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Differential block by troglitazone and rosiglitazone of glibenclamide-sensitive K^+ current in rat aorta myocytes

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Abstract

Thiazolidinediones are insulin-sensitising agents effective in controlling type II diabetes. These compounds also cause vasodilation. We evaluated the effects of the thiazolidinediones troglitazone and rosiglitazone on the glibenclamide-sensitive K^+ current in freshly isolated rat aorta myocytes. Troglitazone inhibited this current in a concentration-dependent manner ($IC_{50} \sim 1$ μM). Rosiglitazone had a similar, but much less potent ($IC_{50} \sim 20$ μM) action. Block of the glibenclamide-sensitive K^+ channels, in particular by troglitazone, may potentially affect the response of arteries to hypoxia and to certain endogenous and exogenous vasodilators.

Keywords: Thiazolidinedione; Troglitazone; Rosiglitazone; BRL 49653; K_{ATP} current; Glibenclamide; K^+ channel

1. Introduction

Thiazolidinediones, including troglitazone and rosiglitazone, are a new class of drugs being utilized for the treatment of noninsulin-dependent diabetes mellitus. Although their efficacy in diabetes is due to their insulin-sensitising property (Saltiel and Olefsky, 1996), thiazolidinediones also lower blood pressure and cause vasodilation (Kotchen et al., 1996, Song et al., 1997). The mechanism of vasodilation is not completely understood, although thiazolidinediones have been reported to inhibit the L-type calcium channel current in vascular smooth muscle cells (Zhang et al., 1994; Song et al., 1997).

Lee et al., (1996) have recently reported that troglitazone inhibited glibenclamide-sensitive K_{ATP} channels in an insulin-secreting cell line. In the vasculature, activation of glibenclamide-sensitive K^+ channels contributes to vasodilation evoked by adenosine and calcitonin-gene-related peptide, and by hypoxia (Nelson and Quayle, 1995). Considering the important role of these channels in controlling vascular tone, we examined the action of troglitazone and rosiglitazone on the glibenclamide-sensitive K^+ channel current in rat aorta vascular smooth muscle cells.

2. Methods

Adult male Wistar rats were killed by cervical dislocation. The thoracic aorta was removed into ice cold physiological saline solution (PSS), cleaned of fat and connective tissue, cut into small pieces and incubated at 37°C in low Ca^{2+} (15 μM) PSS for 15 min. Tissue was then transferred to the same solution containing 0.23 mg/ml of elastase Type I (Sigma) for 20 min. Tissues were then incubated in low Ca^{2+} PSS containing collagenase Type I (1 mg/ml), collagenase Type XI (1 mg/ml), papain (0.5 mg/ml) and dithiolthreitol for a further 20 min. Tissues were then washed in enzyme-free low Ca^{2+} PSS and cells were dispersed by trituration. Cells were stored in low Ca^{2+} PSS at 4°C and used within 5–6 h of their isolation. Whole-cell membrane currents were recorded using standard patch clamp techniques as described elsewhere (Smirnov et al., 1994). PSS contained (mM): NaCl, 130; KCl 5.0; $MgCl_2$, 1.2; $CaCl_2$,1.5; HEPES, 10; glucose, 10. The pH was adjusted to 7.4 with NaOH. Low-Ca^{2+} PSS contained 15 μM Ca^{2+}. Ca^{2+}-free high-K (135 mM) solution was made by replacing NaCl with an equimolar

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concentration of KCl and removing Ca from the PSS. The pipette solution contained (mM): KCl, 110; $MgCl_2$, 2.5; guanosine diphosphate (GDP) sodium salt 1.0; adenosine triphosphate (ATP) magnesium salt, 0.25; HEPES, 10; EGTA, 10 and the pH was adjusted by KOH. In some experiments, GDP was excluded from the pipette solution. Stock solutions of glibenclamide (10^{-2} M), rosiglitazone (6×10^{-2} M) and troglitazone (2×10^{-2} M) were prepared in dimethylsulphoxide. Tetraethylammonium (1 M) was dissolved in double distilled water. Thiazolidinediones were the kind gift of Dr. Robin Buckingham, SmithKline Beecham Pharmaceuticals, Harlow, UK; other chemicals were obtained from Sigma.

3. Results

3.1. Characteristics of glibenclamide-sensitive K^+ channel current in rat aorta

Rat aorta vascular smooth muscle cells were held at −60 mV. The potential was stepped to −80 mV for 50 ms, following which the potential was ramped over 1 s from −80 to +80 mV, at 0.1 Hz. The pipette solution contained 1 mM GDP and 0.25 mM ATP in order to stimulate the glibenclamide-sensitive K^+ current and prevent its rundown. In normal PSS (5 mM K^+), the current amplitude at −80 mV was -16.95 ± 2.97 pA ($n = 19$). The cells were then superfused in Ca^{2+}-free, high (135 mM) K^+ PSS containing 2 mM tetraethylammonium in order to shift the K^+ reversal potential to 0 mV, and to suppress both the Ca^{2+} current and the Ca^{2+}-activated K^+ current, which is prominent in these cells at positive potentials. There was a large inward current in this high K^+ solution at −80 mV of -436.80 ± 66.98 pA (range: −177.63 to −1182.51 pA, $n = 19$) which was reduced to -51.16 ± 9.40 pA ($n = 19$) in the presence of 10 μM glibenclamide. This current was, therefore, almost entirely mediated by glibenclamide-sensitive K^+ channels. A glibenclamide-sensitive current was also observed in cells dialysed with a solution containing neither ATP nor GDP. This tended to be smaller (but not significantly, probably due to the variability in current amplitude) than that seen if the pipette solution contained GDP and ATP (-285 ± 72 pA at −80 mV, range −94 to −599, $n = 6$). Subsequent studies of the effects of thiazolidinediones were carried out using the GDP–ATP containing pipette solution; under these conditions, the current demonstrated negligible rundown. Fig. 1 shows the time-course of the I_{glib} which reached a peak about 2 min after the solution was changed from one containing 5 mM K^+ to the one containing Ca^{2+}-free, 135 mM K^+ PSS with 2 mM tetraethylammonium ($n = 5$). The current in these experiments was measured, following a 5-ms delay to allow the capacitance artifact to decay completely, during the 50-ms step to −80 mV which preceded the voltage ramp.

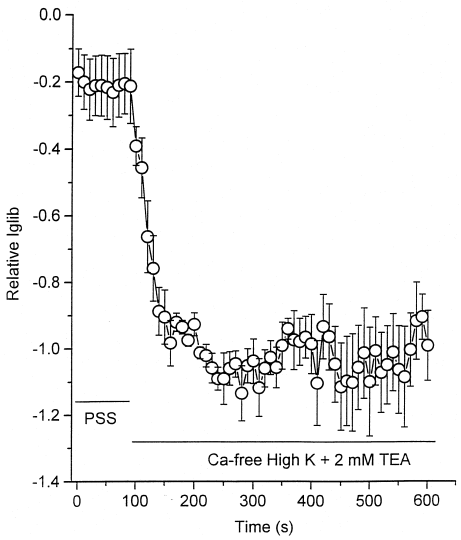

Fig. 1. Stability over time of the inward K^+ current measured at −80 mV in cells bathed first in 5 mM PSS and then in Ca^{2+}-free, 135 mM K^+ solution with 2 mM tetraethylammonium. The ordinate represents the mean ± S.E.M. ($n = 5$) of the normalised amplitude of the current, which in each cell was obtained by dividing the current amplitude (relative to the zero current level) at each time by the maximum inward current observed in that cell in the high K^+/tetraethylammonium solution. As can be noted, the current showed little run down ($n = 5$).

3.2. Effect of rosiglitazone on glibenclamide-sensitive K^+ current

Fig. 2A shows typical current ramps obtained in one cell in normal PSS, in high K^+ solution and then after the addition of 2 and 120 μM rosiglitazone. Rosiglitazone caused a concentration-dependent reduction of current amplitude. The current ramp elicited in high K^+ solution when 10 μM glibenclamide was subsequently applied is also illustrated. This drug profoundly reduced current amplitude over the entire range of the potential ramp, showing that the glibenclamide-sensitive current dominated membrane conductance under these conditions. In several preliminary experiments, glibenclamide had a similar effect on the current in high K^+ solution in cells which had never been exposed to rosiglitazone.

The time-course of the changes in the current observed in this cell at −80 mV during the solution changes described above is shown in Fig. 2B. A stable block of glibenclamide-sensitive current developed 2–3 min after superfusion of rosiglitazone was initiated. Inhibition by 120 μM rosiglitazone was completely reversible on washing. The recovered current was then almost abolished by 10 μM glibenclamide. Fig. 2B also demonstrates the total recovery of I_{glib} from glibenclamide-block on wash.

3.3. Effect of troglitazone on glibenclamide-sensitive K^+ current

Fig. 3A shows typical currents elicited during voltage ramps in the presence of normal PSS, high K^+ solution, 10

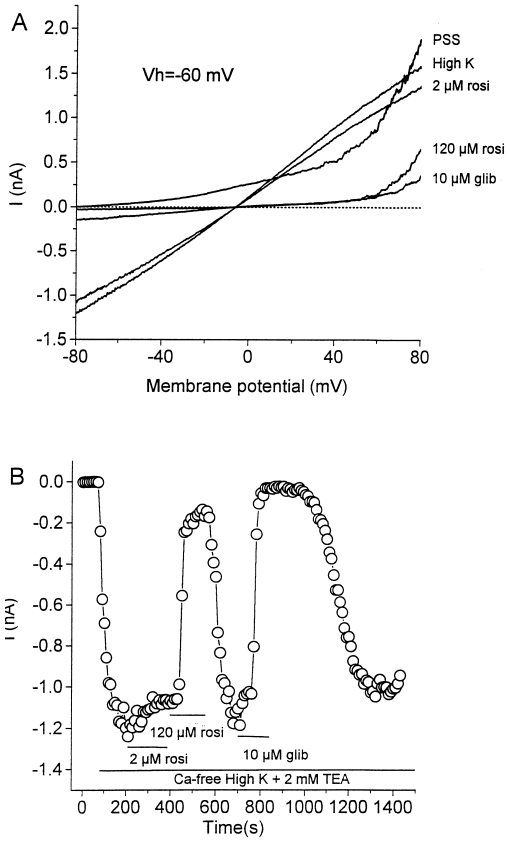

Fig. 2. Effects of rosiglitazone on glibenclamide-sensitive K^+ current. (A) Currents elicited in a typical cell, in: PSS (5 mM K^+ solution), high K^+ (135 mM) solution (containing no added Ca^{2+} and 2 mM tetraethylammonium), high K^+ solution with 2 and 120 μM rosiglitazone and high K^+ solution with 10 μM glibenclamide. (B) Time-course of the current amplitude measured at −80 mV under the conditions indicated.

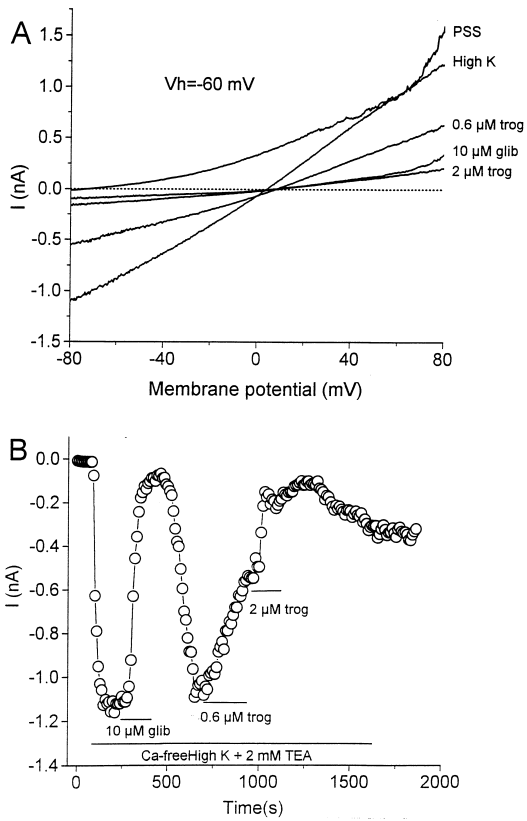

Fig. 3. (A) Currents elicited in a typical cell, in normal (5 mM) K^+ solution, high K^+ (135 mM) solution, high K^+ solution with 10 μM glibenclamide and on recovery from glibenclamide block high K^+ solution containing either 0.6 or 2 μM troglitazone. (B) Time-course of the current amplitude measured at −80 mV under the conditions indicated.

μM glibenclamide and 0.6 and 2 μM troglitazone in the presence of high K^+solution. In this cell, 10 μM glibenclamide completely inhibited the inward-current recorded at the membrane potential of −80 mV from a Vh of −60 mV. Following wash, I_{glib} almost completely recovered from glibenclamide-block and the current was then dose-dependently inhibited by 0.6 and 2 μM troglitazone. The time-course of changes in the current at −80 mV during this experiment is presented in Fig. 3B. As can be seen from this figure, there was poor recovery of I_{glib} from block produced by 2 μM troglitazone. In three cells dialyzed with a pipette solution lacking both ATP and GDP, where the inward current at −80 mV was -318 ± 60 pA, this current was reduced to -33 ± 2 pA by 2 μM troglitazone, and after washout of troglitazone, to -35 ± 7 pA by 10 μM glibenclamide.

The concentration–response curves showing the inhibitory effects of rosiglitazone (2, 10, 60 and 120 μM) and troglitazone (0.2, 0.6 and 2 μM) on the glibenclamide-sensitive K^+ current are depicted in Fig. 4. The current was attenuated by approximately 50% by 1 μM troglitazone and 20 μM rosiglitazone.

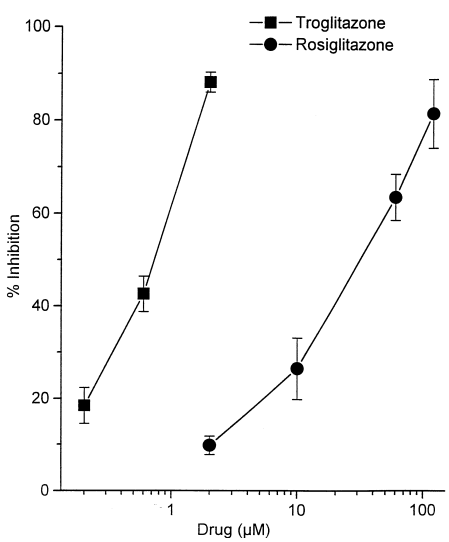

Fig. 4. Effects of several concentrations of troglitazone and rosiglitazone on the glibenclamide-sensitive inward-current measured at −80 mV. Percentage inhibition was calculated as $(I_{con} - I_{drug})/(I_{con} - I_{glib})$, where I_{con} is the control current measured in the high K^+ solution before addition of drug, I_{drug} is the current amplitude in troglitazone or rosiglitazone, and I_{glib} is the current amplitude after glibenclamide was applied ($n = 4$–11 for each concentration of drug).

4. Discussion

Characterisation of glibenclamide-sensitive K^+ currents in the vasculature is incomplete, and there is substantial evidence that these channels are heterogeneous (Nelson and Quayle, 1995; Zhang and Bolton, 1996). It has been widely demonstrated that nucleotide diphosphates such as GDP enhance the glibenclamide-sensitive K^+ currents elicited by ATP depletion, (e.g., Beech et al., 1993; Clapp, 1995). Nevertheless, in comparison to GDP alone, I_{glib} was better maintained if ATP was also present in the pipette solution in long whole-cell recordings (Beech et al., 1993). We also found that including 0.25 mM ATP in 1 mM GDP-containing pipette solution tended to both enhance the glibenclamide-sensitive current and prevent its rundown and as a result we used the ATP–GDP-containing solution routinely when evaluating the blocking effects of the thiazolidinediones. It is noteworthy, however, that 2 μM troglitazone had a similar inhibitory effect on the glibenclamide-sensitive current when no nucleotides were present in the pipette solution.

The results of the present study show that both rosiglitazone and troglitazone were able to markedly attenuate the glibenclamide-sensitive K^+current in rat aorta myocytes. As compared to rosiglitazone, troglitazone was more than 20 times as potent in inhibiting this current. The potency of block of the rat aorta glibenclamide-sensitive current by troglitazone is similar to that observed for the K_{ATP} channel in insulinoma cells (IC_{50} = 0.7 μM, Lee et al., 1996). Troglitazone has been shown to inhibit the Ca^{2+} current over a somewhat higher concentration range (IC_{50} between 6 and 10 μM) in A7r5 cells, and smooth muscle cells from rat tail artery myocytes (Song et al., 1997). In rat aorta, troglitazone and rosiglitazone inhibit the Ca^{2+} current with IC_{50} values of approximately 2 and 20 μM, respectively (Knock et al., 1999), and it is the effect of troglitazone on the Ca^{2+} current which may mediate the increased skin blood flow in the rat in vivo (Fujiwara et al., 1995) and the decrease peripheral vascular resistance in man (Ghazzi et al., 1997). Conversely, the much less potent effect of rosiglitazone on the Ca^{2+} current is consistent with its reported inability to relax human subcutaneous resistance arteries preconstricted with noradrenaline (Walker et al., 1998).

Whether or not the thiazolidinediones have in vivo effects resulting from the inhibition of the glibenclamide-sensitive current described above will depend on their effective concentrations at their site(s) of action. In humans following once a day dosing of 600 mg troglitazone, the median trough concentration was found to be 0.33 μg/ml (746.8 nM; Young et al., 1995). This value is well within the concentration range that inhibits I_{glib} channels in vitro as observed in the present study. Since troglitazone inhibits the glibenclamide-sensitive K^+ current at concentrations even lower than those which attenuate the Ca^{2+} current, it is possible that the former effect may have haemodynamic consequences. This possibility is further supported by the fact that the K_i for binding to the peroxisome proliferator activated receptor γ, the therapeutic target for the antidiabetic action, was found to be 3.0 μM in vitro (Cobb et al., 1997).

Therefore, haemodynamic changes might occur which would be expected to resemble those caused by glibenclamide itself. For example, glibenclamide has been shown to decrease coronary blood flow in dogs (Dunker et al., 1993), to increase resistance in the diaphragmatic, renal and skeletal muscle circulations (Comtois et al., 1994; Gardiner et al., 1996), and to inhibit cerebral (Hong et al., 1994) and coronary autoregulation in vivo (Komaru et al., 1991). It is in addition possible that antagonism of I_{glib} channels could blunt the vasodilator action of endogenous vasodilators such as calcitonin gene-related peptide and adenosine which activate these channels, and might therefore affect reactive hyperaemia. Vasodilation in pathophysiological conditions such as hypoxia (Nelson and Quayle, 1995) could also be reduced. In addition, vasodilator effects of K^+ channel agonists such as minoxidil and nicorandil, and levecromakalin might also be antagonized. The haemodynamic consequences of interactions of thiazolidinediones with vascular ion channels may therefore be more complex than has previously been supposed.

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