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The effects of perhexiline on the rat coronary vasculature

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Abstract

The predominant site and mechanism(s) of perhexiline-induced coronary vasodilatation were investigated in the rat heart. Perhexiline was more potent in the Langendorff perfused heart than in the left anterior descending coronary artery (EC_{50}; 0.27 μM, confidence limits 0.19–0.39: 2.7 μM, 2.0–3.4, respectively). Selective endothelial inactivation with Triton X-100 in the perfused heart, reduced the response to perhexiline 1 μM (76 + 8% to 30 + 3% of control). 1*H*-[1,2,4]Oxadiazolo[4,3-*a*]quinoxalin-1-one (ODQ) 3 μM, *N*ϖ-nitro-L-arginine 100 μM, or a combination of the latter with indomethacin 10 μM, had no significant effect on responses to perhexiline in the perfused heart. Unlike bradykinin-induced vasodilatation, responses to perhexiline were not inhibited by tetrabutylammonium 1 mM, or charybdotoxin 20 nM. SKF525A 5 μM inhibited both perhexiline and bradykinin responses, while apamin 1 μM and glibenclamide 3 μM inhibited neither. Perhexiline exerts partially endothelium-dependent coronary vasodilator effects in the rat, predominantly on small coronary arteries, which appear to be independent of nitric oxide (NO), prostacyclin and the endothelium-derived hyperpolarising factor (EDHF) released by bradykinin.

Keywords: Perhexiline; Coronary circulation; Endothelium; Nitric oxide (NO); EDHF (endothelium-derived hyperpolarising factor)

1. Introduction

Perhexiline is an effective prophylactic anti-anginal agent (Rees, 1983; Horowitz et al., 1995) which has incremental effects over those of other anti-anginal agents in patients with refractory angina (Cole et al., 1990). However, the mechanism(s) of its beneficial effects have remained uncertain. It has been shown to modify myocardial metabolism to produce an oxygen sparing effect via a shift from fatty acid to carbohydrate oxidation (Vaughan-Williams, 1980; Jeffrey et al., 1995), an effect which may be mediated via inhibition of the mitochondrial long-chain fatty acid transporter, carnitine palmitoyltransferase-1 (Kennedy et al., 1996). Perhexiline has also been shown to be a vasodilator in isolated porcine coronary artery strips (Fleckenstein-Grun et al., 1978) an action which may be related to its L-Ca^{2+} channel antagonist action. However it is questionable whether these effects are clinically relevant: the only specific investigation of L-channel antagonism by perhexiline to date, in isolated cardiomyocytes, indicates very weak effects (Barry et al., 1985). As perhexiline induces disproportionate increases in coronary flow relative to negative inotropy compared with verapamil in the Langendorff-perfused guinea-pig heart (Klaus and Guttler, 1978), the issue of the mechanism of vasodilator effect is potentially of clinical relevance. Although not reported to cause the phenomenon of coronary 'steal' characteristic of most selective small coronary artery dilators (Becker, 1978), perhexiline has nevertheless been reported to shown some selectivity for dilating small coronary vessels in the perfused hearts from open chest dogs (Klassen et al., 1976; Forman and Kirk, 1980). Thus the previously described coronary vasodilator effects of perhexiline have never been examined extensively, being superficially 'irrelevant' to its major postulated mechanism of therapeutic effect. However, any coronary vasomotor reactivity may be of considerable importance, particularly in light of the potential use of perhexiline in patients with unstable angina (Stewart et al., 1996) or aortic stenosis (Unger et al., 1997).

To clarify perhexiline's effects in small and large coronary arteries, the present study has examined the potency of perhexiline in isolated rings of the left anterior descending coronary artery of the rat, compared with its potency in the Langendorff-perfused rat heart in which resistance to

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flow is largely determined by the small resistance coronary vessels. Since the relevance of coronary vasodilator actions at therapeutic concentrations of perhexiline has not been explored, except with slow administration of perhexiline (Pepine et al., 1974), these studies included concentrations in the nanomolar to low micromolar range. The potential involvement of the vascular endothelium in responses of coronary arteries to perhexiline was also investigated. Furthermore, in order to identify any potential secondary changes in coronary vasomotor tone resulting from inotropic effects on the heart, developed left ventricular pressure was monitored simultaneously with coronary perfusion pressure in the isolated heart preparations.

2. Materials and methods

2.1. Isolated perfused heart

Male albino Wistar rats weighing 250–350 g were sacrificed under halothane anaesthesia and the hearts were rapidly excised. Experimental animals were treated in conformity with the National Health and Medical Research Council of Australia guidelines for the care of laboratory animals and the protocol was approved by the animal ethics committee of the University of Adelaide. Excised hearts were perfused via the aorta with Krebs solution of the following composition (mM): NaCl (118), KCl (4.7), KH_2PO_4 (1.18), $MgCl_2$ (1.05), glucose (5.55), $NaHCO_3$ (25), EDTA (0.01), $CaCl_2$ (1.25) bubbled continuously with 95% O_2/5% CO_2. The hearts were perfused at constant flow of 10 ml/min with a peristaltic pump (Minipuls 3, Gilson International, WI, USA) and maintained at 37°C. The heart rate was maintained at a constant rate by pacing at 20% above the intrinsic heart rate using platinum electrodes positioned on the right atrium and connected to a stimulator (Grass, Model S6, Grass Instruments, MA, USA). Changes in perfusion pressure were monitored via a Statham pressure transducer (Statham, P23AC) connected to the perfusion line. Intraventricular diastolic and systolic pressure were monitored via a water-filled latex balloon-cannula in the left ventricle and attached to a pressure transducer. Baseline diastolic pressure was adjusted to 5 mm Hg. Perfusion pressure and ventricular pressure changes were recorded on a computerised recording system (MacLab 8e, AD Instuments). The heart rate was obtained from the ventricular pressure trace. The hearts were allowed 20 min to equilibrate prior to drug application. Drugs were introduced to the coronary circulation via the perfusion line.

2.2. Isolated vessel

Hearts were collected into ice-cold Krebs solution. Under a dissecting microscope, the left main coronary artery was located and the left anterior descending coronary artery was dissected free of surrounding myocardial muscle and placed in ice-cold gassed Krebs solution. Vessel rings (approximately 2 mm long) were mounted via wires (40 μm diameter) through the lumen. One wire was fixed and the other attached to a myograph force transducer (DSC-6/MMH). The segments were set up in a 12 ml Mulvany stainless steel microbath (JP Trading, Aarhus, Denmark) containing Krebs bicarbonate solution at 37°C gassed with 95%/5% CO_2. Tension was recorded via a Rikadenki flat bed pen recorder. Baseline tension was 'normalised' using the procedure of Mulvany and Aalkjaer (1990) and Mulvany (1992) so that the internal circumference corresponded to a fixed percentage (90%: IC_{90}) of the calculated circumference (IC_{100}) at an intraluminal pressure of 100 mm Hg. Briefly, the artery was progressively stretched to obtain a length–tension curve which was then analysed by the Basic program, 'Normalisation', to obtain values of the diameter at 100 mm Hg (404 ± 31 μm, $n = 6$) and of the tension corresponding to the IC_{90}.

2.3. Experimental protocols

In the Langendorff-perfused rat heart, a cumulative concentration response curve was established to perfused 9,11-dideoxy-11α,9α-epoxymethano-prostaglandin $F_{2\alpha}$ (U46619). The concentration of U46619 (0.03 to 0.1 μM) which produced a large but submaximal (approximately 80%) response was selected for constriction of the coronary bed prior to testing for vasodilators. Drugs were applied in a cumulative manner to the perfusate. In studies involving inactivation of endothelial function, Triton X-100 was injected as a 0.2 ml bolus (1:300 in Krebs solution at 37°C) essentially as described by Li et al. (1993). This produced a significant sustained increase in diastolic pressure, decreased developed pressure and increase in baseline perfusion pressure. The perfusion pressure was then raised with U46619 to a pressure as close as possible to that in the absence of Triton treatment, prior to assessing vasodilator responses. In order to further define potential endothelium-dependent mediators of vasodilatation, the nitric oxide (NO) pathway was investigated by the effects of the specific NO synthase inhibitor, *N*ϖ-nitro-L-arginine (L-NOARG), and of the specific soluble guanylyl cyclase inhibitor, 1*H*-[1,2,4]oxadiazolo[4,3-*a*]quinoxalin-1-one (ODQ). The role of prostacyclin was tested by examining the effect of the cyclooxygenase inhibitor, indomethacin, while the role of cytochrome P450 monooxygenase metabolites was investigated by examining the effect of SKF525A. The potential role of K_{ATP} channels was examined by testing the effect of the specific blocker, glibenclamide. The role of K_{Ca} channels in endothelium-dependent vasodilator effects was examined by use of the non-specific K_{Ca} channel blocker, tetrabutylammonium, and the specific small- and intermediate-conductance K_{Ca} channel blockers, apamin and charybdotoxin, respectively.

In isolated left anterior descending coronary artery, the contractile response to K^+ was determined at the beginning of the experiment to determine viability by replacing the Krebs solution with one in which the NaCl had been partially replaced by isomolar KCl to a final K^+ concentration of 60 mM. Following washout of the KCl, the arteries were constricted with U46619 applied cumulatively to produce a large but submaximal (approximately 80%) response (0.03 or 0.1 μM U46619). The vasorelaxant response to cumulative doses of acetylcholine was measured as an index of endothelial function. Following washout of the acetylcholine, U46619 was reapplied and cumulative concentration response curves were constructed to perhexiline.

2.4. Analysis of data

Responses to relaxant agents are expressed as percentage decrease in contractile tone (% relaxation) relative to the U46619 control. Peak responses were estimated in the case of all agents which produced sustained responses. However in the case of bolus doses of bradykinin which produced transitory responses and whose duration was affected by drug treatment, responses were estimated in terms of percentage decrease in area under the response. Changes in ventricular developed pressure are expressed as a percentage change from control. Sigmoid concentration response curves were fitted by non-linear regression using Graphpad Prism 2.0, and EC_{50} and E_{max}. were derived from these curves. Responses are presented as mean ± S.E.M. and EC_{50} as geometric means with 95% confidence limits. Multiple comparisons between treatment groups were analysed by one-way analysis of variance (ANOVA). Single comparisons between preparations or treatments were analysed by unpaired Student's *t*-test. Where indicated, Welch's correction was used if variances were not equal. Paired Student's *t*-tests were used for comparison between paired control and drug-treated preparations. A critical value of $P < 0.05$ was used.

2.5. Materials

Acetylcholine chloride, A23187, apamin, bradykinin, glibenclamide, indomethacin, perhexiline maleate, SKF525A (α-phenyl-α-propylbenzeneacetic acid 2-(diethylamino)ethyl ester), sodium nitroprusside, tetrabutylammonium hydrogen sulphate, U46619 (9,11-dideoxy-11α,9α-epoxymethano-prostaglandin $F_{2\alpha}$) from Sigma (St. Louis, MO, USA), (+/−)etomoxir from Research Biochemicals International (Natick, MA, USA), ODQ from Tocris Cookson (Bristol, UK) and charybdotoxin from Austpep (Melbourne, Victoria, Australia). Stock solutions were made up in either ethanol (A23187, glibenclamide, indomethacin, perhexiline, U46619), in dimethyl sulfoxide (ODQ), distilled H_2O (apamin, bradykinin, charybdotoxin, etomoxir, L-NOARG, SKF525A, sodium nitroprusside, tetrabutylammonium) or EDTA 0.01 mM/HCl 0.01 M (acetylcholine).

3. Results

3.1. Perhexiline-induced vasodilatation: effect of vessel calibre

Perhexiline produced a concentration-dependent reduction in perfusion pressure in the perfused rat heart preconstricted with U46619 (Fig. 1) with an ED_{50} of 0.27 (0.19–0.39) μM. Developed left ventricular pressure and diastolic pressure were not altered significantly by U46619 in the doses used. Perhexiline also produced a small but significant increase in left ventricular developed pressure only at 1 μM (8 + 4% increase, $n = 15$, $P < 0.05$), which was associated with maximal vasodilatation. Perhexiline produced a dose-related decrease in tension in the U46619-contracted left anterior descending coronary artery (Fig. 1), but was approximately 10-fold less potent as a vasorelaxant than in the perfused heart preparation, having an ED_{50} of 2.7 (2.0–3.4) μM ($P < 0.05$, compared with isolated heart, unpaired *t*-test, $n = 8$ and $n = 6$ respectively). There was no significant difference between the

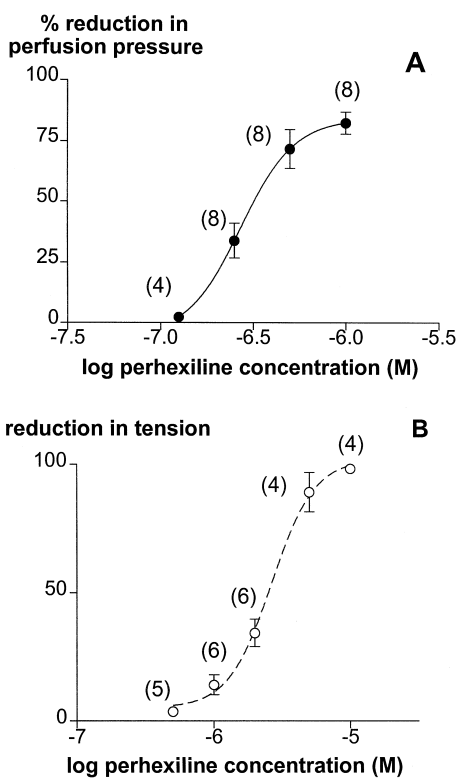


Fig. 1. Vasodilator responses to perhexiline in (A) Langendorff-perfused rat heart and (B) isolated rat left anterior descending coronary artery, as percent reduction in perfusion pressure and percent reduction in tension, respectively, relative to the U46619 contractile response; *n* in parentheses.

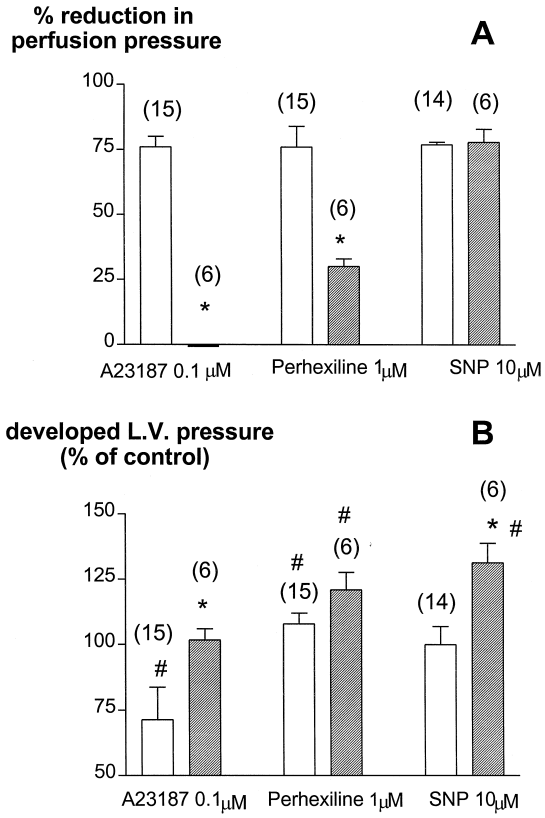


Fig. 2. Effect of Triton X-100 treatment on (A) vasodilator responses and (B) left ventricular developed pressure responses, to A23187, perhexiline and sodium niroprusside (SNP) in Langendorff-perfused rat hearts. Open histograms represent pre-Triton responses; shaded histograms represent post-Triton responses; n in parentheses. $^{*}P < 0.05$, relative to pre-Triton values (Welch's unpaired t-test), $\#P < 0.05$, relative to control LV developed pressure (paired t-test).

E_{max} in the perfused heart and left anterior descending coronary artery, being $84 \pm 9\%$ ($n = 8$) and $102 \pm 8\%$ ($n = 6$), respectively.

3.2. Effect of perhexiline: role of the endothelium in the perfused heart

After a bolus dose of Triton X-100 (1:300 in Krebs, 0.2 ml), basal perfusion pressure increased by $21 \pm 8\%$ and developed left ventricular pressure decreased to a stable level at $50 \pm 9\%$ of the pre-Triton value. After treatment with Triton X-100, and vasoconstriction with U46619, the vasodilator response to the endothelium-dependent agent A23187 in the perfused heart was eliminated, while that to sodium nitroprusside was unaffected (Fig. 2A). The vasodilator response to perhexiline was significantly lower following Triton treatment ($P < 0.002$) being $30 \pm 3\%$ decrease in perfusion pressure compared with $76 \pm 8\%$ in the absence of Triton treatment (Fig. 2A), indicating an endothelium-dependent component in the vasodilator response to perhexiline.

In preparations with intact endothelium, A23187 reduced left ventricular developed pressure by $18 \pm 6\%$ ($n = 15$, $P < 0.005$) relative to control (Fig. 2B). In contrast, perhexiline produced a very small increase of $8 \pm 4\%$ ($n = 15$, $P < 0.05$) and sodium nitroprusside had no significant effect on left ventricular developed pressure. Following endothelial inactivation with Triton X-100, both perhexiline and sodium nitroprusside induced a significant increase in left ventricular developed pressure (by 21 ± 7 and $31 \pm 7\%$, respectively, Fig. 2B, $P < 0.05$, $n = 6$ in each case), while A23187 had no significant effect on left ventricular developed pressure.

3.3. Mechanism of endothelium-dependent perhexiline-induced dilatation

3.3.1. Role of NO and prostacyclin

Pretreatment with L-NOARG 100 μM, either alone, or combined with indomethacin 10 μM, had no significant

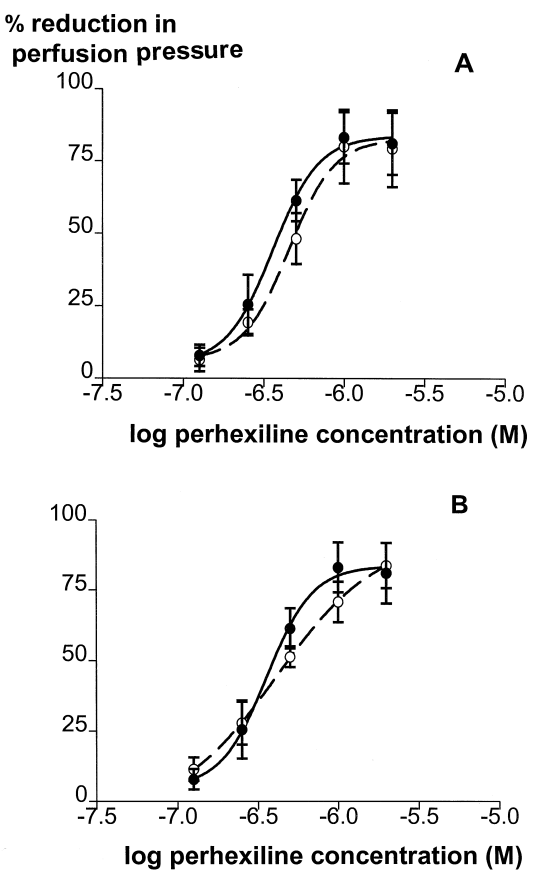


Fig. 3. Effect of (A) L-NOARG 100 μM and (B) L-NOARG 100 μM and indomethacin 10 μM on dose–response curves for perhexiline-induced vasodilatation in Langendorff-perfused rat hearts. ● Control responses, ○ responses following pretreatment with L-NOARG and/or indomethacin, $n = 5$. Control and treated preparations were not significantly different with respect to EC_{50} or E_{max} (one-way ANOVA). EC_{50}s: control 0.36 μM (0.23–0.56), L-NOARG 0.45 μM (0.28–0.71), L-NOARG + Indomethacin 0.42 μM (0.18–0.97); E_{max}: control $84 \pm 8\%$, L-NOARG $83 \pm 10\%$, L-NOARG + Indomethacin $93 \pm 23\%$.

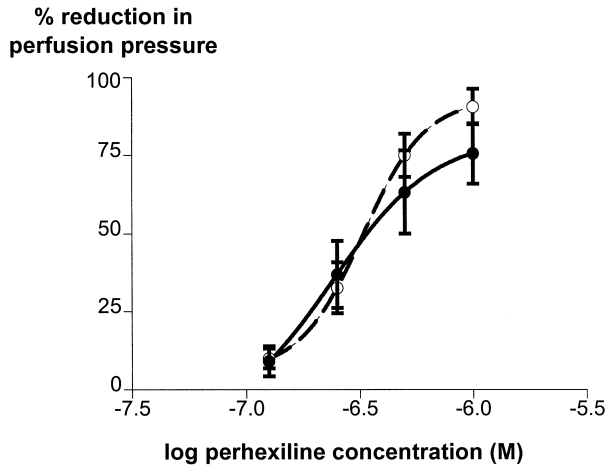


Fig. 4. Effect of ODQ 3 μM on vasodilator responses to perhexiline in Langendorff-perfused rat hearts. ● Responses in presence of dimethylsulphoxide, ○ responses in the presence of ODQ, $n = 5$. The control and treated preparations were not significantly different with respect to EC_{50} or E_{max} (paired t-test). EC_{50}: control 0.24 μM (0.04–1.6), ODQ 0.32 μM (0.23–0.45); E_{max}: control 81 ± 29%, ODQ 93 ± 9%.

effect on the vasodilator dose–response curve to perhexiline in the perfused rat heart (Fig. 3A and B, respectively). Furthermore, inhibition of guanylyl cyclase with ODQ 3 μM (Fig. 4) had no significant effect on the dose–response curve to perhexiline. However, in the same heart preparations, ODQ pretreatment at the same concentration did reduce the response to sodium nitroprusside 10 μM from 78 ± 5% reduction in perfusion pressure to 5 ± 4% ($P < 0.05$, $n = 5$), suggesting a complete inhibition of cGMP-mediated vasodilatation induced by NO released from sodium nitroprusside.

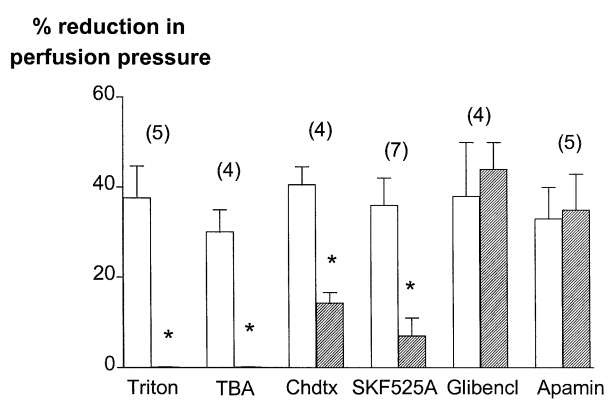


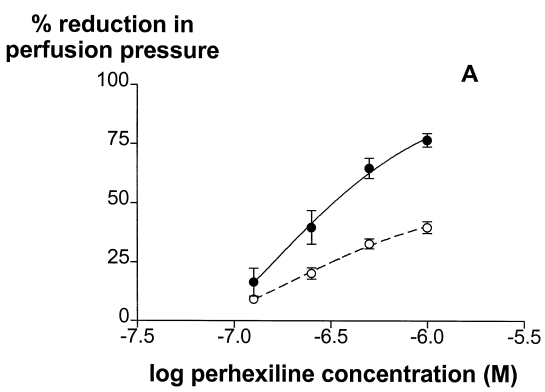
Fig. 5. Effect of inhibitors on vasodilator responses to 100 pmol bolus dose of bradykinin (BK) in the Langendorff-perfused rat heart. $^*P < 0.05$, paired t-test, n in parentheses. Open histograms represent control responses to BK; shaded histograms represent responses following pretreatment with one of the following inhibitors: Triton X-100 0.2 ml bolus (1:300 in Krebs), tetrabutylammonium (TBA) 1 mM, charybdotoxin (Chdtx) 20 nM, SKF525A 5 μM, glibenclamide (glibencl) 3 μM or apamin 1 μM.

3.3.2. Role of endothelium-derived hyperpolarising factor (EDHF)

Vasodilator responses to bradykinin in the perfused heart were maximal at a bolus dose of 100 pmol and were endothelium-dependent as indicated by their abolition following treatment with Triton X-100 (Fig. 5). Vasodilator responses to bradykinin were inhibited by tetrabutylammonium 1 mM, charybdotoxin 20 nM and SKF525A 5 μM, but were unaffected by apamin 1 μM and glibenclamide 3 μM (Fig. 5). In comparison, vasodilator responses to perhexiline were inhibited only by SKF525A (Figs. 6A and 7). SKF525A also inhibited the maximum response to sodium nitroprusside (Fig. 6B) although to a much lesser extent than either bradykinin or perhexiline.

3.3.3. Role of carnitine palmitoyltransferase-1 inhibition

The potent and irreversible carnitine palmitoyltransferase-1 inhibitor, etomoxir, had no significant effect on perfusion pressure at a concentration of 1 μM during a 30 min exposure ($n = 4$ arteries), indicating that the vasodila-



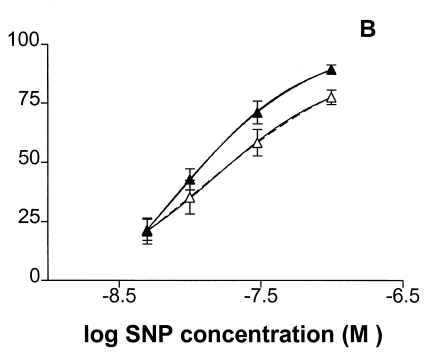


Fig. 6. Effect of pretreatment with SKF525A 5 μM on vasodilator responses to (A) perhexiline and (B) sodium nitroprusside (SNP) in Langendorff-perfused rat hearts. Control dose–response curves are shown by closed symbols and and those following SKF525A pretreatment by open symbols. E_{max}: perhexiline; control 97 ± 8%, SKF 51 ± 4% ($P < 0.001$, $n = 8$, paired t-test) and for SNP; control 100 + 2%, SKF 89 + 2% ($P < 0.02$, $n = 6$, paired t-test). EC_{50}s for both perhexiline and SNP were not significantly affected by SKF525A (paired t-test), perhexiline: control 0.32 μM (0.22–0.45), SKF525A 0.30 μM (0.23–0.40); SNP: control 14.4 nM (8.2–26.0), SKF525A 13.8 nM (6.4–24.0).

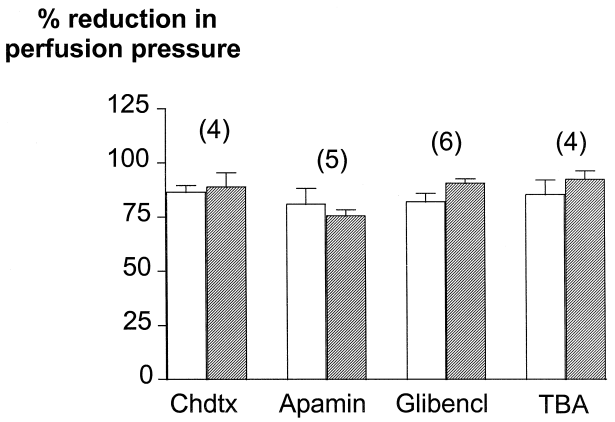


Fig. 7. Vasodilator responses to perhexiline 1 μM in Langendorff-perfused rat hearts, open histograms represent responses before, and shaded histograms represent responses after pretreatment with K^+ channel inhibitors, tetrabutylammonium (TBA) 1 mM, charybdotoxin (Chdtx) 20 nM, glibenclamide (glibencl) 3 μM or apamin 1 μM. n in parentheses. Control and treated preparations were not significantly different, paired *t*-test.

tor effect of perhexiline is unlikely to be related to carnitine palmitoyltransferase-1 inhibition.

4. Discussion

The results of the present study confirms that perhexiline is a coronary vasodilator in the rat heart at concentrations relevant to those achieved in plasma during therapeutic administration of the drug (Horowitz et al., 1986; Cole et al., 1990). It has also identified a selectivity for small resistance coronary arteries over large conduit coronary arteries in this species, since the ED_{50} for perhexiline in the perfused rat heart was approximately one order of magnitude smaller than that in the isolated left anterior descending coronary artery. The potency of perhexiline in the rat left anterior descending coronary artery accords with the data of Fleckenstein-Grun et al. (1978) for isolated porcine left anterior descending coronary artery in which perhexiline produced vasodilatation in the concentration range of 0.5 to 10 μM. Klaus and Guttler (1978) demonstrated an increase in coronary flow in the isolated guinea pig heart in response to perhexiline 0.1 to 1 μM which accords with its potency in the perfused rat heart. Hence selectivity for small vessels occurs in the range of concentrations of perhexiline achieved in plasma in patients treated with the drug viz. 0.5 to 2 μM (Horowitz et al., 1986).

Treatment of the perfused rat heart with a bolus dose of Triton X-100 in order to selectively inactivate/damage the coronary endothelium resulted in a similar effect to those reported by Li et al. (1993) for the Langendorff-perfused rabbit heart. Perfusion pressure rose by approximately 20% and left ventricular developed tension decreased to a stable value at 50% of control values. The increase in baseline perfusion pressure probably reflects the influence of removal of basal release of endothelium-derived vasorelaxant agents. Selective inactivation of endothelium-mediated vasodilatation was confirmed in the present experiments by abolition of the response to the endothelium-dependent agents, A23187 and bradykinin, without any change in the response to the NO donor, sodium nitroprusside which induces an endothelium-independent vasodilatation. Therefore these experiments have for the first time revealed that perhexiline has a coronary vasodilator action which is partially mediated via the endothelium.

The mechanism of its action on the endothelium is unknown but appears to be independent of its carnitine palmitoyltransferase-1 inhibitory actions since another carnitine palmitoyltransferase-1 inhibitor, etomoxir 1 μM, did not cause vasodilatation in the same rat heart preparation. It also appears to be unrelated to its L-Ca^{2+} channel blocking actions since the release of endothelial mediators of vasodilatation appears to depend on increases in intracellular Ca^{2+} concentration via release of intracellular Ca^{2+} followed by Ca^{2+} influx via non-voltage-gated channels (Newby and Henderson, 1990), although the relative importance of these two sources of Ca^{2+} appears to vary depending on the vessel and the agent released (Parsaee et al., 1992; Fukao et al., 1997).

In the rat heart the endothelium-dependent vasodilator action of perhexiline does not appear to be mediated by NO release since the present study has shown that inhibition of NO synthase by L-NOARG pretreatment had no significant effect on the vasodilator concentration response curve to perhexiline. Although 100 μM L-NOARG should induce maximal inhibition of NO synthase, Kemp and Cocks (1997) have recently questioned whether L-NOARG produces maximal inhibition of NO formation since L-NOARG plus oxyhaemoglobin produced incremental inhibition over L-NOARG alone in human coronary arteries. However, the fact that ODQ, a specific inhibitor of guanylyl cyclase (Garthwaite et al., 1995), produced maximal inhibition of the vasodilator response to the NO donor, sodium nitroprusside, without affecting the response to perhexiline, supports the conclusion that perhexiline is acting independently of the NO–cGMP pathway. Prostacyclin formation also does not appear to be involved in the endothelium-dependent component of perhexiline's vasodilator action on the perfused rat heart since indomethacin 10 μM did not inhibit the effects of perhexiline, both in the presence or absence of L-NOARG.

EDHF may play a more significant role as an endothelium-derived vasodilator in small resistance vessels (Garland et al., 1995). Vasodilator responses to bradykinin and perhexiline in the perfused rat heart were inhibited by the non-specific cytochrome P450 inhibitor SKF525A. This effect of SKF525A was not completely specific with respect to inhibition of EDHF formation since responses to sodium nitroprusside were also inhibited, albeit to a smaller degree than those of bradykinin and perhexiline. SKF525A has been reported previously to have additional effects at the concentration used in the present study, including

anti-muscarinic effects (Choo et al., 1986) and inhibition of K^+ entry (Kalsner et al., 1970). Hence although the present data is consistent with endothelium-dependent relaxation by both perhexiline and bradykinin being due to an monooxygenase pathway metabolite, these data need confirmation by the effect of other enzyme inhibitors. The specific K_{ATP} channel inhibitor, glibenclamide, had no significant effect on responses to bradykinin or perhexiline. The non-specific Ca^{2+}-dependent potassium (K_{Ca}) channel inhibitor, tetrabutylammonium, inhibited responses to bradykinin but not perhexiline. Charybdotoxin significantly inhibited responses to bradykinin in the perfused heart, but apamin, a small conductance K_{Ca} channel inhibitor, had no effect, consistent with previous indications that bradykinin mediates vasodilation in this preparation via EDHF acting on intermediate conductance K_{Ca} channels (Quilley et al., 1997). However, perhexiline does not appear to be acting via release of the same endothelium-derived agent as bradykinin in the perfused rat heart since its vasodilator responses were unaffected by both tetrabutylammonium and charybdotoxin. It is possible that some other EDHF is released by perhexiline in the perfused rat heart which acts via a different K^+ channel from those examined in the present study. An effect of perhexiline via K^+ channel-induced hyperpolarisation could also be confirmed by electrophysiological studies of the vascular smooth muscle of small rat coronary vessels.

Observation of a significant although weak positive inotropic effect of perhexiline and sodium nitroprusside in isolated hearts with inactivated coronary endothelium is a novel finding. In isolated cat papillary muscles with damaged endocardial endothelium, a similar positive inotropic effect with sodium nitroprusside and 3-morpholino sindonimine (SIN-1), another NO donor, was reported which was secondary to guanylate cyclase activation (Mohan et al., 1996). However, this does not appear to be the case for perhexiline, as in the present study its effects were not modified by the presence of an NO synthase inhibitor or a guanylyl cyclase inhibitor. Some degree of positive inotropy is consistent with its previously reported effect of increasing cardiac output, for no change in heart rate, in the working rat heart at a concentration of 2 μM (Jeffrey et al., 1995) and with the reported beneficial effects in patients with aortic stenosis (Unger et al., 1997). The small effect on left ventricular developed pressure in this preparation, at a maximal vasodilator concentration of perhexiline, combined with the large endothelium-dependent component, suggests that perhexiline mediates vasodilatation of the rat coronary circulation largely by a primary action, not via autoregulatory responses to increased metabolic demand. The mechanism and physiological relevance for the inotropic effect of perhexiline requires further investigation. In this context, the negative inotropic effect of Triton X-100 perfusion may be distinguished mechanistically from the positive inotropic effect of perhexiline: selective inhibition of release of NO and prostacyclin (Mohan et al., 1995) has been associated with negative inotropic effects, and Li et al. (1993) demonstrated similar effects of Triton in rabbit papillary muscles removed from Langendorff-perfused hearts.

The identity of the endothelium-derived mediator of perhexiline-induced vasodilatation in perfused rat heart requires further study, but the present data indicate that it does not act via NO or prostacyclin release or via carnitine palmitoyltransferase-1 inhibition. Moreover it does not appear to release the same EDHF as bradykinin. In addition, the mechanism(s) of the endothelium-independent component of perhexiline-induced dilatation in both the perfused rat heart and isolated left anterior descending coronary artery have not been identified in the present study.

The selectivity of perhexiline for small coronary arteries revealed in the present study presents a paradox in that perhexiline has well-demonstrated efficacy as an anti-anginal agent in patients (Horowitz et al., 1995), but as a selective small coronary vessel dilator it would be expected to have the potential to produce ‘coronary steal’ (Becker, 1978), a phenomenon which aggravates angina. Potential explanations for this paradox include (1) heterogeneity between species in the selectivity of perhexiline for small coronary resistance vessels and (2) selectivity of perhexiline for ischaemic resistance vessels. Klassen et al. (1976) presented some support for the latter possibility since they demonstrated a preservation by perhexiline of endocardial blood flow relative to epicardial blood flow in canine hearts subjected to graded flow reductions via the left anterior descending coronary artery. It remains uncertain whether perhexiline exhibits microvascular coronary dilator effects in human vessels. This issue and possible variability of perhexiline’s vasodilator effects in ischaemic regions merit further investigation. Finally, it remains uncertain whether such coronary vasomotor effects, even if present in human subjects, are therapeutically relevant. However, with the increasing utilization of perhexiline in acute coronary syndromes and refractory angina, such effects may prove to be of importance.

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