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BRL 34915 (cromakalim) activates ATP-sensitive K^+ current in cardiac muscle

(heart/action potential/voltage clamp/guinea pig/ferret)

MICHAEL C. SANGUINETTI*, ANN L. SCOTT, GLORIA J. ZINGARO, AND PETER K. S. SIEGL

Department of Pharmacology, Merck Sharp & Dohme Research Laboratories, West Point, PA 19486

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ABSTRACT **The mechanism by which the antihypertensive agent BRL 34915 (cromakalim) affects action potential duration (APD) and effective refractory period (ERP) in isolated cardiac muscle was investigated. BRL 34915 (≥3 μM) shortened ERP of ferret (*Mustela putorius furo*) and guinea pig (*Cavia porcellus*) papillary muscles in a concentration-dependent fashion. The reduction in ERP resulted from a decrease in APD. ERP and APD of papillary muscles were also reduced during hypoxia produced by bubbling the physiological bathing solution with N_2 instead of O_2. Reduction of APD during hypoxia has previously been attributed to activation of ATP-sensitive K^+ channels in heart. Glyburide, an inhibitor of ATP-sensitive K^+ channels, prevented or reversed the shortening of ERP and APD produced by hypoxia and BRL 34915, respectively. These results suggest that BRL 34915 acts by opening ATP-sensitive K^+ channels in heart. The actions of BRL 34915 were temperature-dependent, decreasing ERP 64% at 37°C, but having no effect at 22°C. The effect of BRL 34915 on K^+ currents was tested directly in voltage-clamped guinea pig ventricular myocytes. As observed with the papillary muscles, BRL 34915 was without effect at 22°C. At 36°C, BRL 34915 (after a delay) increased outward currents positive to, and less so at potentials negative to, the K^+ current reversal potential. The normal inwardly rectifying current–voltage relationship for peak K^+ currents during 200-msec pulses was changed to one that was nearly ohmic. The current activated by BRL 34915 was blocked by glyburide. The data support the hypothesis that BRL 34915, like hypoxia, activates ATP-sensitive K^+ channels in the heart. Based upon the profound temperature sensitivity of BRL 34915 action, this activation may be indirect, perhaps by means of modulation of an enzymatic activity that regulates gating of these channels. BRL 34915 and glyburide will be valuable tools for studying the role of ATP-sensitive K^+ channels in normal and abnormal cardiac function.**

Potassium (K^+) channels of cardiac muscle cells have many functions, including setting of the resting membrane potential and determining refractoriness. Some of these channels are regulated by neurohormones, such as acetylcholine (1), and others are regulated by intracellular cations, such as Ca^{2+} (2) or Na^+ (3). Another class of K^+ channels is activated when internal ATP levels are low [here referred to as IK(ATP) channels]. These channels are present in at least as great a density (about 2000 per cell) as inward rectifier K^+ channels in cardiac cells (4); however, under normal physiological conditions IK(ATP) channels are closed, due to the blocking effect of normal intracellular levels of ATP. The conductance of IK(ATP) channels is more than twice that of inward rectifier K^+ channels, and the current–voltage relationship does not show marked rectification when measured using physiological concentrations of K^+ (5). Thus, total outward K^+ conductance is dramatically increased when IK(ATP) channels are activated. Activation of these channels, such as during experimentally induced internal ATP depletion, hypoxia, or treatment with uncouplers of the oxidative phosphorylation pathway (4–8), markedly shortens action potential duration (APD). These channels are probably activated during transient periods of ischemia in intact hearts, perhaps protecting ischemic cells from Ca^{2+} overload (9) but also increasing extracellular [K^+] and causing a dispersion in refractoriness, events that can precipitate arrhythmias (10).

Study of IK(ATP) channels has been facilitated by the discovery that sulfonylurea antidiabetic drugs, such as glyburide, are specific blockers of this channel in pancreatic beta cells (11). High-affinity binding sites (K_d = 0.3–2 nM) for these drugs have been identified (12, 13). Also, diazoxide is capable of activating these channels (14). The physiological roles of IK(ATP) channels in a variety of tissues are now being investigated with the aid of these IK(ATP) channel agonists and antagonists.

BRL 34915 [cromakalim, (±)-6-cyano-3,4-dihydro-2,2-dimethyl-*trans*-4-(2-oxo-1-pyrrolidyl)-2*H*-benzo[*b*]pyran-3-ol] is a new antihypertensive agent that directly relaxes vascular smooth muscle when contracted by any one of a variety of agonists (15, 16). This relaxation is associated with hyperpolarization of the membrane potential and an enhanced efflux of ^{86}Rb or ^{42}K, suggesting that the drug acts by opening K^+ channels (17, 18). In cardiac muscle, BRL 34915 causes a marked shortening of APD (19–21). Indirect pharmacological evidence suggests that BRL 34915 activates IK(ATP) channels, since BRL 34915 relaxation of vascular smooth muscle is reversed by glyburide (15, 16). The vasorelaxation caused by BRL 34915 is also antagonized by nonselective K^+ channel blockers (15, 16).

Electrophysiological studies have explored possible mechanism(s) by which BRL 34915 acts to change membrane potential. Results from voltage-clamp experiments suggest that the drug activates Ca^{2+}-activated K^+ channels in aortic smooth muscle cells (22), blocks inward rectifier K^+ current (23), or activates only outward K^+ currents (positive to the equilibrium potential for K^+) in cardiac cells (20). Thus, a consensus on the identity of the K^+ channel(s) activated by BRL 34915 in cardiac and vascular smooth muscle has not been achieved. Determination of the specific channel responsible for the actions of BRL 34915 would spawn interest in rational therapeutic design, since this agent has shown clinical promise.

We have investigated the mechanism of action of BRL 34915 in isolated cardiac tissue and cells. Our results indicate that this compound specifically activates IK(ATP), a mechanism consistent with its known effect on effective refractory period (ERP) and APD of cardiac cells.

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Abbreviations: ERP, effective refractory period; APD, action potential duration; Me_2SO, dimethyl sulfoxide.

*To whom reprint requests should be addressed.

MATERIALS AND METHODS

Tissue and Cell Preparation. Papillary muscles were dissected from the hearts of guinea pigs (*Cavia porcellus*) and ferrets (*Mustela putorius furo*) and placed in a physiological salt solution gassed with 95% O_2/5% CO_2. Ferret papillary muscles were used for measurement of contractile force and ERP because these tissues are especially stable during long periods in isolation. Guinea pig papillary muscles were used for recording of electrical activity (action potentials) since the voltage-clamp experiments were performed on cells isolated from guinea pig hearts. Tyrode's solution used for the guinea pig muscles had the following composition (in mM): 127 NaCl, 4.0 KCl, 0.43 NaH_2PO_4, 1.0 $MgCl_2$, 23.8 $NaHCO_3$, 1.8 $CaCl_2$, 5.5 glucose (37°C, pH 7.4). The Krebs–Henseleit solution used for the ferret muscles was composed of the following (in mM): 118 NaCl, 4.7 KCl, 1.2 KH_2PO_4, 1.2 $MgSO_4$, 23 $NaHCO_3$, 2.0 $CaCl_2$, 11 glucose (37°C, pH 7.4). This latter solution also contained 0.1 μM timolol, a nonselective β-adrenergic antagonist, to block the effects of catecholamines released during electrical stimulation.

Guinea pig ventricular myocytes were isolated by a procedure described by Mitra and Morad (24). Cells were isolated by retrograde perfusion of hearts with a Ca^{2+}-free salt solution containing (in mM) 132 NaCl, 4.8 KCl, 1.2 $MgCl_2$, 5 glucose, and 10 Hepes (pH 7.2); this was followed by perfusion with the same solution containing 300 units of type II collagenase per ml (Cooper Biomedical, Malvern, PA) and 1.6 units of protease type XIV per ml (Sigma). This was followed by washout of the enzymes with the Hepes-buffered saline containing 0.2 mM $CaCl_2$. Cells were stored in 1.8 mM Ca^{2+}, Hepes-buffered salt solution at 24°C until used.

Force Measurement. Isometric force of ferret papillary muscles was monitored for determination of ERP and inotropic state. Papillary muscle tendons were attached with a cotton thread to an isometric force transducer, while the base of the muscle was secured to a rigid tissue holder. The preparation was suspended in a 20-ml organ bath containing oxygenated Krebs–Henseleit solution. The minimum resting tension was applied that produced the maximal developed tension for each muscle. All preparations were allowed to equilibrate for 2 hr. In some experiments the muscles were made hypoxic by gassing the saline bathing the tissues with 95% N_2/5% CO_2. The muscles were paced (1 Hz) with bipolar platinum electrodes at a voltage 30% greater than threshold (1 msec duration). ERP was measured by paired pulse stimuli and was defined as the minimum interpulse interval resulting in a measurable contractile response.

Electrophysiological Measurements. Transmembrane action potentials were recorded from guinea pig papillary muscles using standard techniques (25). The muscles were pinned to the bottom of a tissue chamber superfused with Tyrode's solution (37°C) at a rate of 2.5 ml/min. All muscles were equilibrated for at least 1½ hr before recording data. ERP was measured by a standard two-pulse technique (25) during basal stimulation at 60 pulses per min. As with the ferret muscles, the gas used to aerate the Tyrode's solution was switched from the normal 95% O_2/5% CO_2 to 95% N_2/5% CO_2 to produce hypoxia.

Voltage-clamp experiments were performed on isolated guinea pig ventricular myocytes using the whole-cell suction pipette technique (26). The recording pipettes contained the following (in mM): 110 potassium aspartate, 30 KCl, 2 $MgCl_2$, 11 Hepes, 10 EGTA, and 3 Na_2ATP (pH 7.4 with KOH). The extracellular solution was the 0 Ca^{2+}, Hepes-buffered saline plus 1 mM $CdCl_2$. The resistance of heat-polished and filled electrodes ranged from 1 to 3 MΩ. Series resistance was compensated upon establishment of the whole-cell recording mode. Currents were filtered using an eight-pole Bessel filter with a cut-off frequency (−3 dB) of 3 kHz. Data acquisition and analysis were performed with a Compaq Deskpro 386 personal computer and Pclamp software (Axon Instruments, Burlingame, CA).

Whole-cell K^+ currents were measured during depolarizing and hyperpolarizing pulses (200-msec duration) applied from a holding potential of −40 mV. Na^+ current was voltage-inactivated at this holding potential, and Ca^{2+} current was blocked by addition of 1 mM $CdCl_2$ to the 0 Ca^{2+}-containing saline solution. Under these conditions, inward rectifier and delayed rectifier K^+ currents could be measured without interference from Na^+ or Ca^{2+} currents.

Data are presented as mean ± SEM (n = number of preparations). Analysis of variance with a range test was used to test significance of differences between multiple repeated observations on a single group. Paired t tests were used for other comparisons.

Materials. Glyburide was a gift from Hoechst Roussel Pharmaceuticals (Sommerville, NJ) and BRL 34915 was kindly provided by Beecham Pharmaceuticals (Harlow, Essex, U.K.). A 10 mM stock solution of each drug was prepared in dimethyl sulfoxide (Me_2SO), and final desired concentrations were made by dilution with the appropriate physiological saline solution.

RESULTS

Effect of Hypoxia, BRL 34915, and Glyburide on ERP and APD. ERP of untreated (no drug or [Me_2SO] equivalent to highest used with drug) ferret papillary muscles was shortened by 30% after aerating the Krebs–Henseleit solution with 95% N_2/5% CO_2 for 15 min (Table 1). Isometric force was also markedly depressed during hypoxia, twitch tension decreasing 92% relative to that measured in normoxia. Pretreatment of the muscles with 0.2–10.0 μM glyburide prevented the hypoxia-induced reduction of ERP and partially protected against the decrease in contractile force. As shown in Table 1, the glyburide concentration–response relationship was very steep. The drug had no significant effect at 0.03 μM, and the peak response was observed at 0.2 μM. Glyburide (up to 10 μM) had no effect on ERP of muscles under normoxic conditions. Similar shortening of APD and ERP in guinea pig papillary muscles was observed with hypoxia induced by low glucose (1 mM) and bubbling of the Tyrode's solution with 95% N_2/5% CO_2 (not shown), confirming the findings of several previous studies (27, 28). Glyburide (3 μM) had no effect on action potential configuration of guinea pig muscles in normoxic conditions, but

Table 1. Effect of glyburide on ERP of ferret papillary muscles during hypoxia

		ERP, msec			
Treatment	n	Control	1 hr after treatment	15-min hypoxia	% change
None	7	155 ± 7	156 ± 5	109 ± 6	−30 ± 3*
Me_2SO	7	141 ± 6	141 ± 6	96 ± 6	−31 ± 6*
Glyburide, μM					
0.03	4	143 ± 6	152 ± 8	107 ± 11	−30 ± 5*
0.1	7	141 ± 4	143 ± 6	113 ± 6	−20 ± 6†
0.2	6	142 ± 11	147 ± 4	140 ± 8	−4 ± 2
0.3	7	141 ± 4	141 ± 4	140 ± 7	0 ± 5
1.0	6	137 ± 3	138 ± 2	136 ± 6	−1 ± 4
3.0	6	149 ± 6	141 ± 6	145 ± 3	+4 ± 3
10.0	4	137 ± 3	151 ± 6	151 ± 8	0 ± 7

Hypoxia was produced by bubbling bathing solution with 95% N_2/5% CO_2 in place of 95% O_2/5% CO_2. Data represent mean ± SEM; n = number of preparations.
*Significant decrease, $P < 0.01$.
†Significant decrease, $P < 0.05$.

protected these muscles from the shortening of ERP and APD (n = 4) during 15 min of hypoxia.

Exposure of papillary muscles to BRL 34915 also shortened APD of guinea pig muscles and ERP of ferret muscles and decreased isometric force of ferret papillary muscles in a concentration-dependent manner. These effects were completely reversible upon washout of the drug. Glyburide pretreatments (0.3–1.0 μM) shifted the ERP dose–response curve for BRL 34915 to the right in ferret papillary muscles (Fig. 1). When tissues were exposed to 10 μM BRL 34915 for 30 min, ERP was decreased by 54% and force was decreased by 82%. Glyburide at 3 μM was capable of restoring ERP to 94% and force to 67% of its control value (n = 4, data not shown). Action potential recordings of guinea pig papillary muscles confirmed these findings and showed that the reduction in ERP reflected changes in APD. BRL 34915 appeared to be less potent on guinea pig papillary muscles. ERP was reduced 21% ± 3% by 10 μM and 67% ± 5% by 30 μM BRL 34915 in these muscles (n = 4). In the experiment shown in Fig. 2, 0.3 μM glyburide fully reversed the shortening of ERP and APD measured at 90% repolarization (APD90) caused by 30 μM BRL 34915. In four preparations, 0.3 μM glyburide restored ERP and APD (shortened by 30 μM BRL 34915) to 91% ± 7% of control. These results suggest that BRL 34915 activates IK(ATP) channels, producing mechanical and electrical effects similar to those caused by experimentally induced hypoxia. Activation of IK(ATP) was assessed directly by measuring membrane currents in isolated guinea pig ventricular myocytes.

Temperature-Dependent Effects of BRL 34915. In initial experiments, BRL 34915 had no effects on K^+ currents at concentrations that were effective in the isolated tissue assays. At these concentrations (10–30 μM), BRL 34915 neither increased nor decreased inward rectifier K^+ currents (currents negative to about −40 mV) or delayed rectifier K^+ currents (currents positive to about 0 mV, measured at the end of the 200-msec pulses). However, these initial voltage-clamp experiments were done at room temperature (22–24°C). It was important to determine if the apparent discrepancy between results was due to differences in the assay temperatures.

Ferret papillary muscles were treated with 10 μM BRL 34915 for 1 hr at 37°C. ERP was then measured in control (untreated) and drug-treated tissues. The temperature of the tissue baths was then lowered to 32°C and steady-state conditions were attained before measuring ERP again. This procedure was repeated at 28, 25, and 22°C. As temperature was lowered, ERP was lengthened in treated and untreated tissues. BRL 34915 had a greater effect on ERP at 37°C than at lower temperatures (Fig. 3). BRL 34915 at 10 μM decreased ERP of ferret papillary muscles by 64% when

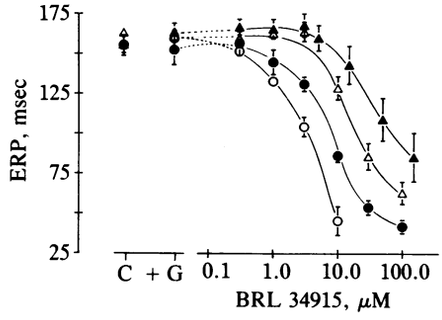

FIG. 1. Effects of glyburide (G) on concentration–effect relationship (ERP) for BRL 34915. Ferret papillary muscles either were not treated (○) or were pretreated with 0.3 μM (●), 1.0 μM (△), or 3.0 μM (▲) glyburide for 1 hr before the effect of BRL 34915 on ERP was determined (n = 7 for each treatment group). C, control.

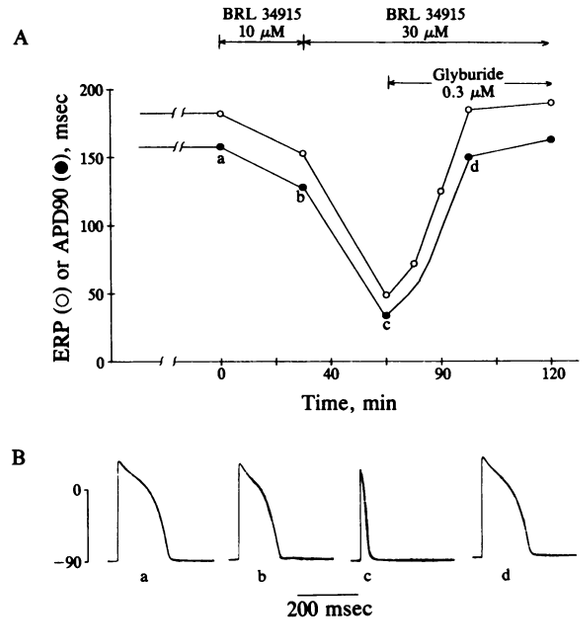

FIG. 2. Glyburide reverses the effect of BRL 34915 on APD measured at 90% repolarization (APD90) and ERP in guinea pig papillary muscle. (*A*) APD90 and ERP recorded during a single microelectrode impalement. The top of the graph shows the order in which the drugs were added. Letters a–d correspond to times when action potentials shown in *B* were recorded.

measured at 37°C, 35% at 32°C, and 13% at 28°C but was without significant effect at temperatures below 25°C. The same temperature dependence was observed when APD and ERP were measured in guinea pig papillary muscles.

Effect of BRL 34915 and Glyburide on K^+ Currents. When the whole-cell voltage-clamp experiments were repeated at 36°C (Fig. 4), BRL 34915 at 10 or 30 μM greatly increased time-independent K^+ currents, changing the normal inwardly rectifying current–voltage relationship for K^+ currents to one that was nearly linear, with the same reversal potential. The action of BRL 34915 always occurred after a delay of 2–4 min (N = 4), and steady-state changes in K^+ currents were obtained in ≈10 min. The effects of BRL 34915 were always studied within a few minutes after establishment of whole-cell clamp mode. Therefore, the increase in K^+ current observed in the presence of drug was not due to the spontaneous activation of IK(ATP) channels known to occur during prolonged (>20 min) cell perfusion at 37°C (29). In addition, the effect of 30 μM BRL 34915 was reversible in the

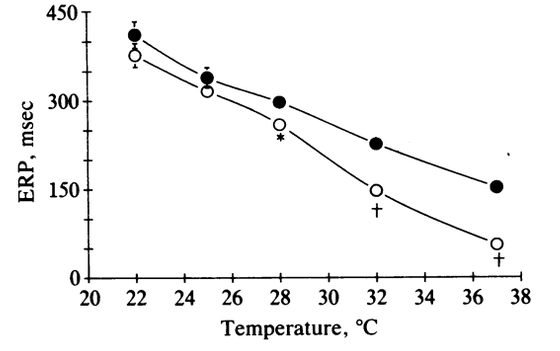

FIG. 3. Temperature-dependent effect of BRL 34915 on ERP measured from ferret papillary muscles (n = 4). ●, Me_2SO; ○, 10 μM BRL 34915. Error bars are not evident on some points because they are smaller than the symbols. Significantly different from Me_2SO control: *, $P < 0.05$; †, $P < 0.001$.

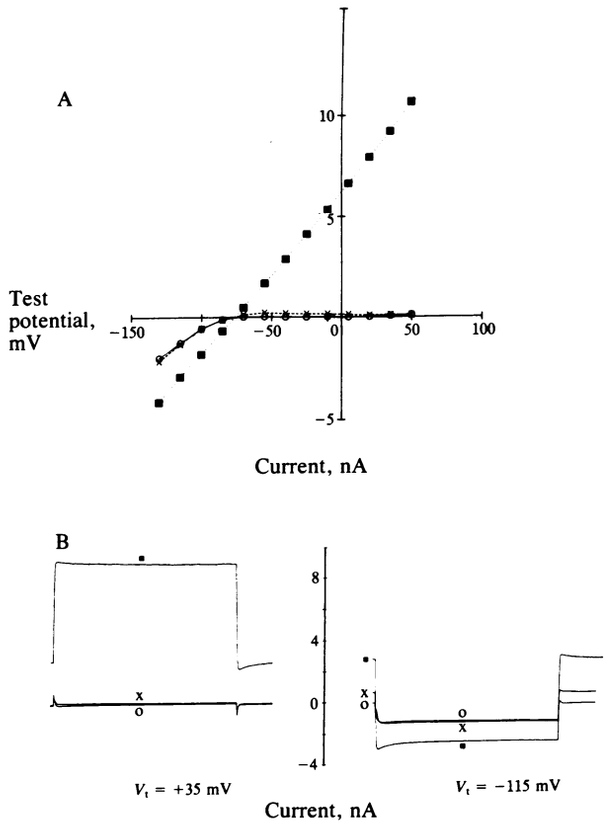

FIG. 4. Activation of K^+ current at 36°C by BRL 34915 and reversal of this effect by glyburide in a guinea pig ventricular myocyte. (*A*) Current–voltage plots. Points represent peak K^+ currents measured during 200-msec pulses from a holding potential of −40 mV. (*B*) K^+ currents measured at test potentials (V_t) of +35 and −115 mV in control (o), 11 min after addition of 10 μM BRL 34915 (■), and 2 min after addition of 10 μM glyburide in the continued presence of BRL 34915 (x).

one experiment in which this was examined. BRL 34915 increased outward more than inward K^+ currents. In two cells, 10 μM BRL 34915 increased outward current measured at a test potential of +50 mV from an average of 0.41 nA to 11.2 nA and increased inward current measured at −115 mV from −1.53 nA to −3.34 nA. In a third cell, 30 μM BRL 34915 enhanced K^+ currents from 0.40 nA to 15.3 nA (+50 mV) and from −2.72 nA to −6.16 nA (−115 mV). The time-independent K^+ currents induced by BRL 34915 are very similar to those in cells perfused for long times with internal solutions containing 0 ATP (29) or after exposure of cells to agents that uncouple the oxidative phosphorylation pathway (4, 7). Glyburide at 10 μM blocked the current activated by 10 μM BRL 34915 and restored the typical inwardly rectifying current–voltage relationship (Fig. 4). The current–voltage relationship shown in Fig. 4 was obtained by first pulsing to +50 mV and then to progressively more negative test potentials at 0.5 Hz. Glyburide completely blocked K^+ current activated by BRL 34915 during the first test pulse (to +50 mV), but block was incomplete at the remaining test potentials that were positive to the K^+ current reversal potential (−75 mV). The current measured at a holding potential of −40 mV became increasingly more outward during this pulse protocol (see current traces in Fig. 4), suggesting that block by glyburide was partially removed. Apparently, block of IK(ATP) by glyburide is voltage- or use-dependent, consistent with its use-dependent effects on APD of hypoxic guinea pig papillary muscles (30). Glyburide did not block inward or delayed rectifier K^+ currents. This can be clearly seen in Fig. 4, where inward and outward currents measured in the presence of BRL 34915 plus glyburide superimpose on those measured during control. The specificity of glyburide for IK(ATP) is consistent with the lack of effect on ERP or the configuration of action potentials when measured during normoxic conditions.

The maximum outward current density measured in the presence of high concentrations of BRL 34915 is similar to that calculated for cells depleted of ATP. For example, in the experiment shown in Fig. 4, the maximum outward current density at 0 mV is 75 $\mu A/cm^2$ after exposure to BRL 34915. This calculation is based on a measured cell capacitance of 83 pF and assuming a specific membrane capacitance of 1 $\mu F/cm^2$. The maximum outward current density at 0 mV for guinea pig myocytes depleted of internal ATP is 110 $\mu A/cm^2$ (29). Therefore, high concentrations of BRL 34915 can activate IK(ATP) to nearly the same maximum measured during depletion of internal ATP.

DISCUSSION

Previous studies have shown that anoxia-induced shortening of APD, and hence ERP, results from an activation of IK(ATP) (4). Ca^{2+} currents are also decreased, but only after longer periods of anoxia (4, 7). Thus, the changes in ERP and APD during the initial stage of hypoxia (i.e., the first 15 min) are primarily due to increased K^+ currents and not decreased Ca^{2+} current. Treatment of tissues with BRL 34915 mimicked the effect of hypoxia on ERP and APD. Glyburide prevented the effects of hypoxia and reversed the actions of BRL 34915 on ERP in guinea pig and ferret preparations. Interpretation of our results relies on the specificity of glyburide as a blocker of IK(ATP). There are several K^+ channels in cardiac ventricular cells, including the inward rectifier, the delayed rectifier, Na_i-activated K^+ channel, and perhaps a Ca^{2+}-activated K^+ channel. At the concentrations used in this study, glyburide had no effect on ERP or action potential configuration of normoxic papillary muscles and did not alter either inward or delayed rectifier K^+ currents in isolated cells (see Fig. 4). There is no evidence that Na_i-activated K^+ channels contribute significantly to net outward currents under any condition in these cells. In the only published report of this current (31), perfusion of cells with high Na^+ only resulted in a modest increase in outward current, far less than that observed after activation of IK(ATP) current. Activation of the Ca^{2+}-activated K^+ channel by BRL 34915 is unlikely since our patch pipettes contained 11 mM EGTA and 0 Ca^{2+}, a condition that would preclude activation of such channels. Thus, there is ample evidence that glyburide, at the concentrations used in our study, does not affect any cardiac K^+ current other than IK(ATP). These results indirectly demonstrate that BRL 34915 shortens APD and ERP of cardiac tissue by means of activation of IK(ATP). Evidence in favor of such an interpretation is that the effects of BRL 34915 on ERP and APD are fully reversed by glyburide and that the currents induced by BRL 34915 are similar to those turned on by low internal concentrations of ATP (29). Glyburide did not fully restore contractile force reduced by BRL 34915. This indicates that BRL 34915 has actions in addition to activation of IK(ATP), such as blockade of Ca^{2+} channels. The activation of IK(ATP) by BRL 34915 is most likely indirect since the compound had no effect at low temperatures, and, at 36°C, its onset of action in single cells was delayed. This temperature dependence and time course of action suggests that BRL 34915 may activate IK(ATP) channels by altering an enzymatic activity that regulates gating of these channels. It has been proposed that BRL 34915 interacts with protein kinase C in mesenteric artery, as assayed indirectly by inhibition of the ^{86}Rb efflux response by certain phorbol esters (32).

Alternatively, the temperature-dependent effect of BRL 34915 may result from a less specific mechanism, such as an alteration of channel gating at low temperatures that prevents a direct action of the drug on the channel.

Our results confirm the findings of others that BRL 34915 increases outward current at 37°C (21), and not at room temperature (23). However, unlike our results, in these studies BRL 34915 reduced inward current negative to the K^+ current reversal potential. A possible explanation for this difference is in the manner in which the K^+ currents were measured. In our experiments, peak K^+ currents were measured during 200-msec pulses, as opposed to steady-state currents measured at the end of 5-sec (23) or 10-sec (21) pulses in previous studies. In smooth muscle cells, BRL 34915 enhanced the activity of high-conductance Ca^{2+}-activated K^+ channels (at room temperature) in cell-attached patches but not in excised patches (22). Voltage-clamp experiments on isolated smooth muscle cells are needed to determine if BRL 34915 activates IK(ATP) channels, Ca^{2+}-activated K^+ channels, or both at relevant concentrations and at a physiological temperature (37°C).

BRL 34915 is not unique with regard to its apparent ability to activate K^+ channels, resulting in hyperpolarization of membrane potential and relaxation of vascular smooth muscle. Pinacidil, minoxidil, and nicorandil have all been proposed to activate an unspecified K^+ conductance in smooth muscle (33–35) and cardiac cells (36–38). It will be of interest to determine if all these compounds, which are chemically unrelated to BRL 34915, can activate IK(ATP) channels in cardiac and vascular cells.

Vascular smooth muscle is about 10–30 times more sensitive to the relaxant properties of BRL 34915 than is cardiac tissue to the effects on ERP (15, 16). BRL 34915 was more potent (about 3-fold) in ferret than in guinea pig papillary muscle. Thus, the potency of BRL 34915 varies between species as well as tissue type. It remains to be determined if the potency difference between vascular and cardiac muscle is related directly to interaction of BRL 34915 with its receptor(s) or represents tissue selectivity secondary to other causes. For example, it is not known what percentage of IK(ATP) channels needs to be activated to observe the physiological responses to BRL 34915 measured in each tissue type. Changes in background K^+ conductance subsequent to the opening of a small number of IK(ATP) channels may lead to sufficient hyperpolarization to cause relaxation in vascular muscle but be without measurable effect on action potential duration of cardiac cells.

BRL 34915 and glyburide represent potent tools for future study of the functional roles played by IK(ATP) in cardiac muscle. The exact mechanism by which BRL 34915 activates IK(ATP) and whether this drug and others (e.g., pinacidil and minoxidil) have the same action in vascular smooth muscle will require further study.

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