British Journal of Pharmacology (1996) 118, 249–254 

# Effects of ranolazine on L-type calcium channel currents in guinea-pig single ventricular myocytes

[1]T.J.A. Allen & R.A. Chapman

The British Heart Foundation Research Group, Dept. of Physiology, School of Veterinary Science, Southwell Street, Bristol, BS2 8EJ

1 Ranolazine has protective effects against ischaemia as exemplified by a reduction of the associated enzyme release and an attenuation of the fall of ATP and other metabolic changes. It has been suggested that ranolazine may affect GTP-binding proteins involved in the β-adrenergic protein kinase A (PKA) cascade by interacting with $G_s$. Calcium channel currents are stimulated by this cascade but the effect of ranolazine upon them is not known. The whole cell patch clamp technique was used to examine the action of ranolazine on basal calcium channel currents and those stimulated by activation at various steps in the PKA cascade.

2 Ranolazine had only a small effect on the basal calcium current (100 μM caused 11.3% inhibition), but markedly attenuated the β-adrenoceptor stimulated current (20 nM isoprenaline increased current by 2.3 fold, 10 μM ranolazine inhibited this increase by 47.6%). When the PKA cascade was activated downstream to the receptor by either G-protein activation with Gpp[NH]p or adenylate cyclase activation with forskolin, the calcium current showed a sensitivity to ranolazine similar to the basal current. Activation of the PKA cascade *via* $H_2$ receptors gave rise to currents which showed an intermediate sensitivity to ranolazine. Ranolazine inhibition of $I_{Ca}$ persisted during muscarinic attenuation of β-adrenoceptor activation.

3 The results indicate that ranolazine, at concentrations which have significantly beneficial effects during ischaemic episodes, only greatly affects whole cell calcium current when facilitated by β-adrenoceptor or histamine receptor activation. Ranolazine would appear to act at the receptor level, rather than at the GTP-binding or $G_s$/adenylate cyclase level. An additional smaller effect is also present, which may be mediated by a direct effect on the channel, or components closely associated with it.

**Keywords:** $Ca^{2+}$ channels; ranolazine; ischaemia; β-adrenoceptor; ventricular myocytes (guinea-pig)

## Introduction

Mobilization of endogenous stores of catecholamines is provoked by ischaemia in the heart (Mukherjee *et al.*, 1979; Hirche *et al.*, 1984) to cause a localized exposure to noradrenaline and a longer term increase in the density of both β-adrenoceptors (canine, Mukherjee *et al.*, 1979; guinea-pig, Maisel *et al.*, 1985) and α-adrenoceptors (canine, Corr *et al.*, 1981; rat, Allely *et al.*, 1993). The role of β-adrenoceptor regulation of the L-type calcium current ($I_{Ca}$) is well defined: receptor binding stimulates the GTP-binding protein, $G_s$, to stimulate adenylate cyclase production of adenosine 3′:5′-cyclic monophosphate (cyclic AMP) and thus activation of protein kinase A and the enhancement of $I_{Ca}$ (PKA cascade; Trautwein & Hescheler, 1990). Whilst α-adrenoceptor activation has little effect on basal calcium currents (guinea-pig and rabbit, Hescheler *et al.*, 1988; rat, Terzic *et al.*, 1992), this pathway appears to interact with and inhibit calcium currents which are pre-stimulated by β-adrenoceptor activation (rat, Boutjdir *et al.*, 1992).

Protection of the ischaemic heart can be provided by a number of manoeuvres, which include adrenoceptor antagonism and calcium channel blockade (Naylor *et al.*, 1985; Toit & Opie, 1992). These mechanisms can reduce symptomatic episodes of myocardial ischaemia in human subjects by reducing some or all of the components of myocardial work; namely, blood pressure, heart rate, and contractility. A new class of compounds appears to work by alternative means, and the piperazine derivative ranolazine ((±)-N-(2,6-dimethyl-phenyl)-4(2-hydroxy-3-(2-methoxy-phenoxy)-propyl)-1-piperazine acetamide dihydrochloride; RS-43285-193) is one such agent. Thus in isolated heart models of ischaemia, ranolazine attenuates enzyme release, improves the preservation of tissue ATP and an improved functional recovery occurs on reperfusion, yet there is little effect on baseline contractile parameters (Clarke *et al.*, 1993 (guinea-pig); Gralinski *et al.*, 1994 (rabbit)). Similar results are seen in *in vivo* animal models of cardiac ischaemia, including prevention of enzyme release and attenuation of metabolite build-up (Allely *et al.*, 1987; Allely & Alps, 1990 (primate)), but with no effects on haemodynamics. In clinical studies of patients with chronic stable angina, ranolazine reduces symptoms without changes in haemodynamics (Cocco *et al.*, 1992; Hayashida *et al.*, 1994). Moreover, its antianginal efficacy has been observed in patients already treated with β-blockers and calcium channel antagonists (Cocco *et al.*, 1992). One recently reported clinical study (Thadani *et al.*, 1994) failed to show efficacy; however, this was probably related to the use of lower doses than the other studies quoted above.

The precise mechanism by which ranolazine protects the ischaemic heart remains to be elucidated. It may act by increasing pyruvate dehydrogenase activity and promoting glucose utilization (Clarke *et al.*, 1993; Gralinski *et al.*, 1994), but it has also been suggested that ranolazine acts as a GTP-binding protein inhibitor (Ferrandon *et al.*, 1992). This latter possibility was suggested by an inhibition of both the ischaemia-induced and the isoprenaline-induced rise in cyclic AMP in rat hearts, and the lack of effect on the rise induced by forskolin (Ferrandon *et al.*, 1992). These effects should be reflected by changes in the regulation of $I_{Ca}$. The present study has therefore investigated the possibility that ranolazine may act at the site between the β-adrenoceptor and adenylate cyclase, using patch clamp recordings of $I_{Ca}$.

[1]Author for correspondence at [1]Present address: Department of Pharmacology, Royal Free Hospital School of Medicine, Rowland Hill Street, London, NW3 2PF.

## Methods

### *Cell isolation*

Ventricular myocytes were isolated from the hearts of male guinea-pigs (250–350 g) as described previously (Rodrigo & Chapman, 1990), except that 40 mM taurine was included in the Tyrode solution into which the myocytes were finally dispersed. The yield of rod-shaped cells was between 50–80%. Myocytes were stored at room temperature and used within 10 h.

### *Experimental procedure*

Myocytes were placed in the recording chamber, which consisted of a 35 mm plastic Petri dish with a Perspex insert forming a central, rectangular channel. The channel volume was ~80 μl and the flow 2.5 ml $min^{-1}$. Solutions were preheated to 34°C by a temperature controller driving Peltier devices (PETC 80-1S, NPI Electronic, Tamme, Germany). The recording chamber was held on an inverting microscope (TMS, Nikon UK, Telford, UK).

Whole-cell voltage clamp was achieved with an Axopatch 1D (Axon Instruments, Foster City, CA, U.S.A.) using the procedure described by Hamill *et al.* (1981). Patch electrodes were fabricated from 2.0 mm o.d. borosilicate glass capillary tubes (Clarks Electromedical Instruments, Reading, UK) and when filled with recording solutions had resistances of 1.5–2.5 MΩ. After formation of giga-ohm seals and membrane rupture, 4–6 min were allowed before whole cell recordings of $I_{Ca}$ began. Mean myocyte capacitance was 80.3±2.25 pF ($n$=43); and for Figure 2c, for example, mean series resistance was 2.7±0.17 MΩ and compensation above 80%.

### *Experimental solutions*

The composition of normal Tyrode solution was (in mM): NaCl 135, KCl 5.4, $NaH_2PO_4$ 0.33, HEPES 10, glucose 10, Na pyruvate 5, $MgCl_2$ 1 and $CaCl_2$ 2; pH was adjusted to 7.3 with NaOH. Enzyme solution was made by adding collagenase (30 mg, Type I, Sigma, Dorset, UK), protease (12 mg, Type XIV, Sigma) and bovine serum albumin (50 mg, Sigma) to 30 ml nominally calcium-free Tyrode solution. For $I_{Ca}$ measurements, normal Tyrode solution was supplemented with 6–7 mM CsCl. The standard internal pipette solution contained (in mM): CsCl 120, HEPES 5, BAPTA 10, $MgCl_2$ 5, $Na_2ATP$ 3 and $Li_4GTP$ 0.5, pH adjusted to 7.3 with CsOH.

### *Data recording and analysis*

Data acquisition and analysis were performed with pCLAMP software (version 5.5; Axon Instruments) on an IBM-compatible AT computer (Elonex 486/50). Clampan was modified (QuickBasic version 4.5) to find the absolute peak inward current. Sampling frequency was 10 kHz, after filtering at 1.5–2 kHz. The holding potential was −80 mV, and the L-type calcium current measured at 0 mV after a 40 ms prepulse to −40 mV in order to inactivate fast $Na^+$ currents. A pulse frequency of 0.3 Hz was used which allowed changes in $I_{Ca}$ induced by pharmacological agents to be determined and longer term change due to current run-down (or run-up) to be monitored. The $I-V$ relationships (after linear leak subtraction calculated from a 10 mV hyperpolarizing pulse from −40 mV) and steady state inactivations were fitted in Fig.P (BioSoft, Cambridge, UK) using modified Boltzmann relationships. For steady state inactivation curves, calcium currents following a 1 s prepulse to various potentials were normalized to those elicited with a prepulse at −50 mV and fitted to the relationship:

$$I/(I_{-50\,mV}) = 1/[1+\exp^{\frac{V-v_i}{K_i}}]$$

where $V$ = preconditioning pulse potential, $v_i$ = half inactivation potential and $K_i$ = slope factor.

Data are shown as means ± s.e.mean. In order to distinguish between ranolazine effects on basal $I_{Ca}$ and effects on the additional current brought about by stimulation (e.g. isoprenaline), we have described the action of ranolazine on the stimulated current as the action on $I_{Ca}$ following stimulation *minus* basal $I_{Ca}$. Statistical analyses have been routinely performed using a parametric (ANOVA) t test (Instat, GraphPad softward, San Diego, CA, U.S.A.). Data are considered significant when $P<0.05$.

### *Solutions*

Isoprenaline ((±)-Isoproterenol, Sigma) was made freshly as a stock solution of 1 mM in de-ionised water with 1 mM ascorbic acid. Ranolazine (Syntex, Edinburgh) was made as a stock solution of 10 mM in Tyrode. Forskolin (Sigma) was made as a stock solution of 5 mM in dimethyl sulphoxide (DMSO). 3-Isobutyl-methylxanthine (IBMX, Sigma) was added directly to the Tyrode solution. Nifedipine (Sigma) was made as a stock solution of 10 mM in absolute ethanol. Acetylcholine (ACh, Sigma) was made as a stock solution in 30 mM. 5'-Guanylyl-imidodiphosphate (Gpp[NH]p) was obtained as the sodium salt from Sigma.

## Results

### *Effects of ranolazine on basal L-type $Ca^{2+}$ current*

The experiment shown in Figure 1 illustrates the procedure used to determine the effects of ranolazine on the basal peak inward calcium current ($I_{Ca}$) when measured at 0 mV. Figure 1a is a plot

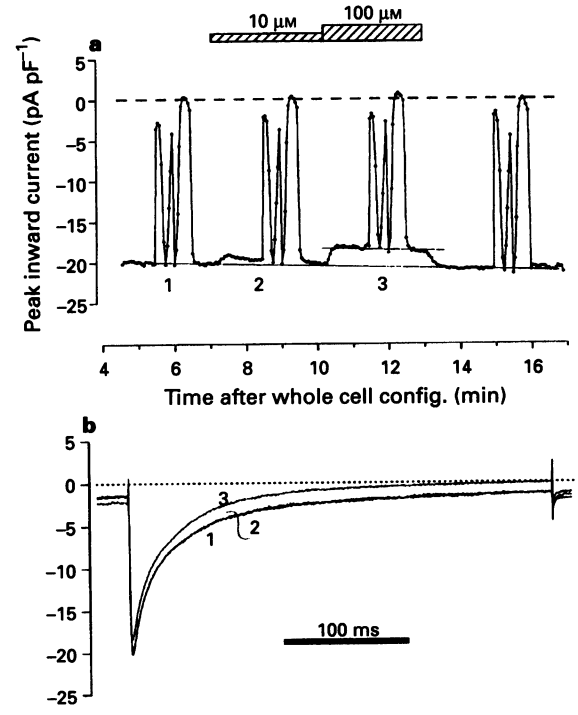


**Figure 1** Effects of ranolazine on basal calcium currents. (a) Peak inward current at 0 mV, determined every 3 s, is plotted against time, after whole cell configuration was established. The hatched boxes indicate periods and concentrations at which ranolazine was applied. The inflections in $I_{Ca}$ are periods when $I-V$ and steady-state inactivation curves were determined. (b) Original currents from the time denoted by numbers in (a): (1) control; (2) in presence of 10 μM ranolazine; (3) in presence of 100 μM ranolazine. Cell capacitance: 84 pF.

of $I_{Ca}$ as a function of time after whole cell configuration and Figure 1b shows examples of original currents taken at the times indicated. The inflections in the peak $I_{Ca}$ trace (Figure 1a) represent periods of $I-V$ and steady-state inactivation relationship determination. (The other figures in this paper have the periods when the $I-V$ curve was determined blanked for clarity.) The application of 10 $\mu$M ranolazine effected a small but consistent inhibition of basal current ($2.4\pm1.24\%$ $n=3$) while 100 $\mu$M caused a more marked $11.3\pm1.09\%$ inhibition ($n=8$; i.e. from $25.96\pm2.14$ pA pF$^{-1}$ to $23.02\pm1.89$ pA pF$^{-1}$: paired $t$ test $P<0.002$). The apparent hastening in the time course of calcium current inactivation in the presence of ranolazine seen in Figure 1b is significant. Inactivation can be fitted by two exponentially decaying components $I_1$ and $I_2$ with time constants $\tau_1$ and $\tau_2$, respectively, and a sustained component $I_0$ (Allen & Chapman, 1995). The first time constant $\tau_1$, changes little from $13.46\pm0.60$ ms to $13.66\pm1.24$ ms on exposure to 100 $\mu$M ranolazine; however, the slower component $\tau_2$ increases significantly from $64.05\pm1.18$ ms to $55.92\pm2.18$ ms ($n=8$, $P<0.01$). This is also reflected by changes in the steady-state inactivation curves; 100 $\mu$M ranolazine effected a 3 mV shift to the left of the curve when compared to basal current (from $-25.07\pm0.72$ mV to $-28.11\pm0.29$ mV; $n=7$ myocytes; paired $t$ test $P<0.005$).

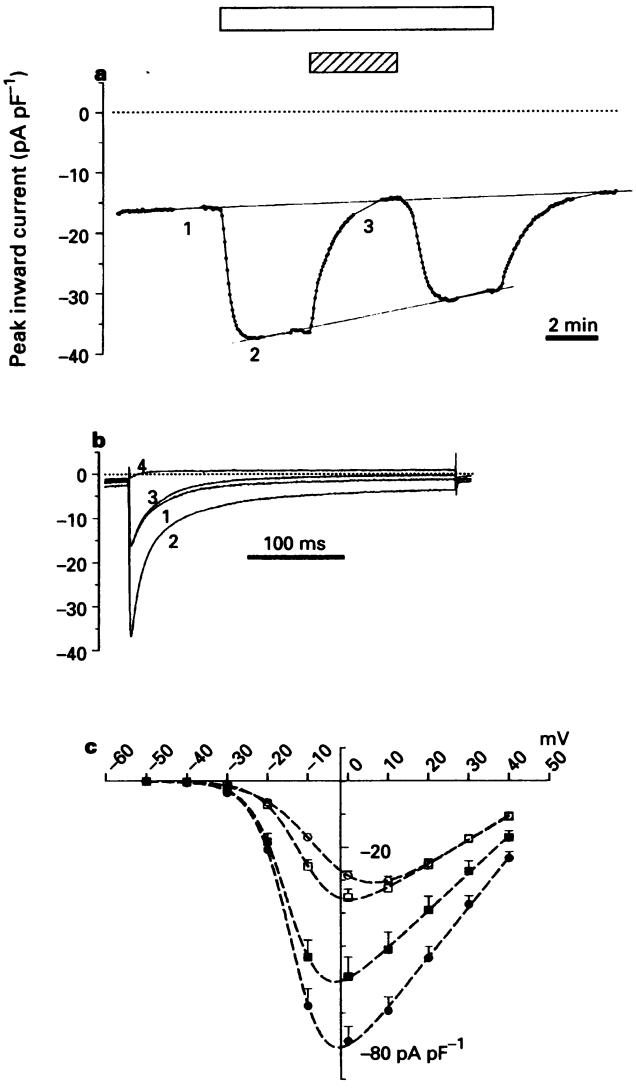


**Figure 2** Effects of ranolazine on isoprenaline-activated calcium currents. (a) Peak inward current is plotted against time after whole cell configuration was established. The open box indicates the period of exposure to 20 nM isoprenaline and the hatched box when 100 $\mu$M ranolazine was present. Cell capacitance: 91 pF. (b) Original currents from the times denoted by the numbers in (a): (1) control; (2) in presence of 20 $\mu$M isoprenaline; (3) in 100 $\mu$M ranolazine; (4) in 5 $\mu$M nifedipine, at end of experiment. (c) Averaged $I-V$ curves from 5 myocytes showing effects of isoprenaline and ranolazine: (○) control; (●) 20 nM isoprenaline; (■) isoprenaline with 10 $\mu$M ranolazine; (□) isoprenaline with 100 $\mu$M ranolazine. Curves are drawn from the modified Boltzmann relationship:

$$I_{Ca} = [G_{max}(V - V_r)]/[1 + \exp^{\frac{V_a - V}{K_a}}]$$

where $I_{Ca}$ is the peak current (pA pF$^{-1}$) at potential V (mV) and $V_a$= potential for maximum activation slope (mV); $V_r$= reversal potential (mV); $K_a$= activation slope factor (mV); and $G_{max}$= macroscopic slope conductance (nS pF$^{-1}$).

### *Effects of ranolazine on the β-adrenoceptor stimulated L-type $Ca^{2+}$ current*

Exposure to the β-adrenoceptor agonist, isoprenaline, increases $I_{Ca}$ *via* the protein kinase A cascade eliciting a 3–4 fold increase (Kameyama *et al.*, 1985; Trautwein & Hescheler, 1990). The effects of ranolazine on the L-type $Ca^{2+}$ current stimulated *via* the β-adrenoceptor pathway were examined after myocytes were exposed to 20 nM isoprenaline (Figure 2). If ranolazine was applied after the response to isoprenaline had stabilized, a reversible inhibition of $I_{Ca}$ was observed, which virtually abolished the activation evoked by isoprenaline. Figure 2b illustrates examples of original traces (as denoted in Figure 2a); it is clear that as 100 $\mu$M ranolazine (trace 3) reduces peak $I_{Ca}$ back close to control (trace 1), a hastening of inactivation of the current also occurs. Figure 2b also illustrates inhibition of $I_{Ca}$ brought about by 5 $\mu$M nifedipine, the L-type calcium channel blocker (trace 4).

In 17 myocytes application of 20 nM isoprenaline increased $I_{Ca}$ by $2.26\pm0.13$ fold; under these conditions 10 $\mu$M ranolazine effected $47.6\pm7.35\%$ inhibition of the increase in calcium current provoked by isoprenaline ($n=8$ myocytes), whilst 100 $\mu$M effected $88.2\pm3.02\%$ inhibition ($n=17$ myocytes; $P<0.001$).

### *Effects of ranolazine on histamine stimulated L-type $Ca^{2+}$ current*

Stimulation of $H_2$ receptors by histamine also elicits an increase in $I_{Ca}$ *via* coupling to the protein kinase A cascade in a manner very similar to β-adrenoceptor stimulation; these two activations are not additive at maximal concentrations (Hescheler *et al.*, 1987; Trautwein & Hescheler, 1990). Stimulation of $H_2$ receptors should indicate if ranolazine acts primarily as a β-adrenoceptor antagonist, since a common pathway is used

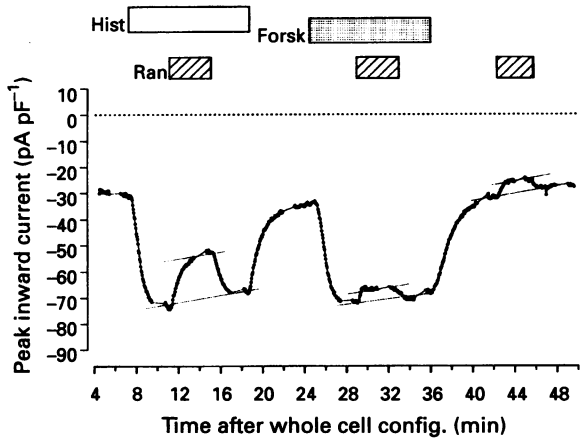


**Figure 3** Effects of ranolazine on $I_{Ca}$ during histamine and forskolin activation. Boxes at top of figure denote periods when myocyte was exposed to: 200 nM histamine (open box) or 1 $\mu$M forskolin (stippled box), and 100 $\mu$M ranolazine (hatched boxes). Cell capacitance: 55 pF.

following receptor activation. In 10 myocytes, 200 nM histamine increased $I_{Ca}$ by 2.68±0.29 fold, which is not significantly different from the activation evoked by 20 nM isoprenaline.

Figure 3 shows an experiment on a myocyte in which $I_{Ca}$ is first stimulated with 200 nM histamine and then exposed to 100 μM ranolazine. This experiment also examines the effects of ranolazine on $I_{Ca}$ stimulated by direct activation of adenylate cyclase with 1 μM forskolin. In both cases ranolazine attenuated $I_{Ca}$ reversibly, but to a much lesser extent in the presence of forskolin. Following histamine activation, 10 μM ranolazine inhibited the stimulated current by 5.1±2.42% ($n=3$) and 100 μM ranolazine by 30.3±4.22% ($n=10$; $P<0.05$ compared to 10 μM). These data illustrate that when $I_{Ca}$ is activated *via* $H_2$ receptors, to a similar magnitude to that elicited by β-receptors, the stimulated current is significantly much less sensitive to 10 μM ranolazine when compared to isoprenaline activation ($P<0.001$); the same follows when 100 μM ranolazine is considered ($P<0.001$).

In one myocyte activation of $I_{Ca}$ by both 20 nM isoprenaline and 200 nM histamine, followed by inhibition with 100 μM ranolazine, were directly compared: although $I_{Ca}$ was increased to a similar extent in this experiment (isoprenaline 2.4 fold, histamine 2.1 fold), ranolazine inhibited the isoprenaline-stimulated current by 89% compared to 28% achieved with histamine.

Direct stimulation of adenylate cyclase can be achieved with micromolar amounts of forskolin, this raises cyclic AMP and activates $I_{Ca}$ to an extent similar to isoprenaline (Hescheler *et al.*, 1986; Trautwein & Hescheler, 1990; Hartzell & Budnitz, 1992). The experiment shown in Figure 3 illustrates that with 1 μM forskolin, although eliciting activation of $I_{Ca}$ similar in magnitude to that achieved by histamine in this experiment, the sensitivity of $I_{Ca}$ to ranolazine is much reduced. In 4 myocytes, 1 μM forskolin increased $I_{Ca}$ by 2.09±0.06 fold; 100 μM ranolazine inhibited this stimulated current by 17.4±4.68% ($P<0.001$ compared to isoprenaline, this is despite a smaller activation of $I_{Ca}$). These data indicate that $I_{Ca}$ sensitivity to ranolazine is reduced when studied downstream to β-receptor activation. This notion is supported by the additional observation that in 3 myocytes exposure to 50 μM IBMX, which inhibits phosphodiesterase activity and thus indirectly raises cyclic AMP *via* basal activity of adenylate cyclase (Hescheler *et al.*, 1986; Trautwein & Hescheler, 1990), only partially increased $I_{Ca}$ by 1.52±0.15 fold, but this activated current was inhibited just 31.36±4.20% by 100 μM ranolazine.

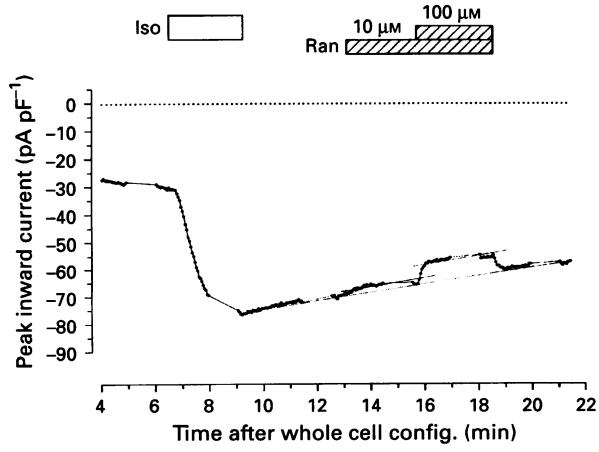


**Figure 4** Effects of ranolazine during activation of calcium currents with Gpp[NH]p. Pipette solution was modified to include 4 mM Gpp[NH]p. Open box denoted period when myocyte was transiently exposed to 20 nM isoprenaline (Iso) to activate $G_s$. Note that $I_{Ca}$ remains activated. Hatched boxes are periods when myocyte was exposed to ranolazine at concentrations indicated. Cell capacitance: 83 pF.

### *Effects of ranolazine on $I_{Ca}$ increased by the GTP analogue, Gpp[NH]p*

Ferrandon *et al.* (1992) suggested that, based on cyclic AMP measurements, the cardioprotective effects of ranolazine may be due to effects on the GTP-binding protein, $G_s$. To examine this possibility, a selective irreversible activation of $G_s$ was provoked by the inclusion of 4 mM of the GTP analogue, Gpp[NH]p, in the patch pipette solution. Once Gpp[NH]p had dialysed into the sarcoplasm, β-adrenoceptor stimulation should release bound GDP from $G_s$ and allow the non-hydrolysable Gpp[NH]p to bind; to result in a persistant activation of $G_s$ (Trautwein & Hescheler, 1990). Indeed exposure to 20 nM isoprenaline caused an increase in $I_{Ca}$, and the current remained activated when isoprenaline was removed (Figure 4). The basal current was not affected by the presence of Gpp[NH]p, possibly due to the presence of 0.5 mM GTP; it appears that only when the GDP/GTP cycle is considerably enhanced by receptor activation, does sufficient Gpp[NH]p bind to $G_s$ to lead to a persistently activated state. Once $I_{Ca}$ is activated, subsequent exposure to ranolazine had relatively little effect on the current, compared to that seen with isoprenaline in the absence of intracellular Gpp[NH]p (compare with Figure 2). In 3 myocytes dialysed with 4 mM Gpp[NH]p, addition of 20 nM isoprenaline increased $I_{Ca}$ by 3.07±0.13 fold. Addition of 10 μM ranolazine attenuated this stimulated current by 5.4±1.10% ($n=2$) and 100 μM by only 16.1±2.73% ($n=3$). At both 10 μM and 100 μM, ranolazine is more effective at inhibiting $I_{Ca}$ stimulated with isoprenaline compared to Gpp[NH]p ($P<0.01$ and $P<0.001$, respectively).

### *Effects of ranolazine on $I_{Ca}$ during muscarinic attenuation of β-adrenoceptor stimulation*

The marked increase in the sensitivity of $I_{Ca}$ to ranolazine on exposure to a β-agonist, compared to histamine activation or manoeuvres which raise cyclic AMP to augment $I_{Ca}$, suggests interaction with receptor binding or receptor/G-protein coupling. One further possibility could be by action similar to muscarinic receptor activation in which adenylate cyclase activity is attenuated by $G_i$ (Hescheler *et al.*, 1986; Trautwein & Hescheler, 1990). This possibility can be examined by addition of ranolazine during muscarinic attenuation of β-adrenoceptor stimulation.

Myocytes were first stimulated by 20 nM isoprenaline and then 9 μM ACh was added to activate the $G_i$ pathway. One such experiment is shown in Figure 5 and demonstrates that both muscarinic and ranolazine attenuation of the isoprenaline-stimulated current are reversible. In 5 myocytes, 9 μM

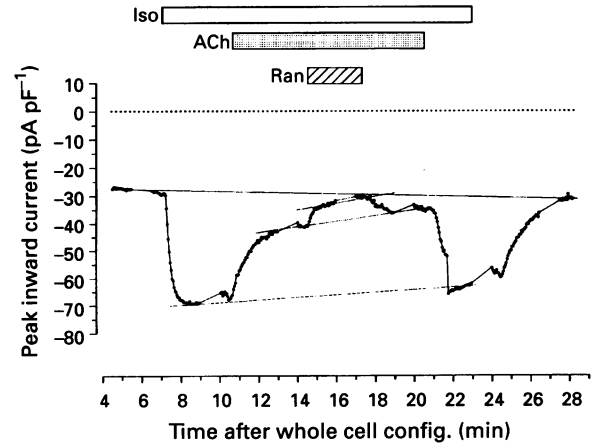


**Figure 5** Effects of ranolazine during muscarinic attenuation of isoprenaline-activated $I_{Ca}$. Boxes at top of figure show periods of exposure to; 20 nM isoprenaline (open box); 20 nM isoprenaline with 9 μM ACh (stippled box); and 20 nM isoprenaline with 9 μM ACh and 100 μM ranolazine (hatched box). Cell capacitance: 65 pF.

ACh attenuated the current stimulated with 20 nM isoprenaline by 65.0±3.71% and 100 μM ranolazine further reduced this to 94.6±2.37% ($P<0.01$ compared to ACh). If this concentration of ACh activates $G_i$, as previous work would suggest (Hescheler *et al.*, 1986), the further reduction observed in $I_{Ca}$ indicates that ranolazine action could potentiate or is additive to $G_i$ activation. A full dose-response relationship would clarify this possibility. In further experiments, basal currents measured in the presence of 30 μM ACh, which had little or no inhibitory effect, were still affected by 100 μM ranolazine which caused an 8.5±0.29% ($n=4$) inhibition in $I_{Ca}$. Thus if ranolazine were exerting an effect primarily *via* $G_i$, then inhibition of basal currents pre-exposed to 30 μM ACh, to maximize any basal $G_i$ activity, would be expected to fail to occur.

## Discussion

We have examined the effects of the antianginal agent, ranolazine, on L-type calcium currents ($I_{Ca}$) in guinea-pig single ventricular myocytes, under control conditions and following activation *via* the protein kinase A cascade at different stages in this pathway. Inhibition of $I_{Ca}$ brought about by ranolazine was dependent on the route of $I_{Ca}$ activation, and not necessarily on the magnitude of activation.

Whilst beneficial effects of ranolazine have been found in patients given a single oral dose (240 mg; Cocco *et al.*, 1992) or intravenous infusion (200–500 μg $kg^{-1}$; Hayashida *et al.*, 1994), studies on isolated hearts in animal models when exposed to ischaemia and reperfusion reveal significantly protective effects of ranolazine at lower concentrations (10 μM; guinea-pig, Clarke *et al.*, 1993, 10–20 μM; rabbit Gralinski *et al.*, 1994). In the data presented in this paper we have demonstrated that ranolazine can significantly inhibit $I_{Ca}$ at similar concentrations.

A small 2.4% and 11.3% inhibition of the basal calcium current was seen with 10 μM and 100 μM ranolazine, respectively; this was associated with a hastening in the time course of inactivation and shift in the steady-state inactivation curve. The inhibitory action of 100 μM ranolazine was markedly enhanced to 88% following β-adrenoceptor activation of $I_{Ca}$ with 20 nM isoprenaline, but reduced to 30% following histamine activation with 200 nM histamine. The sensitivity of $I_{Ca}$ to this concentration of ranolazine is much attenuated down to 16–17%, if stimulated downstream to receptor activation using the GTP analogue Gpp[NH]p, to activate $G_s$, or 1 μM forskolin, to activate adenylate cyclase. These latter observations are despite differences in the magnitude of $I_{Ca}$ activation; 4 mM Gpp[NH]p increasing $I_{Ca}$ some 3.07 fold compared to 2.1 fold with 1 μM forskolin. Although it would be beneficial to provide full dose-response curves for the relative activations in $I_{Ca}$ achieved by these two agents to effect proper comparison, there is already evidence that at maximal doses their relative stimulations are similar (Hescheler *et al.*, 1986; Trautwein & Hescheler, 1990; Hartzell & Budnitz, 1992).

These observations suggest that ranolazine does not act at the GTP-binding protein level (Ferrandon *et al.*, 1992) to affect $I_{Ca}$, because a similar sensitivity to β-adrenoceptor and histamine receptor-stimulated currents would be expected, as they use similar PKA pathways (Trautwein & Hescheler, 1990). That 100 μM ranolazine exerted only a 31% inhibition on the modest 1.5 fold activation of $I_{Ca}$ evoked by 50 μM IBMX, further supports the notion that its major action occurs at the receptor level. A larger portion of the current in this case is basal $I_{Ca}$, which itself is inhibited 11% by this concentration. Together, these data indicate that despite differences in the relative magnitudes of $I_{Ca}$ activation, the inhibitory effects of ranolazine are greater when examined following β-adrenoceptor activation. Ranolazine would seem therefore to affect receptor binding or receptor-GTP binding protein interaction (i.e agonist binding or receptor coupling).

The inhibition of $I_{Ca}$ by ranolazine persists following attenuation of β-adrenoceptor activation *via* muscarinic stimulation. ACh activates $G_i$, the inhibitory GTP-binding protein linked to adenylate cyclase (Hescheler *et al.*, 1986); yet it remains unclear from these observations whether ranolazine action potentiates or is additive to $G_i$. However, if ranolazine did act by potentiating $G_i$ action on $I_{Ca}$, one would expect no or very little effect on basal currents following pre-exposure to 30 μM ACh: under these conditions 100 μM ranolazine still exhibited inhibition. A full dose-response relationship for ACh inhibition of isoprenaline stimulation, and the shift evoked by ranolazine, would clarify this possibility.

The enhanced inhibition of $I_{Ca}$ by ranolazine seen following receptor-activation is greater during β-adrenoceptor activation compared to histamine receptor activation at both 10 μM and 100 μM levels, despite similar activations in $I_{Ca}$ by these agonists. The mobilisation of endogenous stores of catecholamines is provoked by periods of ischaemia in the heart (Mukherjee *et al.*, 1979; Hirche *et al.*, 1984) giving rise to localized exposure to noradrenaline; the same may occur for histamine. The cardioprotective mechanism of ranolazine could involve both β-adrenoceptor and histamine receptor antagonism. From our data on $I_{Ca}$, even a modest release of these agonists during ischaemic episodes (Mukherjee *et al.*, 1979; Hirche *et al.*, 1984) would be affected by doses of ranolazine expected to have beneficial effect, especially as basal $I_{Ca}$ is also affected by these levels.

Although a direct effect on $G_s$, as suggested by Ferrandon *et al.* (1992), is made unlikely by our results, the possibility remains that ranolazine binds to $G_s$ at the same site as β-adrenoceptors and histamine receptors. If this were the case, the difference in the inhibitory action of ranolazine on histamine- and isoprenaline- facilitated currents would indicate differences in efficacy. A further study would aim to include full dose-response curves for these agonists, and perhaps also examine ranolazine action on agents which modulate $I_{Ca}$ but lack interaction with the PKA pathway.

In conclusion, our experiments on patch clamp recordings of whole cell L-type calcium currents suggest that ranolazine causes an inhibition of $I_{Ca}$ *via* two mechanisms; primarily by an effect consistent with an action on receptor-G-protein coupling and second by a direct effect on the channel, or components closely associated with it. Ranolazine may provide some of its cardioprotection during ischaemia by these actions.

Ranolazine was provided by Dr J.G. McCormack, Syntex Research Centre, Edinburgh. We would like to acknowledge the excellent technical assistance of Mrs Dawn Wallace. This work was supported by the British Heart Foundation.

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*(Received October 31, 1995*
*Revised January 11, 1996*
*Accepted January 23, 1996)*