most of the cellular effects of glucagon are mediated by cyclic AMP, increases of $[Ca^{2+}]_{cyt}$ also play an important role, as there is evidence that glucagon is able to induce Ca^{2+} influx in hepatocytes. The nature of this Ca^{2+} influx pathway and the mechanism of its activation, however, are not known.

In the present work we investigated the effects of glucagon on the membrane conductance of rat hepatocytes by patch clamping. For isolation of hepatocytes, fed male hooded Wistar rats were anaesthetised with Nembutal (60 mg/kg, i.p.) and surgically prepared for liver perfusion. The liver was perfused in situ for 15 min with collagenase containing solution and hepatocytes isolated as described previously(1). The animals do not recover consciousness. Patch-clamp recording was conducted in the whole-cell or perforated-patch mode using standard procedures after 24-48 hours.

Glucagon (1-100 nM) activated an outwardly rectifying current after a delay of 40-60s. At membrane potential of 100 mV, the maximal amplitude of the current developed in response to glucagon ranged between 5–50 pA/pF. The current amplitude depended on the Ca^{2+} concentration in the bath, and the current was blocked by the Ca^{2+} channel blocker La^{3+} at 100 nM. Analysis of the current block by La^{3+} suggested that the inward current had two components: one carried by Cl^- and another by Ca^{2+}. Activation of both Ca^{2+} and Cl^- currents in response to glucagon was completely abolished by the phospholipase C inhibitor U73122, while the Cl^- current was partially inhibited by genistein (100 ìM) and a PKC inhibitor chelerythrine (2 ìM). Inhibitors and activators of PKA had little effect on the glucagon response. It is concluded that glucagon activates Ca^{2+} channels by activating PLC (*c.f.* the actions of Ca^{2+} mobilising hormones such as vasopressin and ATP), and Cl^- channels through a PKC-dependent mechanism.

(1) 1. MN Berry, AM Edwards, GJ Barritt, In: RH Burdon, PH van Knippenberg (Eds.), Laboratory Techniques in Biochemistry and Molecular Biology, Vol. 21, Elsevier, Amsterdam, 1991, pp. 15-81.