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Ligustrazine selectively blocks ATP-sensitive K^+ channels in mouse pancreatic β cells

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Ligustrazine potently inhibits hypoxic pulmonary vasoconstriction. This effect can be mimicked by the sulphonylurea tolbutamide, a blocker of ATP-sensitive K^+ channels. Here we demonstrate that ligustrazine is a specific blocker of ATP-sensitive K^+ channels in mouse pancreatic β cells. This suggests that ligustrazine may act in the pulmonary circulation by blocking ATP-sensitive K^+ channels.

Ligustrazine; ATP-sensitive potassium channel; Potassium current; Pancreatic β cell

1. INTRODUCTION

Ligustrazine is a purified and chemically identified component of a Chinese herbal remedy, which is a potent blocker of hypoxic pulmonary vasoconstriction in the rat [1]. Recent studies have shown that tolbutamide, a selective blocker of ATP-sensitive K^+ channels (K-ATP channels) [2], also blocks hypoxic pulmonary vasoconstriction [3] and that high concentrations of diazoxide (an opener of these channels in pancreatic β cells [2]) constricts the vessels of the pulmonary circulation [3]. These findings suggest that K-ATP channels are involved in the responses of the pulmonary vasculature to hypoxia and it is therefore possible that ligustrazine also exerts its effect on the pulmonary vasculature by blocking them. Such a suggestion would be supported by demonstrating that ligustrazine selectively blocks the K-ATP channels of a preparation in which they are well described. We have therefore studied the effects of ligustrazine on the K-ATP (and other) K^+ channels of pancreatic β cells from the mouse.

2. MATERIALS AND METHODS

Single pancreatic β cells of NMRI mice were isolated and maintained in primary tissue culture for up to 3 days as previously described [2,4] and their K^+ currents were recorded using the whole-cell configuration of the patch clamp technique [5]. The extracellular medium contained (in mM); NaCl, 135; KCl, 5; $MgSO_4$, 1.2; $CaCl_2$, 2.5; NaOH-HEPES, 5; 21-24°C, pH 7.4. To study K-ATP currents, the pipette solution contained (in mM); KCl, 107; $CaCl_2$, 1; $MgSO_4$, 2; NaCl, 10; K-EGTA, 11; KOH-HEPES, 11; ATP, 0.3; pH 7.2. The small amount of ATP was included to prevent K-ATP channel run-down [2]. When studying voltage-activated currents the ATP-sensitive component was blocked by increasing the intracellular ATP level to 5 mM, and adding 0.2 mM tolbutamide (Rastinon, Hoechst) to the extracellular medium. Currents were recorded using an Axopatch (Axon Instruments) and analysed as previously described [6]. Diazoxide (Eudemine) was obtained from Allen and Hanbury, Greenford, U.K. Ligustrazine was dissolved in distilled water.

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3. RESULTS

Effect of ligustrazine on K-ATP channels

We measured K-ATP currents as the response to 10 mV depolarizing voltage steps (200 ms duration, 0.5 Hz) from a holding potential of -70 mV: an experimental protocol which measures only current through K-ATP channels [2,7]. Characteristic features of this current were: (i) On first obtaining the whole-cell configuration the currents were small but then increased in amplitude (run-up) as intracellular ATP was dialysed from the cell, reflecting the relief of ATP inhibition of K-ATP channels (fig.1A). A reduction of the negative holding current accompanied this run-up. (ii) The application to the bath of 0.2 mM tolbutamide, a specific and potent blocker of K-ATP channels [2,7], resulted in rapid, reversible inhibition of this conductance and an increase in the holding current (fig.1B). The rate at which tolbutamide inhibited K-ATP currents reflected the rate of exchange of the bath solutions.

Fig.2 shows that 0.74 mM ligustrazine inhibited K-ATP currents (by 34.2 ± 1.3%, mean ± S.E.M., n = 8 cells). The response to ligustrazine was much slower than that to tolbutamide, but similar to that of the more potent sulphonylurea glibenclamide [7]. Doubling the dose of ligustrazine to 1.48 mM did not significantly change the percentage inhibition (32.4 ± 1.3%; n = 6; $P > 0.6$, unpaired Student's t-test) suggesting that the lower dose is maximal. However, 74 μM ligustrazine

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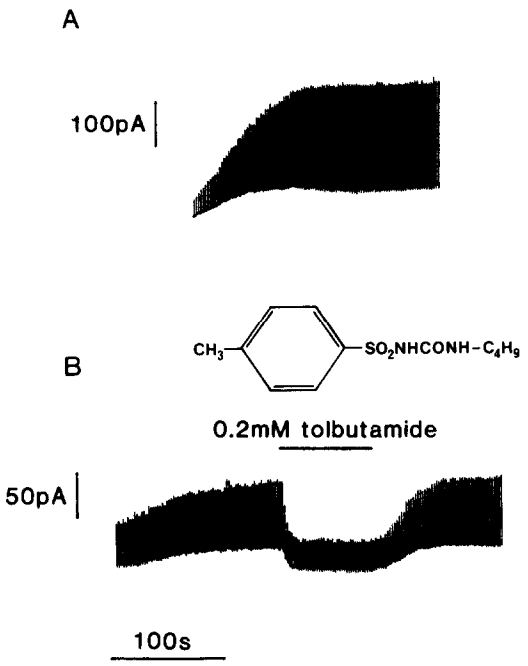

Fig.1. (A) 'Run-up' of K-ATP conductance, 20 s after achieving whole-cell configuration. (B) Inhibition of K-ATP conductance by bath application of 0.2 mM tolbutamide (horizontal bar). Insert shows structure of tolbutamide.

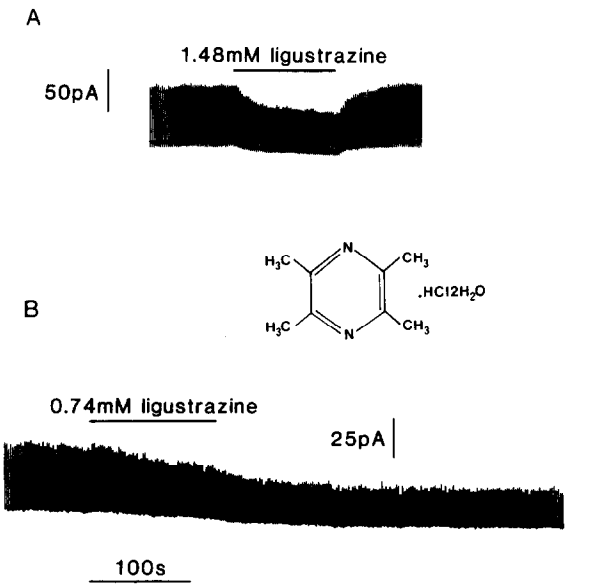

Fig.2. Effects of ligustrazine on K-ATP conductance (applied as shown by horizontal bar). (A) Record from one cell showing reversibility of inhibition, contrasting with (B) showing little or no recovery, recorded in another cell. Insert shows structure of ligustrazine.

was without detectable effect (not shown). As shown in fig.2 recovery of the K-ATP conductance following removal of 0.74 mM ligustrazine from the bath was extremely variable. This variability appeared to be independent of the duration of its application.

Diazoxide is a potent activator of K-ATP channels which reverses the blocking effect of tolbutamide [2,7]. Fig.3 demonstrates that bath application of 0.4 mM diazoxide rapidly reversed the effect of ligustrazine, evoking a current which was in many cases greater than the control level. This effect was observed both during the continued presence of ligustrazine (fig.3A) and also after its removal when the recovery of K-ATP currents was incomplete (fig.3B). This latter finding suggests that the failure of K-ATP currents to recover after ligustrazine was not due to their running-down, as diazoxide does not reactivate channels subject to run-down [7].

Effect of ligustrazine on voltage activated K-currents

Voltage activated K^+ currents elicited by stepping to various depolarized test potentials from a holding potential of -80 mV were similar to those described in the literature [4]. These outward currents arise when delayed rectifier (IK-V) and calcium activated (IK-Ca) potassium channels are activated and they are followed by a slow decay to a new steady state which consists exclusively of IK-V (fig.4A, control; [9]). Fig.4A shows that the addition of ligustrazine to the bath had no effect on the steady state outward current measured at the end of the pulse. Under these experimental conditions the calcium current runs down [8], reducing the contribution of IK-Ca and making it impossible to measure its modulation by ligustrazine accurately. However, in a few cases ligustrazine was added before the IK-Ca component had run down appreciably, and in such experiments ligustrazine had no detectable effect on the peak current amplitude (e.g. fig.4A, upper traces). At

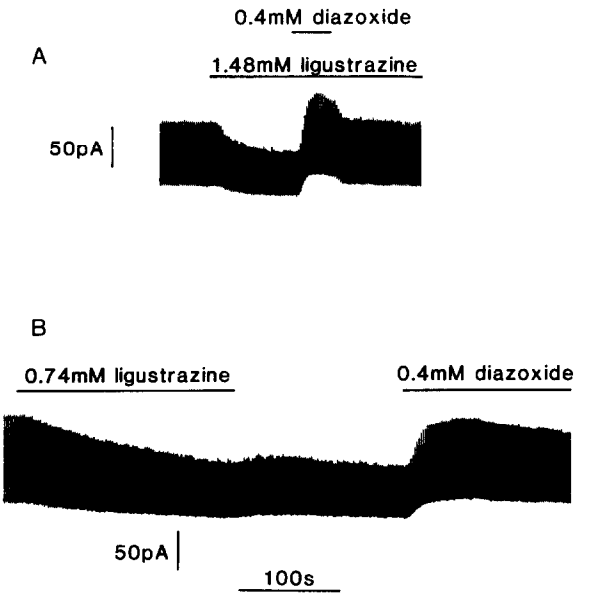

Fig.3. Activation of K-ATP conductance by 0.4 mM diazoxide, applied (A) in the presence of ligustrazine and (B) following removal of ligustrazine in a cell that did not show recovery before application of diazoxide (A and B different cells).

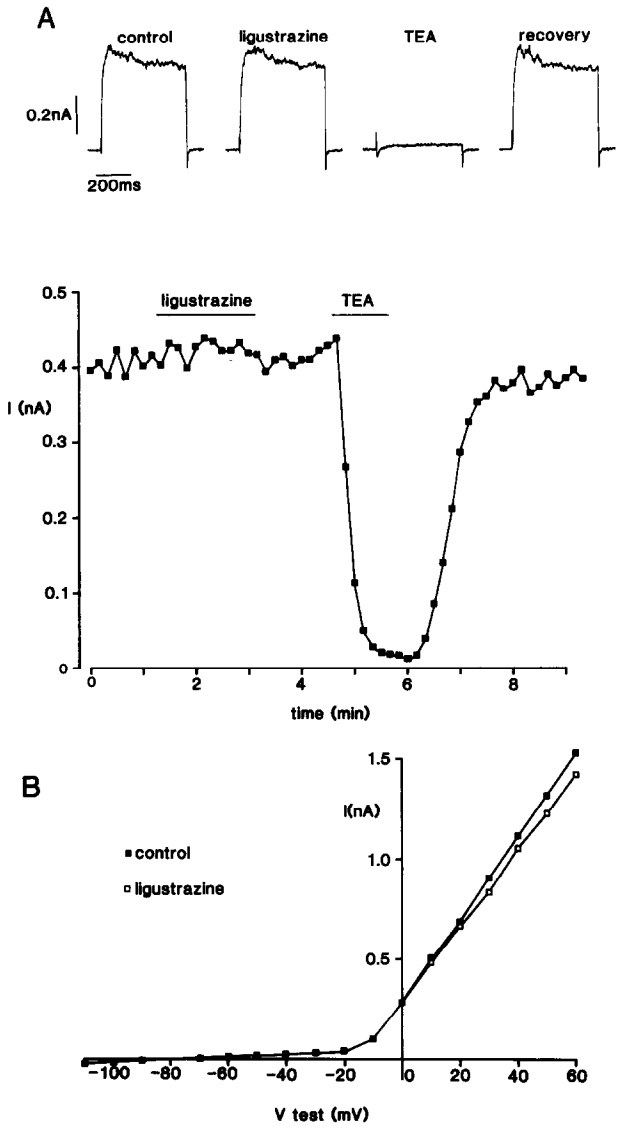

Fig.4. (A) Effects of 0.74 mM ligustrazine and 20 mM tetraethylammonium (TEA) on whole-cell K^+ current amplitudes. with K-ATP channels blocked by 5 mM ATP in the pipette and 0.2 mM tolbutamide in the bath. Upper traces, representative examples of currents activated by 500 ms depolarizing steps to +20 mV. Lower trace, time series of steady state current amplitudes (IK-V), recorded before and during application of 0.74 mM ligustrazine and 20 mM TEA, indicated by horizontal bars. (B) Current-voltage relationship of IK-V in another cell before and during application of 0.74 mM ligustrazine.

the end of 500 ms pulse, IK-Ca is always completely inactivated because its time course closely mirrors that of the transient Ca^{2+} current [9] and this allows a measure of IK-V alone. Ligustrazine (0.74 mM) was virtually without effect on IK-V, unlike 20 mM TEA which caused an almost complete block of the outward current (fig.4A), an observation consistent with its known ability to block IK-V both rapidly and reversibly at this dose [4]. Fig.4B demonstrates that ligustrazine was virtually without effect over the complete range of activating test potentials studied. Similar results were observed in 7 other cells.

4. DISCUSSION

We have demonstrated that ligustrazine is a novel blocker of K-ATP channels in mouse pancreatic β-cells which has almost no effect on IK-Ca or IK-V. Its action therefore resembles that of the sulphonylurea tolbutamide in that it appears to be specific for K-ATP channels and its effects can be reversed by diazoxide. However, ligustrazine differs from tolbutamide in the following respects: (i) its maximum effect is reached more slowly; (ii) it is less potent, maximally suppressing conductance by only 30–35% and (iii) recovery from its block is often incomplete. Such differences are perhaps not surprising in view of their dissimilar structures (figs. 1 and 2). The slow rate of onset of ligustrazine block, and in some cases the duration of its action (e.g. fig. 2B) are reminiscent of the block of K-ATP channels by glibenclamide. However, unlike glibenclamide [7], the effects of ligustrazine are reversed by diazoxide (fig. 3).

Our findings are consistent with the idea that ligustrazine blocks hypoxic pulmonary vasoconstriction by modulating K-ATP channels in the pulmonary vasculature (see section 1). We know of no reports that describe the effects of ligustrazine on blood glucose or insulin levels but suppose that this would be unlikely because it is so much less potent than tolbutamide in β cells. However, the converse is true in the pulmonary vasculature where ligustrazine is a far more potent blocker of hypoxic pulmonary vasoconstriction than tolbutamide [1,3].

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