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In vitro cardiac models of dog Purkinje fibre triggered and spontaneous electrical activity: effects of nicorandil

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1 The effects of nicorandil (30 μM and 100 μM) on two models of triggered activity [early afterdepolarizations (EADs) and delayed afterdepolarizations (DADs)] and on spontaneous automaticity occurring from both normal and depolarized levels of membrane potential were examined in isolated cardiac Purkinje fibres of the dog. Standard intracellular microelectrode techniques were used.

2 Nicorandil (30 μM) abolished EADs provoked by superfusion with Tyrode solution containing 2.7 mM K^+ and 3 mM Cs.

3 DADs were induced by 0.2 μM acetylstrophanthidin in Tyrode solution containing 5.4 mM K^+. Nicorandil (30 μM) significantly reduced the amplitude of these DADs from 12.5 ± 2.5 mV to 5.5 ± 0.2 mV ($P < 0.02$, $n = 6$), while DADs were fully abolished by 100 μM nicorandil.

4 In unstimulated Purkinje strands, superfused with 2.7 mM K^+ containing Tyrode solution having a pH of either 7.4 or 6.8, spontaneous depolarizations developed with a mean maximum diastolic potential (MDP) of -84.6 ± 1.6 mV ($n = 9$) or -54.0 ± 1.2 mV ($n = 9$), respectively. Nicorandil significantly reduced the frequency of this automatic activity and caused its cessation, at either level of MDP. Nicorandil, however, produced significant hyperpolarization only when automaticity occurred from the depolarized level of potential.

5 These results suggest that nicorandil may exert significant antiarrhythmic actions *in vivo* by abolishing both spontaneous and triggered electrical activity.

Introduction

The effects of nicorandil, a potent vascular smooth muscle relaxant (Uchida *et al.*, 1978), on normal cardiac electrical activity have been examined in several mammalian cardiac preparations. Nicorandil (50 μM to 2.5 mM) has been shown to lengthen spontaneous cycle length significantly in rabbit isolated sinus node preparations (Satoh & Hashimoto, 1984). In guinea-pig isolated ventricular muscle, nicorandil shortened action potential duration in a concentration-dependent manner (Kojima & Ban, 1988). In paced Purkinje fibres, nicorandil (10 to 100 μM) caused no significant change in maximum diastolic potential, action potential amplitude and maximal upstroke velocity while it significantly shortened action potential duration (Yanagisawa & Taira, 1981; Imanishi *et al.*, 1983). In isolated spontaneously active Purkinje fibre preparations, nicorandil (between 1 to 100 μM) suppressed automaticity occurring from normal levels of diastolic potential without producing significant hyperpolarization (Yanagisawa & Taira, 1981). Nicorandil did hyperpolarize maximum diastolic potential while blocking automaticity in Purkinje fibres in which spontaneous activity arose from a depolarized (positive to -70 mV) level (Imanishi *et al.*, 1984). These cardiac electrophysiological effects of nicorandil are all believed to be due to an increase in potassium conductances as demonstrated in voltage clamp experiments (Kakei *et al.*, 1986). In addition, it has been suggested that these effects of nicorandil may possibly be antiarrhythmic (Imanishi *et al.*, 1984). The antiarrhythmic effects of nicorandil, however, have not been extensively examined in *in vitro* models of arrhythmogenesis. The goal of the present study, therefore, was to examine the effects of nicorandil in four different *in vitro* arrhythmia models representing possible causes of triggered and spontaneous cardiac electrical activity.

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Methods

Adult mongrel dogs ($n = 17$, weighing 12–15 kg) of either sex were anaesthetized with Na-pentobarbitone (30 mg kg^{-1}, i.v.). After opening the chest, the heart was rapidly removed. Small free-running false tendons (0.4 to 0.6 mm in diameter) were dissected and individually mounted in a plexiglass chamber allowing for continuous superfusion with normal Tyrode solution containing (in mM): Na^+ 153, K^+ 5.4, Ca^{2+} 2.7, Mg^{2+} 1.05, Cl^- 162.9, HEPES 5.4 and glucose 11.0 with a pH of 7.4 ± 0.05 at 36.9 ± 0.2°C while gassed with 100% O_2. During a 1 h equilibration period, preparations were externally paced (MECA, model SIM-2) at 3.3 Hz with 1 ms wide rectangular pulses having an amplitude equal to twice the diastolic threshold and delivered to the preparation through a pair of platinum electrodes. Conventional 3 M KCl filled glass microelectrodes having resistances in the range of 10 to 20 MΩ were used to record transmembrane potential. These electrodes were connected to the input of a high impedence electrometer (WP Instruments, model 750) the output of which was connected to a storage oscillosope (Tektronix, model 5115). These recordings were referenced to a Ag-AgCl-3 M KCl agar electrode placed in the superfusion chamber and connected to ground. Recordings were photographed on Polaroid film (type 667) from which analyses were made. Continuous microelectrode impalements were maintained throughout each experimental protocol. Only one preparation from each heart was used for a particular protocol; therefore, where the number of preparations is indicated, this value also reflects the number of different animals represented.

Student's *t* tests for paired data (Sokal & Rohlf, 1969) were used to determine the statistical significance of the results

which are given as the mean ± its standard error (s.e.). Changes were considered significant when P was found to be less than 0.05.

Nicorandil (Chugai Pharmaceutical Co., Ltd, Tokyo, Japan) was diluted from a 3 mM stock solution to its final concentration of 30 μM or 100 μM before every experiment. Acetylstrophanthidin (Sigma Chemical) and CsCl (Sigma Chemical) were also prepared daily by dilution of stock solutions (0.1 mM and 1 M, respectively).

Results

Effect of nicorandil on normal stimulated action potential parameters

The effects of nicorandil on normal paced Purkinje fibre action potential characteristics were examined in 3 preparations stimulated at a basic cycle length of 700 ms in order to allow comparison of our results to those previously reported. Nicorandil (30 and 100 μM) produced no significant effect on maximum diastolic potential (MDP), action potential amplitude (APA), and the maximum rising velocity of the action potential upstroke ($\dot{V}_{max}$) in these preparations after 15 min of exposure. Action potential durations measured at 50% (APD_{50}) and 90% (APD_{90}) of repolarization, however, were both significantly decreased by nicorandil (30 and 100 μM) following 15 min of exposure. In the presence of 30 μM nicorandil, APD_{50} was decreased to 222.0 ± 62.9 ms from a control value of 271.3 ± 50.6 ms while APD_{90} shortened to 281.7 ± 59.4 ms from a control value of 334.3 ± 50.6 ms. The higher concentration of nicorandil (100 μM) decreased APD_{50} and APD_{90} to 153.3 ± 60.6 ms and 210.3 ± 58.7 ms, respectively. Thus, both 30 and 100 μM nicorandil significantly shortened APD_{50} and APD_{90} ($P < 0.05$; $P < 0.005$) without producing any other significant change in stimulated isolated Purkinje fibre electrical characteristics. The changes in action potential duration were rapidly (< 10 min) reversed by superfusion in drug-free normal Tyrode solution.

Effect of nicorandil on triggered activity

Early afterdepolarizations (EADs) In order to determine the effects of nicorandil on triggered activity occurring before complete repolarization of the action potential, EADs were induced in 5 Purkinje strand preparations in Tyrode solution containing 2.7 mM K^+ plus 3 mM Cs^+. Purkinje strands were driven at 2 Hz during 10 s long trains of stimuli (20 stimuli) separated by a 5 s long period interrupted by a single extrastimulus delivered at a long coupling interval (3000 ms). After several minutes of superfusion with the modified Tyrode solution containing Cs^+, the duration of the action potential evoked by the extrastimulus was increased by approximately 90%. EADs appeared during the action potential evoked by the extrastimulus from a mean take-off-potential of −65.0 ± 1.9 mV (Figure 1b). Nicorandil (30 and 100 μM) rapidly abolished these EADs while shortening the duration of the action potential evoked by the extrastimulus (Figure 1c). Removing nicorandil from the superfusate allowed re-establishment of the triggered activity (Figure 1d). Similar results were obtained in 4 additional Purkinje strand preparations. Nicorandil tended to hyperpolarize MDP while abolishing EADs from −85.8 ± 1.8 to −89.0 ± 2.7 and −91.5 ± 4.6 mV in 30 and 100 μM nicorandil, respectively. This effect, however, was not significant. The effects of nicorandil were rapid, usually occurring within 5 to 10 min of exposure, and easily reversed by superfusion in nicorandil-free modified Tyrode solution.

Delayed afterdepolarizations (DADs) Another form of the triggered automaticity is the occurrence of DADs arising after repolarization of the action potential at normal (i.e., negative) levels of diastolic potential. In this study DADs were induced after 60 min of superfusion with Tyrode solution containing 0.2 μM acetylstrophanthidin in 6 preparations each obtained from a different dog heart. The preparations were stimulated during 8 s long trains of stimuli delivered at 2 Hz. These trains of stimuli were separated by 5 s periods during which stimulation was discontinued in order to allow development of DADs. After 30 min of exposure to 0.2 μM acetylstrophanthidin, DADs, 12.5 ± 2.5 mV in amplitude, developed at a mean coupling interval of 800 ± 150 ms (Figure 2b). Subsequent addition of 30 μM nicorandil significantly reduced ($P < 0.05$) the amplitude of these DADs to 5.5 ± 2.2 mV without significantly affecting their coupling interval (Figure 2c). The higher concentration of nicorandil (100 μM) completely abolished the acetylstrophanthidin-induced DADs (Figure 2d). After washout of nicorandil in acetylstrophanthidin-containing Tyrode solution, DADs reappeared with an amplitude of 12.7 ± 2.3 mV (Figure 2e). This amplitude was not significantly different from the amplitude of DADs observed before nicorandil treatment and the coupling interval was not significantly altered by the previous exposure to nicorandil. The maximum diastolic potential was slightly reduced by acetylstrophanthidin alone (from −80.0 ± 0.26 mV to −77.8 ± 1.0 mV). This depolarization was reversed and MDP returned to −79.3 ± 1.1 mV and −80.2 ± 1.0 mV following exposure to 30 μM and 100 μM nicorandil, respectively. Following the nicorandil washout in acetylstrophanthidin, MDP returned to −76.7 ± 1.1 mV.

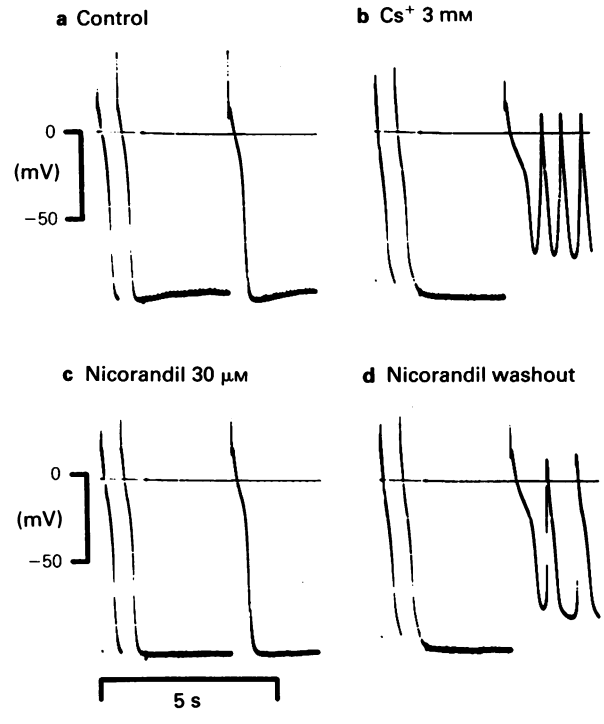

Figure 1 Effect of 30 μM nicorandil on early afterdepolarizations (EADs) induced by 3 mM Cs^+: (a) control recordings; (b) effect of 15 min exposure to 3 mM Cs^+; (c) effect of the addition of 30 μM nicorandil; (d) 10 min washout of nicorandil in Cs^+-containing Tyrode solution. Each panel shows the last 2 action potentials evoked during the train of 20 stimuli delivered at a frequency of 2 Hz and that evoked by the single extrastimulus (S2) delivered after a pause of 3 s following the train. Calibration scales for the transmembrane potential are presented to the left of each row, while the time scale for all panels is given at the bottom left of the figure.

Spontaneous electrical activity

Two groups of Purkinje strands, consisting of 9 preparations each, were superfused with Tyrode solution containing 2.7 mM K^+. Following the equilibration period, stimulation (2 Hz) was terminated to allow development of spontaneous activity. In the first group of preparations, the pH was kept at the

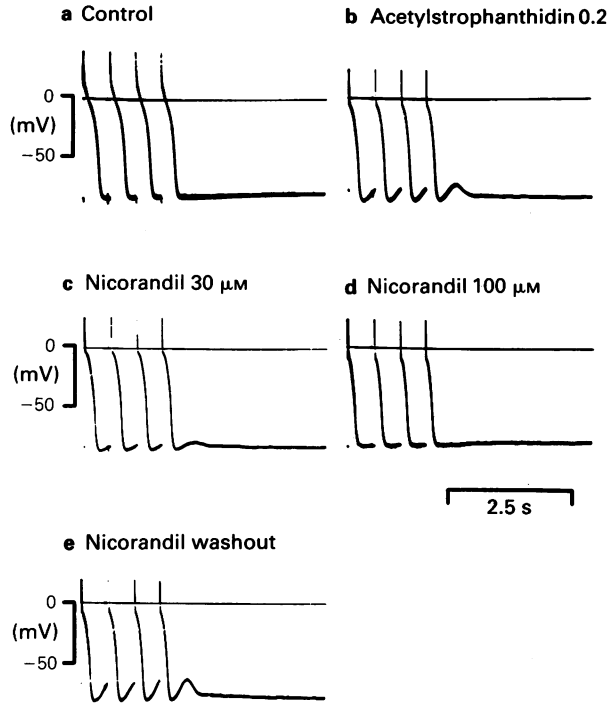

Figure 2 Effect of nicorandil on delayed afterdepolarizations (DADs) induced by 0.2 μM acetylstrophanthidin: (a) control recordings; (b) effect of 70 min exposure to 0.2 μM acetylstrophanthidin; (c) effect of 15 min exposure to 30 μM nicorandil in the continued presence of acetylstrophanthidin; (d) 15 min of addition of 100 μM nicorandil; (e) 30 min washout of nicorandil in acetylstrophanthidin-containing Tyrode solution. Each panel shows the last 4 action potentials evoked during the train of 16 stimuli delivered at a frequency of 2 Hz and the initial portion of the 5 s pause between trains. Calibration scales for the transmembrane potential are presented to the left of each row, while the time scale for all panels is shown at the bottom right of the panel (d).

normal value of 7.4, while in the second group pH was adjusted to 6.8 by addition of HCl.

Normal pH Maximum spontaneous diastolic potential remained negative (-84.6 ± 1.6 mV, $n = 9$) in the group of preparations in which the pH was kept at 7.4. The automatic frequency developed in these preparations was 0.342 ± 0.06 Hz. The low concentration of nicorandil (30 μM) significantly slowed ($P < 0.005$) spontaneous activity to 0.278 ± 0.06 Hz from 0.414 ± 0.06 Hz without altering MDP in 6 preparations. In 5 of the 6 preparations in which 30 μM nicorandil slowed spontaneous activity, 100 μM nicorandil was also applied in order to determine the concentration-dependent effects of the drug. Spontaneous activity was completely abolished by 100 μM nicorandil in 4 of these preparations. In the remaining one preparation, spontaneous frequency was slowed from 0.408 Hz to 0.208 Hz by 100 μM nicorandil. In 3 preparations, 30 μM nicorandil alone blocked spontaneous activity. The higher nicorandil concentration (100 μM) was not applied in these preparations.

Low pH Maximum diastolic potential depolarized to -54.0 ± 1.2 mV in 9 other preparations superfused with 2.7 mM K^+ containing Tyrode solution having a pH of 6.8. These preparations developed a more rapid spontaneous frequency (1.35 ± 0.11 Hz) than those superfused with Tyrode solution having a pH of 7.4. The low concentration of nicorandil (30 μM) slowed automatic frequency from 1.44 ± 0.18 Hz to 1.07 ± 0.18 Hz in 4 preparations. In the remaining 5 preparations, this nicorandil concentration (30 μM) blocked automaticity. The higher nicorandil concentration (100 μM) was subsequently applied to 4 of these 5 preparations. The effects of nicorandil on the diastolic membrane potentials are tabulated in Table 1. In this table, the data are divided into two subgroups based upon the effect of 30 μM nicorandil on automatic activity (i.e., those that were only slowed and those that were blocked). The Acid I group represents data obtained from the 4 preparations slowed by 30 μM nicorandil, and the Acid II group, data from 4 fibres whose spontaneous activity was blocked by 30 μM nicorandil. In the Acid I group, 100 μM nicorandil produced no significant effect on diastolic potential; while in the Acid II group the higher concentration of nicorandil (100 μM) caused the diastolic potential to hyperpolarize. The effects of 30 and 100 μM nicorandil on a preparation from the Acid II group are illustrated in Figure 3.

Discussion and conclusions

In the present study, nicorandil shortened Purkinje fibre APD without altering other action potential characteristics when the preparations were stimulated at a constant basic cycle length of 700 ms. These results confirm those of previous reports (Yanagisawa & Taira, 1981; Imanishi *et al.*, 1983) which also demonstrated that nicorandil over the range of 10 to 100 μM shortens Purkinje fibre APD while not significantly affecting its amplitude, maximal rate of rise or diastolic potential during stimulation. This effect of nicorandil on APD is believed to be due to an increase in a K^+ conductance (Yanagisawa & Taira, 1981; Imanishi *et al.*, 1983; 1984). Which specific cardiac K^+ channel conductances are affected by nicorandil is still controversial.

In terms of what changes an increase in outward current through an increased K^+ conductance may be expected to produce on the types of arrhythmogenic electrical activity examined in this study, we can predict that nicorandil should block the development of EADs that depend on reduction of

Table 1 Effect of nicorandil on Purkinje fibre diastolic potential at pH 6.8

	$[K^+]_o$ 5.4 mM (BCL = 500 ms)	$[K^+]_o$ 2.7 mM (Automatic)	*Nicorandil* 30 μM	*Nicorandil* 100 μM	*Nicorandil washout*
Acid I					
MDP (mV)	-87.9 ± 2.0	-54.5 ± 1.0^a	-54.0 ± 1.0^a	—	-53.3 ± 0.5^a
E_m (mV)	—	—	—	-53.3 ± 4.2^a	—
Total number of preparations (n) = 4					
Acid II					
MDP (mV)	-89.5 ± 1.5	-54.5 ± 1.7^a	—	—	-52.5 ± 2.0^a
E_m (mV)	—	—	-47.0 ± 3.9^{ab}	-79.3 ± 4.2^{ab}	—
Total number of preparations (n) = 4					

BCL = basic pacing cycle length; MDP = maximum diastolic potential; E_m = diastolic potential following block of spontaneous activity.

[a] Significant difference from value obtained during stimulation in 5.4 mM $[K^+]_o$ ($P < 0.05$); [b] significant difference from value obtained in 2.7 mM $[K^+]_o$, pH = 6.8, alone ($P < 0.05$).

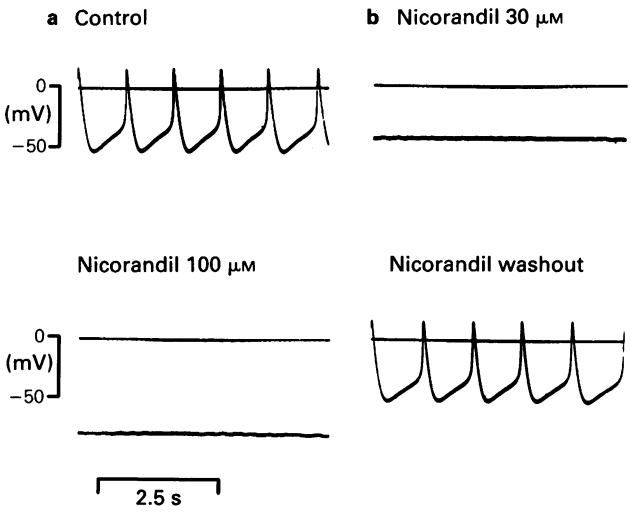

Figure 3 Effect of nicorandil on the spontaneous electrical activity in a Purkinje fibre preparation superfused with 2.7 mM $[K^+]_o$ at a pH of 6.8: (a) 15 min 2.7 mM $[K^+]_o$ at pH = 6.8, alone (control); (b) 10 min 30 μM nicorandil; (c) 10 min superfusion with 100 μM nicorandil; (d) 10 min washout of nicorandil in 2.7 mM $[K^+]_o$. Calibration scales for the transmembrane potential are presented to the left of each row, while the time scale for all panels is presented at the bottom left of the figure.

I_K and I_{K1} (Noble & Tsien, 1968; Hauswirth *et al.*, 1969). Indeed, in the present study nicorandil (30 and 100 μM) reduced and blocked caesium-induced EADs.

Delayed afterdepolarizations (DADs) are also believed to be arrhythmogenic (Cranefield, 1977) playing an important role in the genesis of glycoside-induced ventricular arrhythmias (Ferrier *et al.*, 1973; Zipes *et al.*, 1974). It has been suggested that DADs result from activation of non-specific ion channels as a result of the phasic release of Ca^{2+} from the sarcoplasmic reticulum in Ca^{2+} overloaded Purkinje fibres (Kass *et al.*, 1978). In the present study, nicorandil also reduced the amplitude of, and blocked development of, acetylstrophanthidin-induced DADs. The mechanism responsible for this effect is unknown. A nicorandil-induced increase in a K^+ conductance and resulting outward shift in the current-voltage relation, however, may have been expected to reduce the contribution of inward (i.e., depolarizing) current through the non-specific ion channels activated as a result of acetylstrophanthidin administration. Such an hypothesis may be supported by the slight nicorandil-induced hyperpolarization observed in our studies.

We also demonstrated that nicorandil blocked automaticity in unstimulated Purkinje fibres exposed to Tyrode solution containing 2.7 mM K^+ at a pH of either 7.4 or 6.8. The spontaneous electrical activity thus induced occurred from two different levels of maximum diastolic potential; one occurring at a normal level of approximately −85 mV at pH 7.4, the other from a depolarized level of approximately −55 mV at pH 6.8. Both types of automaticity have been implicated in the genesis of ventricular arrhythmias (Hoffman & Rosen, 1981) and may be due to different mechanisms (Elharrar & Zipes, 1980).

Purkinje fibre automaticity, occurring at normal (i.e., negative) diastolic potentials, is believed to be due to: (1) a time-dependent reduction in the magnitude of I_K activated during action potential depolarization (Noble & Tsien, 1969), (2) voltage-dependent activation of inward current (I_f, carried primarily by Na^+) on return of the membrane potential to negative diastolic levels (DiFrancesco, 1981a,b), and (3) activation of inward Ca^{2+} current through T-type calcium channels on depolarization of the membrane beyond approximately −70 mV (Hagiwara *et al.*, 1988). This type of spontaneous activity in Purkinje fibres has been referred to as normal automaticity (Hoffman & Dangman, 1987). An outward shift in the current-voltage relation produced by increasing a K^+-channel conductance between −90 and −70 mV may also be expected to reduce the contribution of I_f and prevent activation of T-type Ca^{2+} channels.

Automaticity in depolarized Purkinje strands usually exhibits a more rapid spontaneous frequency than that occurring at normal levels of diastolic potential (Elharrar & Zipes, 1980), as demonstrated in the present study with preparations exposed to pH 6.8. This type of spontaneous electrical activity has been termed 'abnormal automaticity' (Hoffmann & Rosen, 1981). We sought to depolarize the membrane by reducing external K^+ (from 5.4 to 2.7 mM) which is known to increase 'paradoxically' Purkinje fibre membrane sodium/potassium permeability ratio (Sheu *et al.*, 1980) and shift the steady-state current voltage relation in the inward direction (Gadsby & Cranefield, 1977). In addition, we lowered the pH of the superfusate to 6.8. Low pH has been suggested to reduce the activity of the Na^+-K^+ pump in low $[K^+]_o$ (Brown *et al.*, 1978), an effect that would also be expected to depolarize the diastolic potential. The mechanism responsible for spontaneous diastolic depolarization at these depolarized levels (−60 to −50 mV) is believed to be primarily due to voltage- and time-dependent reduction in the delayed rectifier K^+ conductance which is activated during action potential depolarization (Hauswirth *et al.*, 1969). Nicorandil slowed and blocked spontaneous Purkinje fibre depolarizations occurring from a maximum diastolic potential of approximately −55 mV in the present studies. The nicorandil-induced hyperpolarization observed in the second group of acidic preparations may have resulted from an increase in a K^+ conductance which allowed the membrane potential to move toward E_K [negative to −90 mV when $[K^+]_o$ = 2.7 mM (Sheu *et al.*, 1980)].

In conclusion, we have demonstrated that nicorandil shortened stimulated Purkinje fibre APD as previously described. In addition, we have demonstrated that nicorandil blocked Cs^+-induced EADs, acetylstrophanthidin-induced DADs, and Purkinje fibre automaticity occurring both at normal and depolarized levels of membrane potential. Each of these types of 'automatic' Purkinje fibre activity have been implicated in the genesis of *in vivo* cardiac arrhythmias (Hoffman & Dangman, 1987). As a potent vasodilator, nicorandil may also be expected to increase blood flow to ischaemic myocardium. This effect combined with the electrophysiological effect produced may allow nicorandil to provide significant anti-arrhythmic effects in ischaemic heart disease. Nakamura *et al.* (1984), however, failed to demonstrate a beneficial effect of nicorandil infusion (500 $\mu g\,kg^{-1}\,h^{-1}$) in reducing the dose of ouabain required to produce ventricular arrhythmias in pentobarbitone-anaesthetized dogs. In addition, Komori *et al.* (1985) reported that single bolus injections of nicorandil (1 $mg\,kg^{-1}$ and 3 $mg\,kg^{-1}$) failed to reverse ouabain-induced ventricular arrhythmias in intact, anaesthetized, dogs. The concentrations of nicorandil used in the present study were higher than the serum concentrations observed in patients ranging from approximately 2.5 to 5 μM (Nakaya *et al.*, 1979). The antiarrhythmic efficacy of nicorandil in relation to its vasodilator effects should, therefore, be carefully examined in intact animal arrhythmia models.

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