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Short communication

WAY 120,491 activates ATP-sensitive potassium channels in rat tail artery

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WAY 120,491 lowers blood pressure in a number of animal models. Its mechanism of action appears to be the selective opening of K^+ channels in vascular smooth muscle cells. The patch clamp technique was used to determine the specific K^+ channel affected by WAY 120,491 in isolated tail artery cells of the rat. In the inside-out configuration, WAY 120,491 caused a dose dependent partial reversal of the ATP-induced inactivation of channel activity. Glyburide (10 μ M) almost completely inhibited the effect of WAY 120,491. WAY 120,491 had no effect on Ca^{2+} -activated K^+ channels inactivated by the absence of Ca^{2+} . In the whole cell configuration, 10 μ M WAY 120,491 increased outward K^+ current. 10 μ M glyburide completely reversed the increase in current. WAY 120,491 appears to be a relatively specific activator of ATP-sensitive K^+ channels in vascular smooth muscle.

K^+ channels; ATP; Smooth muscle (vascular); Patch clamp

1. Introduction

During the past several years several chemically distinct compounds, e.g., pinacidil, diazoxide and cromakalim, have been shown to lower blood pressure by reducing peripheral resistance (Falotico et al., 1989). The main mechanism of action of these drugs appears to involve an increase in the efflux of K^+ ions from vascular smooth muscle cells and the resultant cell membrane hyperpolarization. Specifically, these compounds increase the open probability of ATP-sensitive K channels (K_{ATP}) (Standen et al., 1989; Furspan and Webb, in press).

WAY 120,491 [(–)-(3S-trans)-2-[3,4-dihydro-3-hydroxy-2,2-dimethyl-6-(trifluoromethoxy)-2H-1-benzopyran-4-yl]-2,3-dihydro-1H-isoindol-1-one] is a novel benzopyran derivative that has been shown to lower blood pressure in a number of animal models (Oshiro and Colatsky, 1991). Pre-clinical data on this compound indicate that, as is the case for the drugs mentioned above, its mechanism of action is the selective opening of K^+ channels in vascular smooth muscle cells. WAY 120,491 increases the efflux of both ^{86}Rb

and ^{42}K from rabbit aorta and rat portal vein (Edwards et al., 1990; Lodge et al., 1991). This effect could be blocked by glyburide, a specific antagonist of K_{ATP} . On the basis of this ability of glyburide to antagonize the relaxant effect of WAY 120,491 in vitro, it has been postulated that this drug interacts with the ATP-sensitive K^+ channel.

In this study the patch clamp technique was used to determine the specific K channel affected by WAY 120,491 (supplied by Wyeth-Ayerst Research, Princeton, N.J.) in isolated tail artery cells of the rat.

2. Materials and methods

Adult male and female Wistar-Kyoto (WKY) rats (16–20 weeks old) were obtained from rat colonies maintained in the Department of Anatomy and Cell Biology, University of Michigan.

To isolate vascular smooth muscle cells, tail artery segments were incubated in digestion medium (mg/ml): collagenase (0.5), trypsin inhibitor (0.3), papain (0.4), dithiothreitol (0.3), bovine serum albumin (7.5); pH 7.4 in physiological salt solution (PSS) [(mmol/l): NaCl 140; KCl 5.4; KH_2PO_4 0.44; NaH_2PO_4 0.42; $NaHCO_3$ 4.17; $CaCl_2$ 0.5; HEPES 5; dextrose 5.55; pH adjusted to 7.4 with 1 N NaOH] for 90 min at 37°C. Cells were then dispersed by trituration with fire-polished Pasteur pipettes of decreasing tip diameter.

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Single channel and whole cell K^+ currents were measured with standard patch clamp technique. A Dagan 8900 Patch Clamp/Whole Cell Clamp (Minneapolis, MN USA) was used to voltage clamp membrane in the whole cell and inside-out configurations. Single channel data were digitized using a modified digital audio processor (Sony PCM-701ES, Medical Systems Corp, Greenvale, NY USA) and recorded on videocassette tape for analysis. Whole cell data were stored on computer hard disk. Electrodes were prepared from borosilicate glass (Kimble R-6, Richland Glass, Richland, NJ USA).

The bath in the whole cell configuration and the pipette in the inside-out configuration contained (in mM): 145 KCl, 10 HEPES (N-[2-hydroxyethyl]piperazine-N'-(2-ethanesulfonic acid)), 10 glucose and KOH to bring pH to 7.4. A Ca^{2+} -buffered solution was used as the transmembrane solution for both configurations. It contained (in mM): 113 KCl, 2.02 EGTA, 20.2 K_2 PIPES (piperazine-N,N'-bis[2-ethanesulfonic acid]); pH 7.0. For the whole cell experiments the pipette solution contained 1 mM ATP. Sufficient $CaCl_2$ was added to yield a calculated free Ca^{2+} concentration of 100 nM as described by Chang et al. (1988).

Computer analysis of single channel records was performed on a Gateway 2000 computer using

pCLAMP software (Axon Instruments) and auxiliary programs designed to read pCLAMP files. The auxiliary programs were used to calculate mean current from single channel records (Richards et al., 1989).

Mean current was defined as the total charge carried through the channel(s) in the patch divided by the total time of the current record. Segments of data files containing digitized current records were identified which represented closed channel baseline. Mean current was then defined as the mean deviation from the defined baseline over the course of the record. Appropriate scaling factors were used to express the mean current in picoamperes (pA). This analysis can be applied to any patch in which a stable closed current baseline can be identified and therefore be used to analyze records containing more than one active channel and other current records for which an idealized record cannot be easily constructed.

3. Results

3.1. Inside-out configuration

A 1 min baseline record was obtained at +20 mV prior to adding to the bath in a cumulative fashion 1

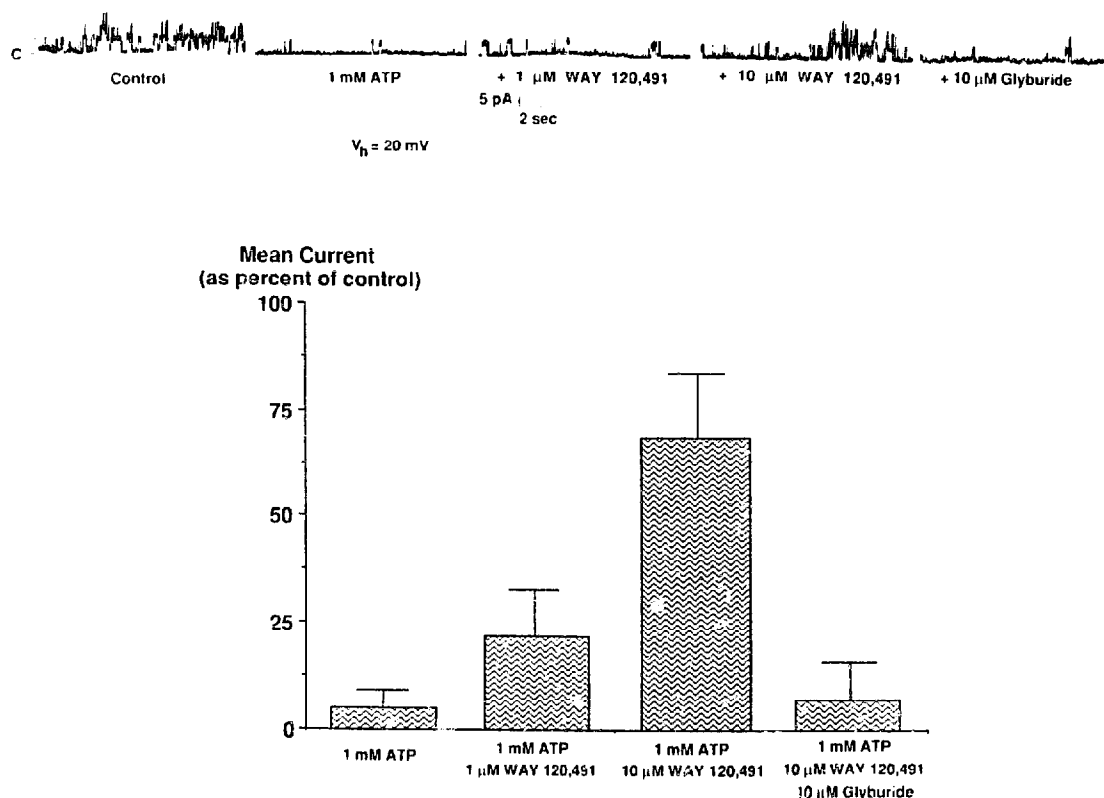


Fig. 1. Single channel currents of K_{ATP} recorded from inside-out patches of rat tail artery cell membrane. Top: representative tracings of K_{ATP} under control conditions (see text) and after the cumulative addition of ATP, WAY 120,491 and glyburide to the bath. Bottom: average response of K_{ATP} to ATP, WAY 120,491 and glyburide presented as percent of control mean current \pm S.E.M. Sample size is 6.

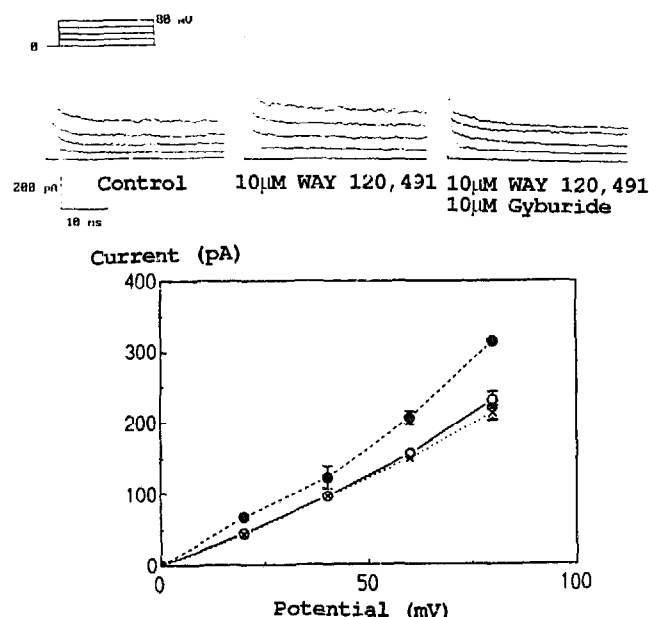


Fig. 2. Outward whole-cell K^+ current in rat tail artery cells. Top: representative tracings showing outward K^+ current in response to depolarizing command potentials from 0 to 80 mV in 20 mV increments under control conditions and in the presence of 10 μ M WAY 120,491 and glyburide. Bottom: mean current-voltage relationships of outward K^+ current ($n = 5$ in each group) under control conditions (open circles) and in the presence of WAY 120,491 (filled circles) and glyburide (\times). Values are the means \pm S.E.M. * $P < 0.05$, different from control.

nM ATP, 1 μ M WAY 120,491, 10 μ M WAY 120,491, 10 μ M glyburide. Activity was recorded for 1 min after each addition. In one patch from each of four rats, WAY 120,491 caused a dose-dependent partial reversal of the ATP-induced inactivation of channel activity (fig. 1). Glyburide (10 μ M) almost completely reversed the activation of channel activity caused by WAY 120,491 (fig. 1). WAY 120,491 had no effect on Ca^{2+} -activated K^+ channels inactivated by the absence of Ca^{2+} (data not shown).

3.2. Whole cell configuration

Stable baseline current-voltage relationships were obtained after an initial 3 min equilibration period (the decrease in K^+ current observed during this period was probably due to the reduction in free Ca^{2+} caused by equilibration of the cytoplasm with the Ca^{2+} -buffered pipette solution). Figure 2 (top) presents the results of a representative experiment in which outward K^+ current was measured under control conditions and after the addition of 10 μ M WAY 120,491 and 10 μ M glyburide. The mean response to these compounds is presented in the current vs voltage plot in the bottom part of fig. 2.

4. Discussion

The results of this study support and extend previous work on the mechanism of the vasodilatory and antihypertensive action of WAY 120,491. It is now apparent that the WAY 120,491-induced increase in K^+ efflux observed in previous studies occurs via an increase in the open probability of ATP-sensitive K channels. This mechanism of action is the same as that of cromakalim, a structural relative. Standen et al. (1989) reported a direct activating effect of cromakalim on a K_{ATP} in mesenteric artery from the rabbit that was inhibited by glyburide.

In the whole cell configuration, the increase in outward K^+ current induced by 10 μ M WAY 120,491 was eliminated by the specific antagonist of K_{ATP} , glyburide. This result parallels work done at the tissue level in which WAY 120,491 increased ^{42}K and ^{86}Rb efflux from rabbit aorta and rat portal vein, and glyburide inhibited the increase (Edwards et al., 1990; Lodge et al., 1991).

The single channel data provide direct confirmation that WAY 120,491 activates a K_{ATP} in vascular smooth muscle. In this respect, it is similar to its structural relative, cromakalim.

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