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SPECIAL REPORT

Induction of a glibenclamide-sensitive K-current by modification of a delayed rectifier channel in rat portal vein and insulinoma cells

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In insulinoma cells (RINm5F), the glibenclamide-sensitive K-current ($I_{K(ATP)}$) which developed spontaneously or after exposure to levcromakalim or to butanedione monoxime was always accompanied by a reduction in the delayed rectifier current ($I_{K(V)}$). At potentials over which $I_{K(V)}$ was fully activated, the total outward current remained constant. In rat portal vein, the delayed rectifier channel inhibitor, margatoxin, reduced the combined induction of $I_{K(ATP)}$ and inhibition of $I_{K(V)}$ by levcromakalim. These data suggest that the ATP-sensitive K-channel, $K_{(ATP)}$, is a voltage-insensitive state of the delayed rectifier, K_V.

Keywords: Levcromakalim; RINm5F; K_V; K_{ATP}; K-channel; portal vein

Introduction In rat portal vein, we have demonstrated that K-channel openers or procedures designed to reduce channel phosphorylation stimulate a non-inactivating K-current ($I_{K(ATP)}$) and simultaneously inhibit a delayed rectifier K-current ($I_{K(V)}$). We thus concluded that the effects of levcromakalim were consistent with an action on K_V via removal of its voltage-sensitivity (Edwards *et al.*, 1993).

We have now studied the possible conversion of $I_{K(V)}$ into $I_{K(ATP)}$ in insulinoma cells and extended our observations on portal vein to include margatoxin, a potent inhibitor of K_V (Garcia-Calvo *et al.*, 1993).

Methods Single rat portal vein cells were isolated and recordings made as previously described (Ibbotson *et al.*, 1993). RINm5F cells were maintained in culture. The composition of the solutions used was (mM): pipette – NaCl 5, KCl 120, $MgCl_2$ 1.2, K_2HPO_4 1.2, glucose 11, HEPES 10, EGTA 1.2, oxalacetic acid 5, sodium pyruvate 2, sodium succinate 5, pH 7.3; bath (oxygenated) – NaCl 125, KCl 4.8, $MgCl_2$ 3.7, K_2HPO_4 1.2, glucose 11, HEPES 10, EGTA 1, pH 7.3. All experiments were performed at room temperature (22–25°C).

Results When RINm5F cells were voltage-clamped at −90 mV and stepped in 10 mV increments to test potentials more positive than −40 mV, a delayed rectifier K-current ($I_{K(V)}$) was observed. At potentials more negative than −40 mV only a non-inactivating cation current (I_{NI}) was present. When cells were clamped for 1 min at −10 mV, $I_{K(V)}$ inactivated, leaving only I_{NI}. With time, an additional non-inactivating K-current developed (peak approximately 12 min) and simultaneously $I_{K(V)}$ declined (Figure 1a). The non-inactivating K-current ($I_{K(ATP)}$) was totally inhibited by 10 μM glibenclamide (data not shown). $I_{K(V)}$ was obtained by digital subtraction of the non-inactivating currents ((I_{NI} or $I_{NI} + I_{K(ATP)}$) from currents obtained at similar test potentials after stepping from −90 mV. At the more positive test potentials, total current remained constant with time (Figure 1b).

In some cells under conditions in which the extracellular glucose concentration was increased to 22 mM, $I_{K(ATP)}$ did not develop. Application of levcromakalim (30 μM) or the 'chemical phosphatase', butanedione monoxime (BDM, 5 mM), induced $I_{K(ATP)}$ and inhibited $I_{K(V)}$ (Figure 1c,d).

Applying the same voltage-step protocols to rat portal vein cells, only $I_{K(V)}$ and I_{NI} were observed under control conditions. Levcromakalim (1 μM) induced $I_{K(ATP)}$ and simultaneously inhibited $I_{K(V)}$. Margatoxin (100 nM) had no effect on $I_{K(V)}$. However, after pre-exposure to margatoxin and in its continued presence, the ability of levcromakalim to induce $I_{K(ATP)}$ and to inhibit $I_{K(V)}$ were both markedly reduced (Figure 2).

Discussion Recent experiments on rat portal vein cells have suggested that K_{ATP} is a partially-dephosphorylated state of

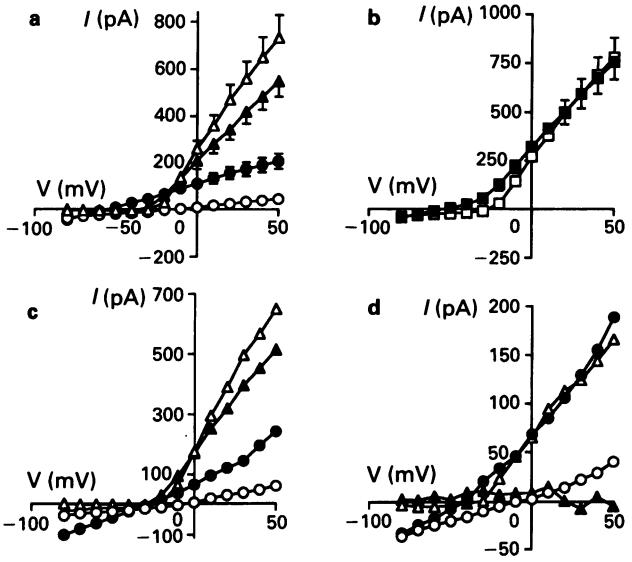

Figure 1 Effect of time on whole-cell currents in RINm5F cells: (a) 14 min after formation of the whole-cell recording configuration a non-inactivating current ($I_{K(ATP)}$, ●) had developed and $I_{K(V)}$ (▲) was reduced. However, the total current at this time (■) was similar to the initial total current (□) (b). Levcromakalim (10 μM, c) and butanedione monoxime (5 mM, d) similarly induced $I_{K(ATP)}$ (●) and inhibited $I_{K(V)}$ (▲); (△) and (○) indicate control $I_{K(V)}$ and non-inactivating currents, respectively. In (a) and (b), each point represents the mean ± s.e.mean, $n = 3$. (c) and (d) each represent the result from a single cell.

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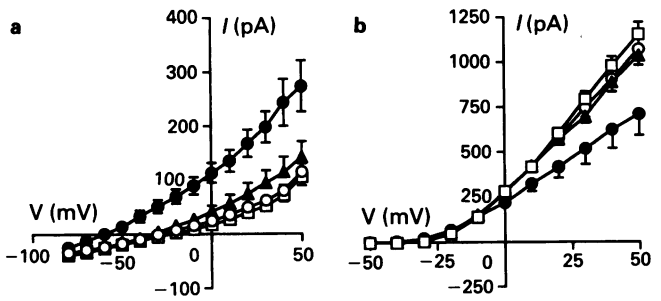

Figure 2 Effect of margatoxin on response to levcromakalim in rat portal vein cells. Levcromakalim (1 μM, ●) induced $I_{K(ATP)}$ (a) and inhibited $I_{K(V)}$ (b). Margatoxin alone (100 nM, □) had no effect but inhibited the effects of levcromakalim (1 μM, ▲); (○) indicates control currents. Each point represents the mean ± s.e.mean, $n = 5$.

K_V, consistent with findings that inhibitors of $I_{K(V)}$ also inhibit $I_{K(ATP)}$ (see Edwards *et al.*, 1993). A key physiological role of K_{ATP} is the regulation of insulin secretion in pancreatic β-cells. We therefore examined the relationship between $I_{K(V)}$ and $I_{K(ATP)}$ in an insulinoma cell (RINm5F). In this cell, as in the β-cell, K_{ATP} is considered to be almost maximally inhibited by physiological concentrations of intracellular ATP ($[ATP]_i$), and only to open as $[ATP]_i$ falls (Harding *et al.*, 1993).

Immediately after cell breakthrough only $I_{K(V)}$ and I_{NI} were detected in RINm5F cells, with no evidence of glibenclamide-sensitive currents. However, the magnitude of $I_{K(V)}$ declined with time and simultaneously a glibenclamide-sensitive K-current ($I_{K(ATP)}$) developed. In the potential range + 30 to + 50 mV, at which $I_{K(V)}$ was probably fully activated, the total outward current after development of $I_{K(ATP)}$ (i.e. $I_{K(V)} + I_{NI} + I_{K(ATP)}$) was essentially identical to that immediately after breakthrough ($I_{K(V)} + I_{NI}$). Furthermore, in RINm5F cells in which the spontaneous induction of $I_{K(ATP)}$ was suppressed by additional glucose, levcromakalim and a 'chemical phosphatase', BDM, each induced a glibenclamide-sensitive K-current and simultaneously inhibited $I_{K(V)}$. Again, the total outward current at the most positive test potentials remained constant. In rat portal vein cells, margatoxin, a potent inhibitor of voltage-dependent K-channels ($K_{V1.3}$; Garcia-Calvo *et al.*, 1993), surprisingly had no effect on $I_{K(V)}$. Nevertheless, this agent antagonized the combined induction of $I_{K(ATP)}$ and the inhibition of $I_{K(V)}$ by levcromakalim.

We cannot yet totally exclude the possibility that K_V and K_{ATP} are two independent K-channel types. However, in both RINm5F cells and in rat portal vein cells, evidence is accumulating which suggests that K_{ATP} and K_V are not separate entities. $K_{(V)}$ and $K_{(ATP)}$ possess almost identical conductances and, in general, share a common pharmacology (see Edwards *et al.*, 1993). Although glibenclamide is a reasonably selective inhibitor of K_{ATP}, high concentrations of this agent do inhibit K_V (Beech *et al.*, 1993). Furthermore, glibenclamide binding is inhibited under phosphorylating conditions (Schwanstecher *et al.*, 1991).

We thus conclude that modification of K_V (probably by partial dephosphorylation as $[ATP]_i$ falls or by levcromakalim or BDM) removes its voltage-sensitivity and simultaneously imparts increased glibenclamide-sensitivity. It is thus possible that insulin secretion in β-cells is not regulated by the voltage-insensitive channel, K_{ATP}, but rather by a modification of the voltage-sensitivity of K_V in response to fluctuating concentrations of $[ATP]_i$. Such a modification can be effected by a metabolic change or by agents such as levcromakalim or BDM which probably also reduce the degree of phosphorylation of K_V.

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