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Electrophysiological and antiarrhythmic actions of the κ agonist PD 129290, and its **R,R** (+)-enantiomer, PD 129289

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1 The **S,S** (−)-enantiomer PD 129290, a κ agonist, and its corresponding inactive **R,R** (+)-enantiomer (PD 129289) were studied in rat isolated hearts and in intact rats for cardiovascular and antiarrhythmic actions. The electrophysiological actions of PD 129290 were also studied in rat isolated cardiac myocytes using voltage clamp.

2 Ventricular pressure, heart rate and ECG were studied in isolated hearts while blood pressure, heart rate and ECG were studied in pentobarbitone-anaesthetized rats. In the latter, responses to electrical stimulation and coronary occlusion were also investigated.

3 In isolated hearts both enantiomers, over the concentration range 2–16 μM, dose-dependently reduced systolic ventricular pressure and heart rate while prolonging the P–R and QRS intervals of the ECG.

4 At doses of 1–32 μmol kg^{-1} both enantiomers reduced blood pressure and heart rate in anaesthetized rats. In addition, both enantiomers increased the size of the RSh and increased P–R, QRS, and Q–T intervals of the ECG. The thresholds for premature beats and ventricular fibrillation were dose-dependently increased by PD 129289. At lower doses PD 129290 decreased thresholds. These decreases were blocked by naloxone to reveal underlying increases similar to those seen with PD 129289. Both enantiomers increased refractory periods.

5 Naloxone (8 μmol kg^{-1}) did not alter any of the actions of PD 129290, except to abolish the initial decreases in thresholds in intact rats seen with lower doses of PD 129290.

6 Both enantiomers (2 and 8 μmol kg^{-1}) equally reduced arrhythmias in anaesthetized rats subject to occlusion of a coronary artery.

7 In rat isolated cardiac myocytes 20 μM PD 129290, in the presence and absence of naloxone decreased the amplitude of the transient sodium current by about 50% without affecting the voltage-dependence of activation or inactivation of this current.

8 The antiarrhythmic actions of both enantiomers appear to primarily result from their Class I (sodium channel blockade) properties which are independent of κ agonism.

Keywords: Opioid; antiarrhythmic; sodium channel; κ agonist

Introduction

A range of different studies in rats have demonstrated that a wide spectrum of opioid drugs reduce arrhythmias induced by occlusion of a coronary artery (e.g. Sitsapesan & Parratt, 1989; Pugsley *et al.*, 1992b). The degree of antiarrhythmic protection varied with the opioid drug studied and there was no simple correlation between opioid type (agonist or antagonist) and antiarrhythmic activity. After examining a series of agonist and antagonist opioid drugs, Sarne *et al.* (1991) came to the provisional conclusion that sodium channel blockade was responsible for observed antiarrhythmic actions.

We recently studied the antiarrhythmic actions of the κ agonist, U-50,488H in rats and were able to relate its antiarrhythmic actions to sodium channel blockade (Pugsley *et al.*, 1992a,b) and not to κ agonism. PD 129290, an arylbenzacetamide similar in chemical structure to U50,488H, is a newly developed κ agonist (Hunter *et al.*, 1990). PD 129290 is an **S,S** (−)-enantiomer whereas PD 129289 is the corresponding **R,R** (+)-enantiomer which has less than 1/1,000 of the κ receptor binding activity of PD 129290. Its affinity for μ receptors is 0.5 that of the **S,S**-enantiomer (Halfpenny *et al.*, 1990). In view of the large difference in κ agonist activity between the two enantiomers it was apparent that a comparison of the pharmacological and antiarrhythmic profiles of PD 129289 and PD 129290 might help resolve the mechanisms by which such structurally similar, but pharmacologically different, drugs exert an antiarrhythmic action.

We have examined the actions of PD 129289 and PD 129290 in isolated hearts and in intact rats. The latter were subjected either to coronary occlusion, or to electrical stimulation of the left ventricle. The present studies are analogous to those recently completed with U50,488H (Pugsley *et al.*, 1992a,b); similar doses and concentrations were used in both studies. In the U50,488H studies possible opioid receptor-dependent (κ and μ) effects *in vivo* and *in vitro* were abolished with naloxone. In the present study we used naloxone for the same purpose and in addition used the enantiomer PD 129289 which is inactive at κ opioid receptors. We have also examined the inhibitory effects of PD 129290 on sodium currents recorded in isolated cardiac myocytes by use of the whole-cell variant of the patch clamp technique.

Methods

General

Male Sprague-Dawley rats (Charles River Laboratories, Montreal, Quebec) weighing 150–200 g were used for all experiments conducted at UBC. The isolated cardiac myocyte experiments conducted at Canberra used the available male Wistar (200–300 g) strain rats. All experiments conducted at U.B.C. were according to guidelines enforced by the U.B.C.

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Animal Care Committee while those performed in Australia were according to the guidelines used by the Australian National University.

Intact rats were anaesthetized with pentobarbitone (60 mg kg^{-1}, i.p.) and the trachea cannulated for artificial ventilation at a stroke volume of 10 ml kg^{-1}, 60 × min^{-1}. Body temperature was monitored by a rectal thermometer and maintained between 36–37°C with a heating lamp.

Isolated hearts

Hearts were mounted on a modified Langendorff apparatus (Curtis *et al.*, 1986) and perfused at a pressure of 100 mmHg with Krebs-Henseleit solution (pH 7.4), bubbled with 5% CO_2 and O_2 and maintained at a temperature of 35°C. A compliant saline-filled balloon was inserted into the left ventricle and inflated with approximately 0.5 ml saline to a diastolic pressure of 10 mmHg. The developed pressure in the balloon was recorded together with the maximum rate of intraventricular pressure development ($+dp.dt_{max}^{-1}$) by means of a Grass differentiator, model 7P20C. ECGs were recorded (band width of 0.1–40 Hz) from silver-ball electrodes placed on the epicardial surfaces of the right atrium and left ventricle.

Hearts weighing between 1.2 and 1.6 g were, according to a random design, perfused for 5 min at each concentration with saline or either of PD 129289 or PD 129290 at 1, 2, 4, 8 and 16 μM.

Intact rats

Dose-response curves were constructed for the effects of PD 129289 and PD 129290 (cumulative doses of 0.5, 1.0, 2.0, 4.0, 8.0, 16.0 and 32.0 μmol kg^{-1}, i.v.) in pentobarbitone-anaesthetized and artificially ventilated rats ($n = 6$ per group). A carotid artery and jugular vein were cannulated for recording blood pressure and administering drugs, respectively. Drug was given over a 2 min period and recordings made 5 min later, i.e. just before the next dose. In a separate experiment naloxone (8 μmol kg^{-1}) was administered just before dosing with PD 129290. This dose of naloxone was the largest which could be given without influencing ECG, blood pressure or heart rate.

P–R, QRS and Q–T intervals of the ECG were measured. Since the Q–T interval in rats does not change with rate (unpublished observations), it was not corrected for heart rate. A new ECG measure, RSh (mV), was used as a reflection of possible sodium channel blockade (Penz *et al.*, 1992). RSh is the difference in mV between the peak of the R-wave and the trough of the S-wave.

In intact rats, electrical stimulation of the left ventricle was performed via two Teflon-coated silver wire stimulating electrodes inserted through the chest wall and implanted in the left ventricle (Walker & Beatch, 1988); the inter-electrode distance was approximately 1 mm. Square wave stimulation was used to determine the threshold current (i_T–μA) and pulse width (t_T–ms) for induction of extrasystoles, threshold currents for induction of ventricular fibrillation (VF_T–μA), effective refractory period (ERP–ms) and maximum following frequency (MFF–Hz) (Howard & Walker, 1990). Although MFF is related to ERP there are differences in their sensitivity to drugs (Walker & Beatch, 1988) and so both measures were made.

Coronary occlusion studies

The surgical procedures used were those of Au *et al.* (1979) and Paletta *et al.* (1989). In brief, rats were anaesthetized with pentobarbitone and artificially ventilated as described above. A carotid artery cannula was used to record blood pressure and to withdraw blood samples for determination of serum K^+ concentrations (Ionetics Potassium Analyzer). A polyethylene occluder was used to occlude the main left coronary artery in animals whose chest cavity was loosely closed after the occluder had been implanted. Rats were allowed to recover for 30 min after surgical preparation.

All rats were given an initial injection of saline except for one group which received 8 μmol kg^{-1} naloxone. In a random and double-blind manner a second injection of saline, or enantiomer was given 5 min later. Blood pressure and ECG were recorded 5 min after injection and a blood sample (0.25 ml) taken. The occluder was then tightened so as to produce occlusion.

ECG, arrhythmias, blood pressure, heart rate and mortality were monitored for 30 min after occlusion. Arrhythmias, consisting of ventricular premature beats (VPB), ventricular tachycardia (VT) and ventricular fibrillation (VF) were recorded and summarized as an arrhythmia score (AS) according to the system of Curtis & Walker (1988). After 30 min, a second blood sample was taken in surviving animals. Hearts were then removed and perfused via the Langendorff technique with cardiogreen dye (1 mg ml^{-1}) to reveal the underperfused occluded zone (zone-at-risk). The treatment groups were a saline control, PD 129290 (2 or 8 μmol kg^{-1}), PD 129289 (2 or 8 μmol kg^{-1}) and PD 129290 (8 μmol kg^{-1}) after pretreatment with naloxone 8 μmol kg^{-1}. Experimental design and analyses were performed according to the Lambeth Conventions (Walker *et al.*, 1986) with replacement of lost animals to maintain balance between groups. Rats were only included in the study providing that: (1) Mean arterial blood pressure did not fall below 25 mmHg for more than 5 min before and after occlusion. (2) Pre-occlusion ECG had well-defined P, QRS and T waves, was in normal sinus rhythm, and the S–T segment was below the R-wave peak. (3) Arrhythmias before occlusion were less than 15 VPB and no VT or VF occurred. (4) Serum potassium was between 2.9 and 3.9 mM. (5) Occlusion produced an increase in R-wave and elevation of the S–T segment (Johnston *et al.*, 1983). (6) Occluded zone size was 25–50% of total left-ventricular weight.

Statistical analyses

Statistical analyses were performed by use of the NCSS statistical package (Hintze, 1987); values are given as mean ± s.e.mean (n = group size). A general linear model ANOVA was used and multiple comparisons were made by Duncan's test. The statistically significant differences in group incidences of ventricular tachycardia (VT) or ventricular fibrillation (VF) were determined by reference to Mainland's contingency tables (Mainland *et al.*, 1956). The number of VPB was $\log_{10}$ transformed before parametric statistical analyses.

Electrophysiological studies of PD 129290 in isolated myocardial cells

In view of a limited supply of the **R,R**-enantiomer (PD 129289) it was only possible to perform electrophysiological studies on isolated cell using the **S,S**-enantiomer, PD 129290. Single cardiac myocytes were prepared by enzymic dissociation of rat isolated hearts; the method has been described in detail elsewhere (Saint *et al.*, 1992). Isolated cells were allowed to settle onto a glass slide coated with poly-l-lysine in a chamber of 1 ml volume which was perfused at 1 ml min^{-1}.

Evoked currents were recorded by conventional whole-cell patch clamp techniques. The bathing solution (temperature 34°C) had the following composition in mM: NaCl 130, KCl 5.4, $MgCl_2$ 1.0, $CaCl_2$ 2.0, $CoCl_2$ 5.0, CsCl 5.0, tetraethyl sulphonic acid 10.0, NaOH 5.0, glucose 10.0 with the pH adjusted to pH 7.4. The composition of the electrode solution in mM was: CsF 50.0, NaF 70.0, K-EGTA 20.0, $CaCl_2$ 2.0, tetraethyl sulphonic acid 10.0, ATP-Na_2 5.0, ATP-Mg 5.0, with the pH adjusted to 7.4 with KOH. Microelectrodes were fabricated from borosilicate glass and typically had a resis-

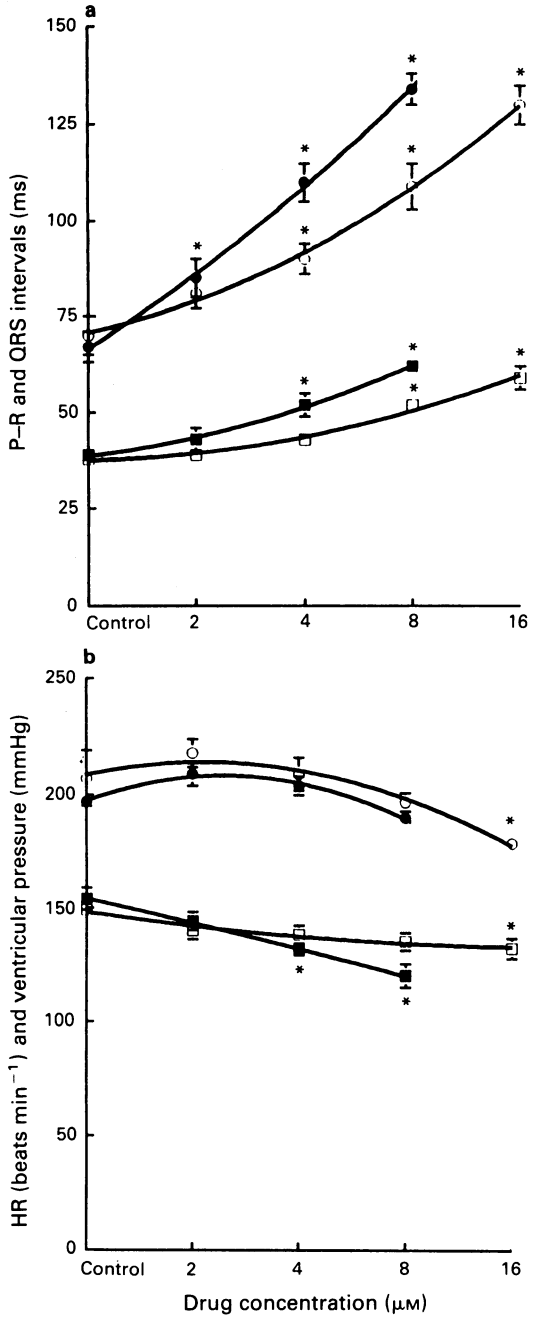

Figure 1 Concentration-response effects of PD 129289 and PD 129290 on ventricular pressure, heart rate and the ECG of rat isolated hearts. Effects of different concentrations of the two enantiomers, PD 129289 (filled symbols) and PD 129290 (open symbols) are shown as changes in ventricular pressure (□), heart rate (○) (b) and the ECG intervals of QRS (□) and P–R (○) (a) from control values after 5 min exposure to different concentrations of drug. Values are mean and vertical lines s.e.mean for $n = 5$. Control values were 154 ± 5 mmHg for ventricular pressure, 197 ± 3 beats min^{-1} for heart rate and 39 ± 2 ms for QRS and 69 ± 4 ms for P–R. *Indicates $P < 0.05$ for statistically significant differences from control.

tance of 10 MΩ. Cells were voltage clamped by use of an Axopatch 1D amplifier. Currents were filtered at 5 KHz, digitized at 10 KHz and stored in a microcomputer which also generated the voltage pulse protocol. Leakage and series resistance compensation was achieved by using the amplifier controls. Voltage pulses were given at 2 s intervals. PD 129290 was applied by adding aliquots of the stock solution of drug to the solution bathing the cells. Naloxone (1 μM) was used to block opioid receptors.

Drugs

PD 129289 (CAM 569; [5**R**-(5α,7α,8β)]-N-methyl-N[7-(1-pyrrolidinyl)-1-oxaspiro [4.5]dec-8-yl]-4-benzofuranacetamide HCl) and PD 129290 (CAM 570 = CI-977), the corresponding **S,S**-enantiomer, were generously supplied by Dr J. Hughes, Parke-Davis Research Institute, Cambridge. Naloxone was from DuPont. All drugs were made up in 0.9% NaCl solution for *in vivo* studies and in appropriate bathing solutions for *in vitro* studies.

Results

Isolated heart studies

Both PD 129289 and 129290 had dose-related effects on the ECG recorded from rat isolated perfused hearts. Figure 1 shows that both enantiomers prolonged P–R and QRS intervals although PD 129289 was somewhat more potent (dose-ratio approximately 2.0) than PD 129290; 8 μM of PD 129289 produced an approximate doubling of P–R and a lesser effect on QRS. Heart rate was reduced by PD 129290 but the change was only statistically significant for the highest concentration (Figure 1b). Dose-related bradycardia was not seen with PD 129289. Both enantiomers tended to reduce ventricular pressure (Figure 1b) and $+dp.dt^{-1}_{max}$ (data not shown). For example with PD 129289 the control peak ventricular systolic pressure of 154 ± 5 mmHg was reduced to 120 ± 5 after 8 μM ($P < 0.05$); similar changes occurred with PD 129290.

The experiments in isolated hearts demonstrated that both enantiomers had direct actions on the ECG. ECG indices were more sensitive to the drugs than either systolic ventricular pressure or heart rate.

Effects in intact rats

Blood presure, heart rate and ECG In saline treated intact rats, blood pressure and heart rate were stable over the measurement period whereas both enantiomers produced falls in blood pressure and heart rate. The fall in blood pressure was dose related for PD 129289. With the initial dose of 0.5 μmol kg^{-1} the change in pressure was − 5 ± 10 mmHg from the pre-drug value of 115 ± 8 mmHg. The fall in pressure increased with dose and was − 35 ± 7 mmHg after 32 μmol kg^{-1}. With PD 129290 the fall after 0.5 μmol kg^{-1} was − 25 ± 6 from a control of 121 ± 5 and − 27 ± 3 from 110 ± 6 mmHg in the presence of naloxone pretreatment. Falls after 32 μmol kg^{-1} were − 34 ± 6 and − 33 ± 7 mmHg in the presence and absence of naloxone, respectively. Between the 0.5 and 32 μmol kg^{-1} doses, falls in blood pressure were not obviously dose-related for PD 129290.

Heart rate in intact rats showed dose-related bradycardia with increasing doses of either enantiomer in the presence or absence of naloxone. A fall in heart rate was seen with PD 129289 after a cumulative dose of 2 μmol kg^{-1} and had fallen 98 ± 17 beats min^{-1} (predrug value 355 ± 27) after 32 μmol kg^{-1}. For PD 129290 in the absence of naloxone the same pattern was seen. The heart rate fell 83 ± 13 from a control of 406 ± 20 beats min^{-1} after 32 μmol kg^{-1}. In the presence of naloxone pretreatment the fall was 32 ± 12 from a control of 391 ± 18 beats min^{-1}.

ECG measures were influenced in a dose-related manner by both compounds (Figure 2). Saline was without effect in that the ECG did not change significantly with time over the experimental period. To an approximately equal extent PD 129289 and PD 129290 (in the presence and absence of naloxone) produced dose-related increases in RSh. For both enantiomers a dose between 1 and 2 μmol kg^{-1} produced a 10% increase in RSh with PD 129289 perhaps being more potent (potency ratio < 2.0).

In terms of effects on QRS duration, PD 129289 (the **R,R**

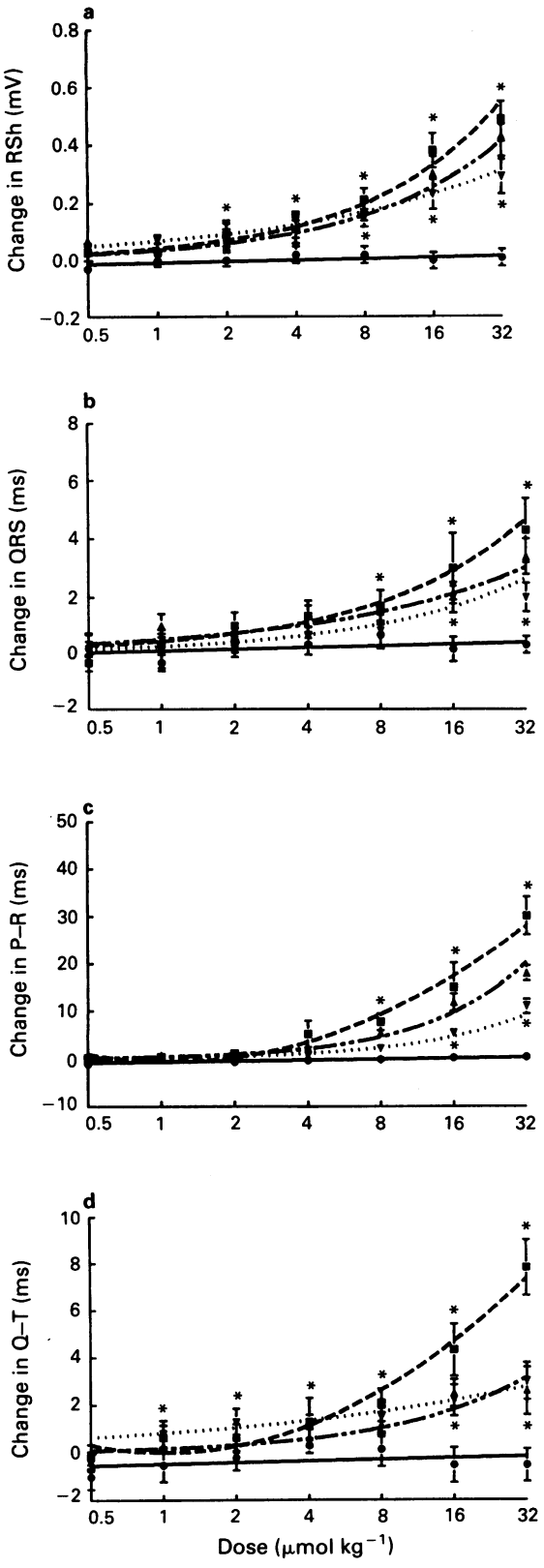

Figure 2 Dose-response curves for the effects of PD 129289 and PD129290 on the ECG of anaesthetized rats. The effects of the two enantiomers in the absence of naloxone, and PD 129290 in the presence of naloxone, are shown as changes in (a) RSh, (b) QRS, (c) P–R and (d) Q–T from control values induced 5 min after administration of a bolus of drug at 1, 2, 4, 8, 16, or 32 μmol kg^{-1}. Values are mean and vertical lines s.e.mean ($n = 8$) for (●) saline, (■) PD 129289, (▲) PD 129290 and (▼) PD 129290 in the presence of naloxone 8 μmol kg^{-1}. Control values were 0.49 ± 0.08 mV for RSh, 28 ± 1 ms for QRS, 53 ± 1 ms for P–R and 38 ± 1 for Q–T. *Indicates $P < 0.05$ for statistically significant differences from control.

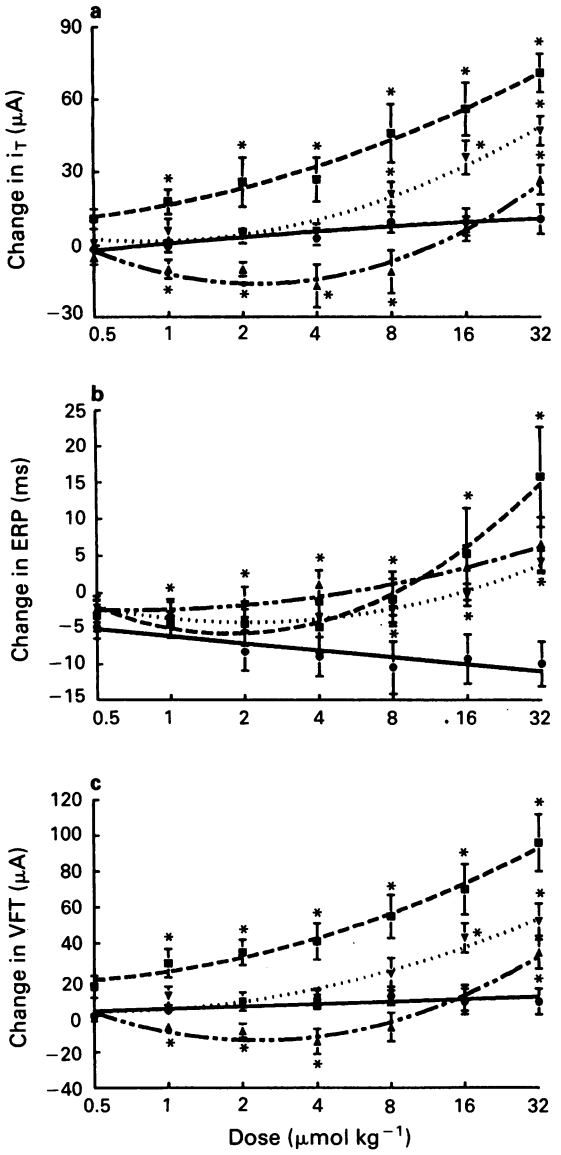

Figure 3 Dose-response curves for the effects of PD 129289 and PD129290 on sensitivity to ventricular electrical stimulation in anaesthetized rats. The effects of the two enantiomers in the absence of naloxone, and PD 129290 in the presence of naloxone, are shown as changes in (a) threshold current (i_T), (b) effective refractory period (ERP) and (c) threshold current for ventricular fibrillation (VF_T) occurring 3 min after administration of either compound at cumulative doses of 1, 2, 4, 8, 16, or 32 μmol kg^{-1}. Values are mean and vertical lines s.e.mean ($n = 6$) for (●) saline, (■) PD 129289, (▲) PD 129290 and (▼) PD 129290 in the presence of naloxone 8 μmol kg^{-1}. Control values were 51 ± 3 μA for i_T, 89 ± 6 μA for VF_T and 54 ± 2 ms for ERP. *Indicates $P < 0.05$ for statistically significant differences from control.

(+)-enantiomer) was slightly more potent than PD 129290 in the presence or absence of naloxone. The dose producing a 10% increase in QRS lay between 16 and 32 μmol kg^{-1}. A similar pattern was seen with P–R interval in that PD 129289 was again more potent (potency ratio < 2.0) than PD 129290 in the presence or absence of naloxone. ED_{10} values for both enantiomers were between 8 and 16 μmol kg^{-1}.

The ECG measurement least influenced by either drug was the Q–T interval. This interval was statistically significantly increased only after the higher doses, such that the approximate ED_{10} was 16 μmol kg^{-1} for PD 129289 and > 32 μmol kg^{-1} for PD 129290, with or without naloxone.

Table 1 Cardiovascular and ECG effects of 2 and 8 μmol kg^{-1} doses of PD 129290 and PD 129289 – antiarrhythmic study

Dose (μmol kg^{-1})	BP	HR	ECG P–R	ECG QRS	ECG Q–T
Saline	105 ± 6	377 ± 12	53 ± 2	29 ± 0.5	38 ± 0.8
PD 129290 2	88 ± 4*	356 ± 16	53 ± 2	31 ± 1.0	37 ± 1.0
PD 129290 8	88 ± 5*	331 ± 11*	62 ± 2*	31 ± 0.3	41 ± 0.4
PD 129290 8 (+N)	76 ± 5*	298 ± 9*	57 ± 2*	33 ± 0.8*	41 ± 1.0
PD 129289 2	71 ± 2*	318 ± 14*	55 ± 1	32 ± 1.0	41 ± 0.6
PD 129289 8	77 ± 6*	338 ± 17*	65 ± 2*	31 ± 0.6	41 ± 0.9

The effects of PD 129290 alone, or in the presence of naloxone (8 μmol kg^{-1}, + N), and PD 129289 are expressed as mean ± s.e.mean ($n = 9$) for the variable indicated. BP = mean blood pressure in mmHg; HR = heart rate in beat min^{-1}; P–R, QRS and Q–T are ECG intervals in ms.
*$P < 0.05$ for comparison with saline.

Table 2 Antiarrhythmic effects of 2 and 8 μmol kg^{-1} doses of PD 129290 and PD 129289 against coronary artery occlusion-induced arrhythmias

Drug and dose (μmol kg^{-1})	Group Incidence VT	VF	VT and/or VF	Arrhythmia score
Saline	8/8	7/8	8/8	5.0 ± 0.6
PD 129290 2	3/8	3/8	3/8	1.1 ± 0.6*
PD 129290 8	4/8	0/8*	4/8	1.5 ± 0.6*
PD 129290 8 (+N)	2/8*	0/8*	2/8*	0.8 ± 0.4*
PD 129289 2	6/8	3/8	6/8	3.0 ± 0.7
PD 129289 8	4/8	0/8*	0/8*	1.3 ± 0.6*
Naloxone 8	6/8	3/8	6/8	3.4 ± 0.2

The antiarrhythmic actions of PD 129290, alone or in the presence of naloxone (8 μmol kg^{-1}), as well as PD 129289, are expressed in terms of the group incidence of one or more episodes of the major arrhythmias of ventricular tachycardia (VT) or ventricular fibrillation (VF), $\log_{10}$ of ventricular premature beats (VPB) and arrhythmia score expressed as mean ± s.e.mean ($n = 9$).
*$P < 0.05$ for comparison with saline.

Effects of treatment on electrical stimulation Figure 3 illustrates the effects of the compounds on sensitivity to electrical stimulation. The control values for thresholds for capture (i_T) and VF (VF_T) were constant over the treatment period whereas those for ERP tended to fall throughout the treatment period. In a clearly dose-related manner PD 129289 increased thresholds and effective refractory periods. PD 129290 in the absence of naloxone initially tended to decrease thresholds (i_T and VF_T) with minima occurring between 2 and 4 μmol kg^{-1}. Thereafter, thresholds increased with increasing doses until finally they rose above control values in the range of 16–32 μmol kg^{-1}. The minimum values with a cumulative dose of 4 μmol kg^{-1} PD 129290 was − 21% from control for i_T and − 14% for VF_T. On the other hand, in the presence of naloxone pretreatment, the i_T and VF_T dose-response curves for PD 129290 were similar in shape to those for PD 129289 and showed no tendency to decrease below control levels. Interestingly in the presence of naloxone the potency of PD 129290 was less than PD 129289 (potency ratio approximately 4.0). Changes in t_T (data not shown) were less consistent.

ERP, and its related variable MFF (not shown), were not markedly influenced by low doses of either drug. The saline control showed a reduction with time for ERP and this was accompanied by the expected increase in MFF. Lower doses of both enantiomers did not have any significant effects on MFF and ERP when compared with saline. At higher doses there were dose-related increases in ERP and corresponding falls in MFF. Notably, responses to PD 129290, in the presence and absence of naloxone, were not markedly different from those for PD 129289.

Antiarrhthmic study In the coronary occlusion-arrhythmia study both 2 and 8 μmol kg^{-1} doses had the ECG and cardiovascular effects (Table 1) expected from the foregoing study. The lower dose (2 μmol kg^{-1}) minimally changed QRS and Q–T intervals. The most noticeable effect of the low dose was on the P–R interval. The large dose (8 μmol kg^{-1}) produced its expected effects on blood pressure, heart rate and ECG. None of the changes induced by the (−)-enantiomer (PD 129290) were reversed by naloxone pretreatment.

Both doses of PD 129289 and PD 129290 statistically significantly reduced arrhythmias induced by coronary occlusion (Table 2). The high dose (8 μmol kg^{-1}) was more antiarrhythmic than the low dose (2 μmol kg^{-1}). Naloxone had no effect on the antiarrhythmic effects of 8 μmol kg^{-1} PD 129290 although, on its own, naloxone appeared to reduce arrhythmias but not to a statistically significant extent.

The antiarrhythmic activity of either enantiomer could not be ascribed to changes in either occluded zone size or serum potassium concentrations. Group mean occluded zone size varied from a group minimum of 30.0 ± 1.2 (% total ventricular weight) to a maximum of 32.5 ± 1.6% (not statistically significant). Similarly, serum potassium concentrations were not influenced by the treatments. Group mean serum potassium concentrations varied from a low of 3.2 ± 0.2 mM to a high of 3.9 ± 0.4 mM and were not related to treatment.

Whole cell voltage clamp studies

Concentrations of PD 129290 (10 and 20 μM) which produced marked effects on isolated hearts (Figure 1) also

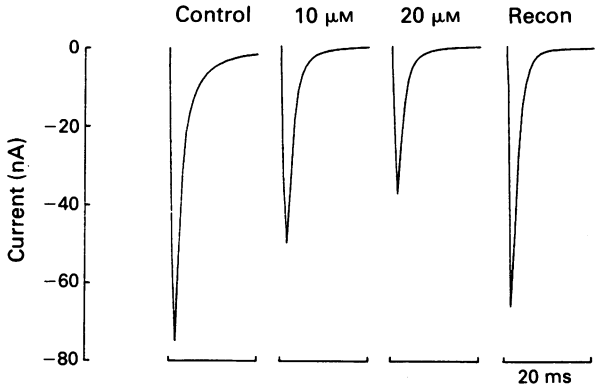

Figure 4 Sodium currents and the effect of PD 129290. Sodium currents were evoked in a single cardiac myocyte by a depolarizing step to − 40 mV from a holding potential of − 140 mV. The current under control conditions had a peak amplitude of 75 nA. This was reduced to 48 nA by 10 μM and to 38 nA by 20 μM PD 129290. These effects could be reversed by wash-out as shown by the re-control (Recon) current obtained 4 min after returning to the control solution. Some decrease in the control sodium current is seen during the course of the experiment, a finding typical of whole-cell records.

reduced sodium currents recorded in whole cell voltage clamp studies (Figure 4). These concentrations are 100–1,000 times greater than those producing κ agonism. The higher concentration (20 μM) of PD 129290 reduced current amplitude by about 50%, without perceptible changes in kinetics. These effects were readily reversible and could not be blocked by naloxone (data not shown).

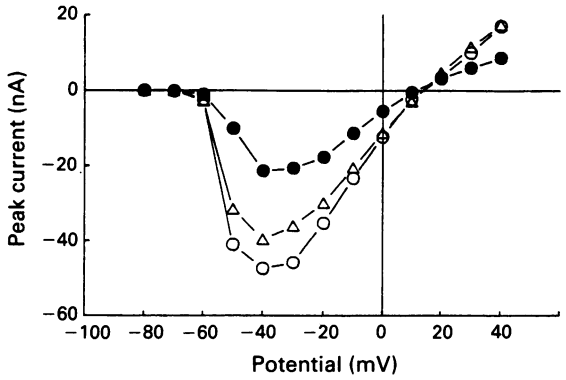

Figure 5 Current-voltage relation for the sodium current and the effect of PD 129290. Peak sodium current evoked by depolarizing steps to various voltages from a holding potential of − 140 mV is shown plotted as a current-voltage relationship. Maximal sodium current of 50 nA was evoked by a step to − 40 mV in control solution (○). The maximal current in the presence of 20 μM PD 129290 was also evoked at − 40 mV, but had a magnitude of only 22 nA (●). The voltage at which the current reversed was not appreciably changed in the presence of PD 129290. It was 14 mV for both curves. E_{NA} was calculated as 14 mV for the solutions used. Recontrol data, after about 4 min of recovery in control solution are shown by the open triangles.

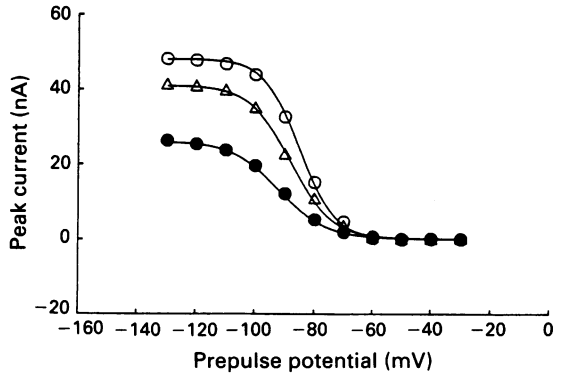

Figure 6 Inactivation curves for the sodium current and the effect of PD 129290. Sodium currents were evoked by a depolarizing step to − 40 mV from prepulses of various potentials. The magnitude of the sodium current is shown plotted against prepulse potential. The inactivation curve so produced was fitted by the Boltzman equation, shown by the line and of the form $y = I_{max}/1 + (e^{((v-v')/k)})$ where v is the prepulse potential, v′ is the voltage at which 50% inactivation occurs and k is a slope factor. The best fits to the data were obtained with values of max = 48 nA, v′ = − 85 mV and k = 6 for the control curve (○), and max = 26 nA, v′ = − 92 mV and k = 8 for the curve obtained in the presence of 20 μM PD 129290 (●). Recontrol data obtained after 4 min of recovery are shown by the triangles.

Figure 5 shows the effect of 20 μM PD 129290 on the current-voltage relationship for the sodium current. Sodium current amplitude was maximum at − 40 mV in control solution and the current reversed at 14 mV. Neither of these values was appreciably altered by the application of 20 μM PD 129290, although maximum sodium current was reduced by about 60%. PD 129290 (20 μM) did not affect the voltage-dependence of inactivation of the sodium current, as shown in Figure 6. Thus, although 20 μM PD 129290 caused large decrease in maximum current amplitude, this was not due to a shift in the voltage-dependence of either the current-voltage relation or inactivation. Thus, in rat isolated heart cells PD 129290 added to the bathing solution was capable of blocking sodium currents.

Discussion

Overall, the actions of the S,S (−)-enantiomer (PD 129290) in these studies were remarkably similar to those of the arylbenzacetamide κ agonist, U50,488H, which we have tested previously under similar conditions (Pugsley *et al.*, 1992a,b). Thus, both U50,488H and PD 129290 (κ agonists) prolonged the P–R and QRS intervals of the ECG in isolated and P–R, QRS, RSh and Q–T in intact hearts. In addition they lowered blood pressure and heart rate and increased threshold currents in the presence of naloxone. Both drugs had lesser effects on the other stimulation variables measured and both were antiarrhythmic against occlusion-induced arrhythmias.

Notably, as was the case with U50,488H, the only unequivocal effect of naloxone pretreatment on the actions of PD 129290 was to antagonize the decrease in threshold currents seen at low doses. Such evidence suggests that most of the antiarrhythmic and other actions of U50,488H and PD 129290 can be ascribed to effects unrelated to κ agonism.

The evidence for the above events being unrelated to the κ receptor is strengthened by findings with PD 129289, the **R,R** (+)-enantiomer of PD 129290, which has very low affinity for κ receptors. Measurement of the binding constants for PD 129289 and PD 129290 on κ receptors indicate at least a 1,000 fold selectivity for PD 129290 (the **S,S** (−)-enantiomer). Thus, it is reasonable to assume that the effects of PD 129290 on RSh, P–R, QRS and Q–T intervals, which were not blocked by naloxone and are similar to those of PD 129289, are probably independent of κ receptors. Similarly, increases in thresholds seen with all doses of PD 129289 and with PD 129290 at higher doses (or at lower doses in the presence of naloxone) may also be independent of κ receptors. Furthermore, PD 129289 was as equally effective as PD 129290 in its antiarrhythmic actions.

Although PD 129289 has very little activity at the κ receptor it has appreciable actions on μ receptors. Thus PD 129289 has almost the same μ receptor binding as PD 129290, and PD 129289 is effective in the hot plate test for analgesia, a test believed to depend upon μ recptors. The ED_{50} for PD 129290 in this test was 2.5 mg kg^{-1} (approx 6 μmol kg^{-1}), i.e. within the dose range tested in this study.

Table 3 Potencies of PD 129290 and PD 129289 with respect to blood pressure (BP), heart rate (HR), and ECG intervals, sensitivity to electrical stimulation (ES) and antiarrhythmic actions

				ECG			*ES*		*Antiarrhythmic*		
Drug	*BP*	*HR*	*P–R*	*QRS*	*Q–T*	i_T	VF_T	*ERP*	*VT*	*VF*	*AS*
PD 129289	16	8	8	>32	>32	8	0.5	3	<2	<2	<2
PD 129290	0.5	0.5	16	>32	>32	32	4	6	2	2	2
PD 129290 (N)	16	32	32	>32	>32	8	4	8	8	<8	<8

In the above table estimates for ED_{25} values (μmol kg^{-1}) have been extrapolated from the data given in the Tables and Figures. PD 129290 (N) refers to PD 129290 in the presence of naloxone. For abbreviations used see text in Methods section.

However naloxone is even more effective in blocking μ receptors than κ receptors (Rees & Hunter, 1990) thus μ effects cannot account for the action of PD 129290 after naloxone treatment.

With respect to the mechanisms underlying the antiarrhythmic actions of the two enantiomers, Table 3 lists the doses of PD 129289 and PD 129290 (in the presence or absence of naloxone) required to produce 25% changes in blood pressure, ECG variables, electrical stimulation variables and antiarrhythmic effects. From this Table it is apparent that marked antiarrhythmic effects occurred at doses which produced limited but consistent changes in the other variables.

In our studies with U50,488H (Pugsley *et al.*, 1992a,b) we suggested that the antiarrhythmic, ECG and most of the electrical stimulation effects of U50,488H were due to sodium channel blockade. In view of the similar actions of PD 129289 and PD 129290 (not blocked by naloxone) it would be reasonable to suggest that sodium channel blockade occurs with all three drugs and that such an action accounts for their antiarrhythmic actions. For PD 129290 we have direct proof that the compound blocks sodium currents in rat isolated cardiac myocytes and that such an action is not blocked by naloxone. Such blockade occurs at concentrations similar to those which produce ECG effects in rat isolated hearts and are consistent with the doses administered to intact rats to produce similar ECG effects. Sodium channel blockade explains the observed P–R prolongation, QRS widening and the increase in threshold currents, all of which occurred at doses which conferred antiarrhythmic actions. However, it should be noted that changes in the Q–T interval of the ECG seen at higher doses suggest that a limited degree of potassium channel blockade may occur with these compounds. It is possible that this action may also contribute to antiarrhythmic actions.

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