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Inhibition of Carnitine Palmitoyltransferase-1 in Rat Heart and Liver by Perhexiline and Amiodarone

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ABSTRACT. The mechanism of the anti-anginal effect of perhexiline is unclear but appears to involve a shift in cardiac metabolism from utilization of fatty acid to that of carbohydrate. We tested the hypothesis that perhexiline inhibits the enzyme carnitine palmitoyltransferase-1 (CPT-1), which controls access of long chain fatty acids to the mitochondrial site of β-oxidation. Perhexiline produced a concentration-dependent inhibition of CPT-1 in rat cardiac and hepatic mitochondria *in vitro*, with half-maximal inhibition (IC_{50}) at 77 and 148 μmol/L, respectively. Amiodarone, another drug with anti-anginal properties, also inhibited cardiac CPT-1 (IC_{50} = 228 μmol/L). The rank order of potency for inhibition was malonyl-CoA > 4-hydroxyphenylglyoxylate (HPG) = perhexiline > amiodarone = monohydroxy-perhexiline. Kinetic analysis revealed competitive inhibition of cardiac and hepatic CPT-1 by perhexiline with respect to palmitoyl-CoA but non-competitive inhibition with respect to carnitine. Curvilinear Dixon plots generated "apparent inhibitory constant (K_i)" values for perhexiline, which indicated a greater sensitivity of the cardiac than the hepatic enzyme to inhibition by perhexiline. Perhexiline inhibition of CPT-1, unlike that of malonyl-CoA and HPG, was unaffected by pretreatment with the protease nagarse. These data establish for the first time that two agents with proven anti-anginal effects inhibit cardiac CPT-1. This action is likely to contribute to the anti-ischaemic effects of both perhexiline and amiodarone. BIOCHEM PHARMACOL 52;2:273–280, 1996.

KEY WORDS. perhexiline; amiodarone; anti-anginal; CPT-1; malonyl-CoA

Perhexiline is a prophylactic anti-anginal agent that is utilized most commonly for the treatment of otherwise intractable angina pectoris in patients already receiving optimal treatment with Ca^{2+} channel blockers, nitrates, and β-adrenoceptor antagonists [1, 2]. Perhexiline exerts minimal negative inotropic effects (via weak L-channel calcium antagonism) and does not affect systemic vascular resistance markedly [3–5]. Hence, its anti-ischaemic actions cannot be ascribed to hemodynamic changes. Its mechanism of action on the heart has been hypothesized to be due to a shift from predominantly metabolism of fatty acids towards increased utilization of carbohydrates [6]. Since fatty acids require approximately 10–15% more oxygen than carbohydrates for the formation of an equivalent amount of ATP [7], such a shift in metabolism may provide an oxygen-sparing effect under conditions of reduced coronary flow. Increased lactate utilization has been demonstrated in rat heart *in vivo* [8], and treatment of rat hepatocytes with perhexiline has been shown to reduce β-oxidation of fatty acids after 72 hr of exposure *in vitro* [9]. Recently, perhexiline has been shown to increase cardiac efficiency while simultaneously reducing fatty acid utilization and increasing that of lactate in the isolated working rat heart [10]. Nevertheless, the precise cellular site of the action of perhexiline is still unclear.

Amiodarone is a prophylactic anti-arrhythmic agent that prolongs action potential duration [11] but also blocks sodium channels, β-adrenoceptors, and L-type calcium channels [12–14]. Originally, amiodarone was introduced as a prophylactic anti-anginal agent. Like perhexiline, amiodarone exerts minimal hemodynamic effects [15], and has been shown to inhibit hepatic mitochondrial β-oxidation of fatty acids [16].

Previous studies have indicated that several agents may act as inhibitors of CPT-1§ (EC 2.3.1.21), a mitochondrial enzyme responsible for long chain acyl carnitine formation, which provides access of long chain fatty acyl-CoA to the site for β-oxidation in the mitochondrial matrix. Recent evidence suggests that CPT-1 is located in the outer mitochondrial membrane with the catalytic site facing the intermembrane space [17]. Of the various agents previously shown to be CPT-1 inhibitors, oxfenicine, which is converted to the active CPT-1 inhibitor HPG, has been in-

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§ *Abbreviations:* CPT, carnitine palmitoyltransferase; HPG, 4-hydroxyphenylglyoxylate; and POCA, 2(5[4-chlorophenyl]pentyl)oxyrane-2-carboxylate.

vestigated previously as a potential anti-anginal agent [18]. Other CPT-1 inhibitors, such as etomoxir and POCA, induce metabolic changes that have been studied in animal models in order to identify possible useful effects in the treatment of diabetes [19, 20], cardiac ischaemia [21, 22], or arrhythmia [23]. However, although exhibiting beneficial anti-ischaemic effects in isolated rat hearts [21, 22], neither POCA nor etomoxir has been tested for anti-anginal effects.

Although no clinically useful anti-anginal agent has been identified previously as a CPT-1 inhibitor, the previously described metabolic effects of perhexiline and amiodarone raised the issue of their hitherto uncertain mechanisms of action. The aim of the present study was to investigate whether inhibition of cardiac CPT-1 is a potential mechanism of anti-ischaemic action of perhexiline and amiodarone.

MATERIALS AND METHODS

Drugs and Chemicals

Palmitoyl-CoA free acid, *l*-carnitine, perhexiline maleate, reduced glutathione free acid, fatty acid-free BSA, malonyl-CoA and nagarse (subtilisin) were obtained from Sigma (St. Louis, MO, U.S.A.). Perhexiline HCl was a gift from Marion Merrell Dow (Australia), amiodarone from Sinofi/Winthrop (Australia), and HPG from Hoffmann-La Roche (U.S.A.). [^{3}H]-*l*-Carnitine (sp. act. 77 Ci/mmol) was obtained from Amersham International (Aylesbury, Bucks, U.K.). All other chemicals were reagent grade purity.

Tissue Preparation

Male Sprague–Dawley rats of 300 g body weight were killed by exsanguination under light ether anaesthesia. The hearts and livers were removed rapidly and placed on ice. Homogenates and mitochondria were prepared essentially as described by Kiorpes *et al.* [24], based on the method of Johnson and Lardy [25]. Briefly, hearts or livers were homogenized at 4° in isolation buffer (10 mmol/L Tris-HCl, pH 7.4, containing 250 mmol/L sucrose and 1 mmol/L EDTA) at 1:5 (w/v), using a Polytron PT-3000 (Kinematica). The post-nuclear supernatant was obtained after centrifugation for 10 min at 600 *g*. In some experiments, the supernatant was used as such, while in the majority of experiments a mitochondrial pellet was obtained by centrifugation at 4° for 10 min at 7000 *g*. The pellet was washed by resuspension to the original volume in isolation buffer and centrifugation at 7000 *g* for 10 min, followed by resuspension in isolation buffer to approximately 40 mg/mL protein. In some experiments the mitochondria were pretreated with nagarse, 5 μg/mL, for 10 min at 37°, followed by addition of 200 μL of 20% BSA. Then the mitochondria were pelleted and washed as above by centrifugation at 7000 *g*. Following protein estimation by a Bio-Rad protein assay kit using BSA standard, the homogenate and mitochondrial preparations were diluted to an appropriate protein concentration for the CPT-1 assay.

CPT-1 Assay

CPT-1 activity was measured by the formation of palmitoyl-[^{3}H]-carnitine from palmitoyl-CoA and [^{3}H]-*l*-carnitine, essentially as described by McGarry *et al.* [26], with the exception that a preincubation period was used prior to enzyme estimation as described by Kiorpes *et al.* [24]. The incubate consisted of 0.4 mL of incubation buffer (pH 7.4) containing 6.25 μmol Tris-HCl, 72 μmol KCl, 2.85 μmol reduced glutathione, 1.45 μmol KCN, 1 μmol $MgCl_2$, 5 μL ethanol, 2.7 mg defatted BSA, and potential inhibitors as indicated below. Preincubation in a shaking water bath at 37° was initiated by the addition of 50 μL of homogenate or mitochondrial preparation. For assays of the cardiac or liver enzyme, 50 μg of mitochondrial protein and 150 μg of homogenate protein were utilized per assay to ensure linearity with protein concentration. The reaction was initiated by the addition of 50 μL of substrate mix containing palmitoyl-CoA and [^{3}H]-*l*-carnitine. Unless otherwise indicated, the final concentrations were 50 or 100 μmol/L palmitoyl-CoA and 400 μmol/L carnitine. The reaction was terminated by the addition of 50 μL of concentrated HCl. Samples were diluted with 1.45 mL of distilled water, and then the product, [^{3}H]-palmitoyl carnitine, was extracted with 1 mL *n*-butanol as described by Kiorpes *et al.* [24]. Blanks were identical in composition to reaction tubes except that concentrated HCl was added prior to addition of substrate mix. The incubation time was 4 min

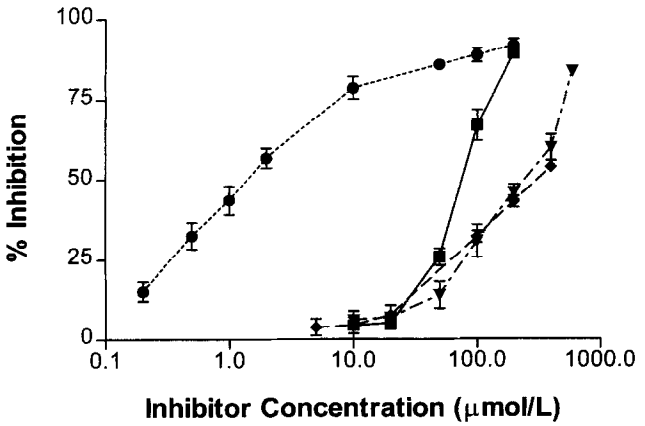


FIG. 1. Inhibition of CPT-1 in rat cardiac mitochondria. Enzyme preparations were preincubated for 15 min with inhibitors before estimating CPT activity by incubation with palmitoyl-CoA, 100 μmol/L, and *l*-carnitine, 400 μmol/L, for 4 min. Key: malonyl-CoA (●----●), perhexiline (■—■), amiodarone (♦--♦), and OH-perhexiline (▼--▼). Values are means ± SEM; N = 4–7. CPT-1 activity in the presence of the ethanol vehicle was 30.4 ± 2.0 nmol/mg protein/min and in control mitochondria was 33.1 ± 3.0 nmol/mg protein/min (not significantly different, N = 7).

TABLE 1. Potency of inhibition of cardiac and hepatic CPT-1

	IC_{50} (μmol/L)			
	Cardiac		Hepatic	
Agent	Mitochondria	Homogenate	Mitochondria	Homogenate
Malonyl-CoA	1.23* (0.76–1.97)† (7)‡	0.92* (0.49–1.75) (5)		
HPG	42 (12–148) (4)			
Perhexiline	77 (63–96) (6)	65§ (54–79) (6)	148‖(91–238) (5)	90‖ (57–142) (5)
Amiodarone	228* (160–325) (4)	197* (187–207) (3)		
OH-Perhexiline	264* (199–349) (5)	205* (159–265) (4)	192 (148–249) (3)	156* (99–249) (3)

Values are geometric means.
* $P < 0.05$, compared with perhexiline, unpaired *t*-test.
† Ninety-five percent confidence limits.
‡ Number of determinations.
§ Not significant, $P = 0.08$, compared with mitochondrial preparation.
‖ $P < 0.05$, compared with cardiac enzyme, unpaired *t*-test.

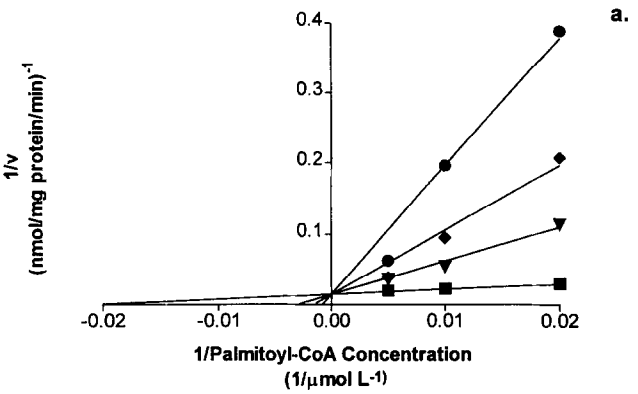
for concentration-curves to inhibitors. However, for kinetic studies utilizing lower concentrations of palmitoyl-CoA and *l*-carnitine, the incubation time was shortened to 1.5 min since preliminary studies indicated that the rate of palmitoyl carnitine formation was linear at all substrate concentrations up to 2 min.

Treatment of Data

The data are presented as means ± SEM, unless otherwise indicated. The IC_{50} and K_i values are presented as the geometric mean with 95% confidence limits. Kinetics of enzyme inhibition have been presented as Lineweaver–Burk and Dixon plots, and interactions between inhibitors by Yonetani–Theorell plots [27]. Data were compared by Student's *t*-test, and a critical value of $P = 0.05$ was used throughout.

RESULTS

The results in Fig. 1 indicate that palmitoyl carnitine formation in rat cardiac mitochondria is inhibited by perhexiline maleate. The percent inhibition increased with increasing time of preincubation (from 33 ± 5% at 5 min to 80 + 7%, N = 4, at 15 min with 100 μmol/L perhexiline), so that all subsequent experiments utilized a 15-min preincubation routinely. The inhibition was mediated by the perhexiline moiety since perhexiline HCl had effects identical to those of the maleate salt (data not shown). The parent compound was significantly more potent than the main hydroxylated products of perhexiline in both cardiac mitochondrial and homogenate preparations (Table 1). The IC_{50} values for perhexiline in cardiac mitochondrial and homogenate preparations were not significantly different ($P = 0.08$). Amiodarone also inhibited palmitoyl carnitine formation but was less potent than perhexiline. The rank order of potency for inhibition was malonyl-CoA > HPG = perhexiline maleate > amiodarone = monohydroxy perhexiline (OH-perhexiline) in both homogenates and mitochondrial-enriched preparations, as indicated by the IC_{50} values (Table 1). Concentration-response curves were steeper for perhexiline than for the other inhibitors (Fig. 1). In excess of 90% of total cardiac mitochondrial enzyme



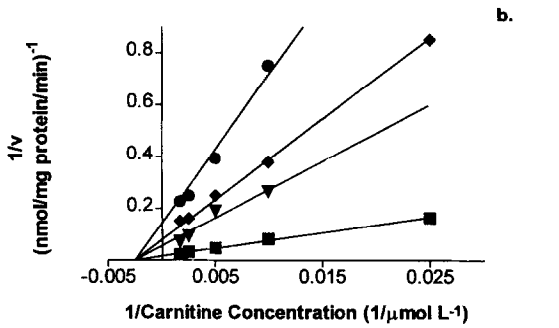


FIG. 2. Lineweaver–Burk plot of inhibition of cardiac mitochondrial CPT-1 by perhexiline. The enzyme preparations were preincubated with perhexiline, or vehicle, for 15 min and then incubated for 1.5 min at 37° with (a) various concentrations of palmitoyl-CoA in the presence of 400 μmol/L carnitine, or (b) various concentrations of carnitine in the presence of 100 μmol/L palmitoyl-CoA. Key: control (■); perhexiline, 100 μmol/L (▼); perhexiline, 150 μmol/L (◆); and perhexiline, 200 μmol/L (●). Example of one of three experiments.

TABLE 2. Effect of perhexiline on kinetic parameters in cardiac CPT-1

	K_m Pal-CoA (μmol/L)	V_{max}* (nmol/mg protein/min)	K_m Carnitine (μmol/L)	V_{max}† (nmol/mg protein/min)
Control	79 (42–150)	52 ± 8	358 (312–403)	56 ± 6
Perhexiline (100 μmol/L)	261‡ (101–670)	51 ± 9	351 (286–420)	17 ± 3‡
Perhexiline (200 μmol/L)	928‡ (770–1130)	53 ± 7	369 (330–413)	6.3 ± 1.2‡

K_m values are geometric means (95% confidence limits); V_{max} values are means ± SEM, N = 3.
* Estimated in the presence of 400 μmol/L carnitine.
† Estimated in the presence of 100 μmol/L palmitoyl-CoA.
‡ $P < 0.05$, compared with control, paired *t*-test, N = 3.

activity was inhibitable by malonyl-CoA (Fig. 1), indicating that predominantly CPT-1 was estimated by the enzyme reaction. Essentially similar data were obtained using cardiac post-nuclear homogenates with 95% of enzyme activity inhibitable by malonyl-CoA (data not shown). With 2 min of preincubation and 50 μmol/L palmitoyl-CoA, the IC_{50} for malonyl-CoA was lower, 0.42 (0.38 to 0.44) μmol/L, N = 4. The corresponding IC_{50} values for perhexiline in liver mitochondrial and homogenate preparations were significantly higher than those in cardiac preparations (Table 1).

The results of kinetic studies of cardiac CPT-1 are shown in Fig. 2, a and b. As can be seen from the Lineweaver–Burk plots from mitochondrial-enriched preparations of rat heart, perhexiline maleate displayed competitive inhibition with respect to palmitoyl-CoA (Fig. 2a), since the K_m for palmitoyl-CoA increased with increasing perhexiline concentrations but the V_{max} remained constant (Table 2). In contrast, perhexiline displayed non-competitive inhibition with respect to carnitine (Fig. 2b), the V_{max} declining with increasing perhexiline concentrations with the K_m for carnitine remaining constant (Table 2). The curvilinear Dixon plots for both cardiac and hepatic CPT-1 revealed that inhibition was greater at higher perhexiline concentrations than could be described by simple Michaelis–Mentin kinetics (Fig. 3, a and b). Results were essentially similar regardless of whether studies were carried out in mitochondrial-enriched preparations or in post-nuclear homogenates except that the "apparent K_is" estimated from these plots were 86 (74–99) and 69 (62–79) μmol/L, respectively, for cardiac CPT, and 146 (127–169) and 86 (75–100) μmol/L, respectively, for hepatic CPT.

The data in Fig. 4 indicate that pretreatment of cardiac mitochondria with the protease nagarse markedly reduced the degree of inhibition of cardiac CPT-1 by both malonyl-CoA and HPG, but had no significant effect on the inhibition caused by perhexiline. Nagarse treatment did not affect the basal CPT-1 activity significantly in these preparations. These data indicate a difference between the inhibitory site for perhexiline on the one hand, and that for malonyl-CoA and HPG on the other. Nevertheless, the ability of perhexiline to interfere with the binding of both malonyl-CoA and HPG to cardiac CPT-1 is demonstrated by the Yonetani–Theorell plots of Fig. 5, a and b, respectively. Perhexiline (60 μmol/L) competed with HPG for inhibition of cardiac CPT-1, as demonstrated by the parallel plots for HPG in the presence and absence of perhexi-

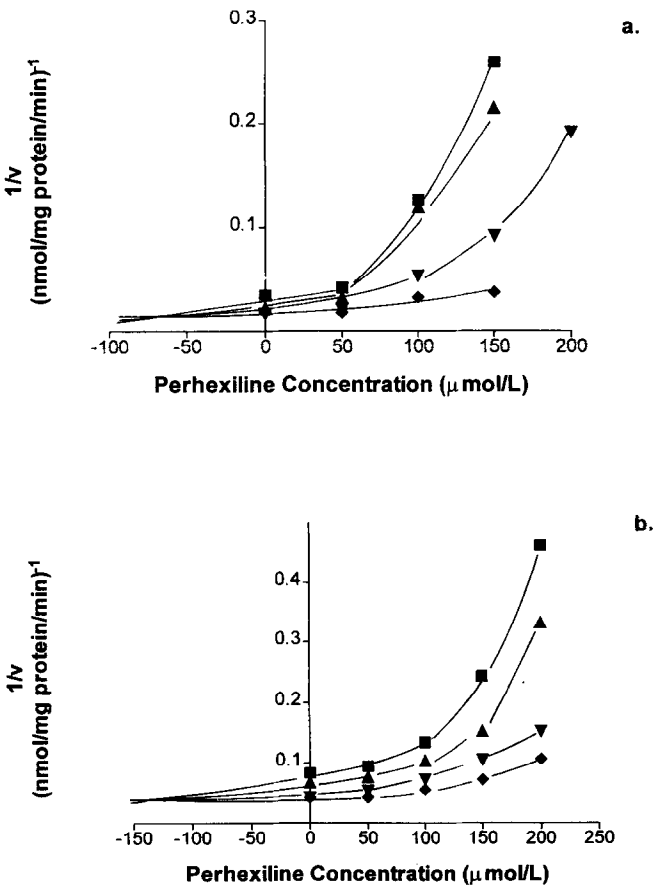


FIG. 3. Dixon plot of inhibition of mitochondrial CPT-1 in (a) cardiac and (b) hepatic preparations by perhexiline at various palmitoyl-CoA concentrations. The enzyme preparations were preincubated with perhexiline or vehicle for 15 min and then incubated with 400 μmol/L carnitine and palmitoyl-CoA at 25 (■), 50 (▲), 100 (▼), and 200 (◆) μmol/L. Example of one of three experiments. K_i values: (a) 86 (74–99), and (b) 146 (127–169) μmol/L, N = 3.

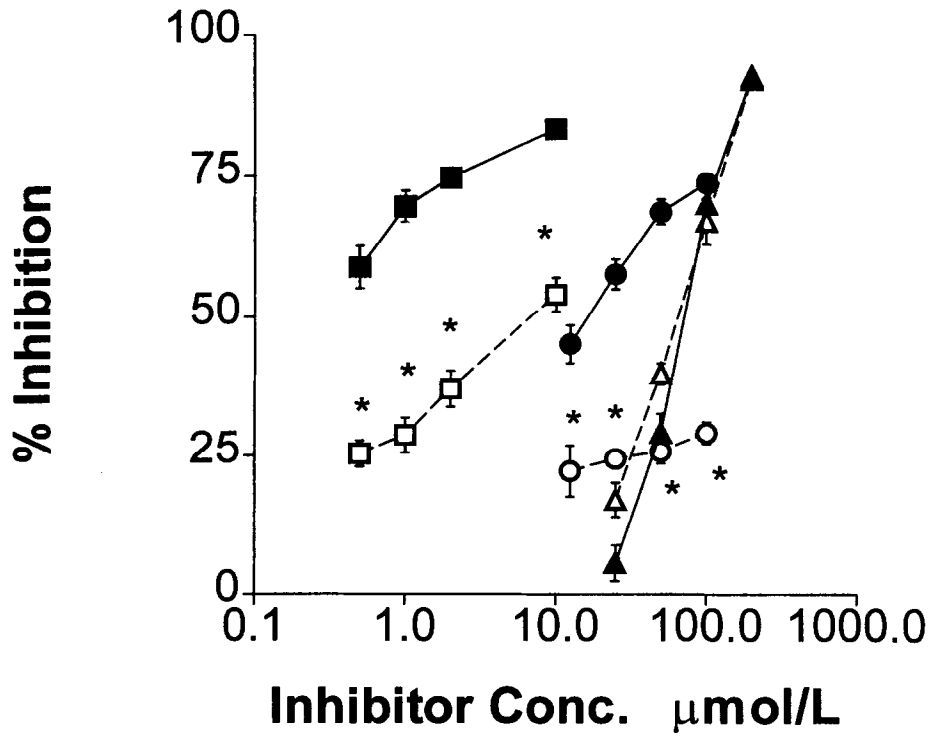


FIG. 4. Effect of nagarse on inhibition of cardiac CPT-1. Mitochondria were incubated with 5 μg/mL nagarse (open symbols) or vehicle (closed symbols) for 10 min at 37°. Following treatment with 20% BSA (w/v), and washing, the mitochondria were preincubated for 15 min with inhibitors and then incubated for 4 min with palmitoyl-CoA (50 μmol/L) and carnitine (400 μmol/L) at 37°. Key: malonyl-CoA (■), HPG (●), and perhexiline (▲); and nagarse pretreated, malonyl-CoA (□), HPG (○), and perhexiline (△). Values are means ± SEM, N = 4. Statistical analysis: (*) $P < 0.05$, compared with no nagarse treatment, paired *t*-test. CPT-1 activity was 19.3 ± 1.4 nmol/mg protein/min in the control and 18.3 ± 1.2 nmol/mg protein/min in the nagarse-pretreated mitochondria (N = 4).

line. However, perhexiline appeared to inhibit the effect of lower concentrations of malonyl-CoA on CPT-1, and the effect did not appear to conform to either a competitive or non-competitive interaction. In contrast, perhexiline displayed a non-competitive interaction with Co-A, indicating that it acts at a separate site from Co-A on cardiac CPT-1 (Fig. 5c).

DISCUSSION

We have provided evidence that perhexiline and amiodarone are inhibitors of cardiac and hepatic CPT-1. The cardiac enzyme appears to be more sensitive to inhibition by perhexiline based on respective IC_{50} and K_i values. Such an action provides an explanation for the ability of perhexiline to decrease fatty acid metabolism in favour of carbohydrate metabolism and thereby increase cardiac efficiency [7]. Amiodarone was significantly less potent (on a molar basis) than perhexiline.

It is not known what local tissue drug concentrations are achieved in the vicinity of the mitochondrial membranes in patients treated with perhexiline or amiodarone. However, both perhexiline and amiodarone are highly concentrated in tissues [28, 29] so that local concentrations in the vicinity of CPT-1 in the heart would be expected to be high relative to plasma concentrations. Therapeutic plasma concentrations of perhexiline range from 0.5 to 2 μmol/L [1], while those of amiodarone range from 1.5 to 4 μmol/L [30]. Our data (unpublished) indicate that perhexiline is concentrated at least 20-fold in rat heart 3 hr after oral administration, whereas amiodarone has been reported to be concentrated 90-fold by the dog heart 2–6 hr after administration [29]. Hence, although it is less potent as a CPT-1 inhibitor than perhexiline, greater myocardial concentrations of amiodarone may be expected per unit plasma concentration. In addition, recent evidence indicates that both perhexiline [9] and amiodarone [31] are particularly concentrated by mitochondria. Hence, the concentrations of both perhexiline and amiodarone required for inhibition of CPT-1 in a broken cell preparation will quite likely be achieved in cells *in vivo* at therapeutic circulating concentrations of these drugs. Nevertheless, confirmation of the effects of perhexiline and amiodarone on CPT-1 in preparations of tissues from animals treated with perhexiline and amiodarone is required.

Despite the finding that perhexiline displayed competitive inhibition with respect to palmitoyl-CoA, the current experiments do not permit determination of the site of binding of perhexiline to CPT-1. Previous data suggest that malonyl-CoA, the principal endogenous inhibitor of CPT-

1, interacts with two sites. Although malonyl-CoA is structurally similar to palmitoyl-CoA and inhibits CPT-1 competitively, most of its inhibitory effect is exerted remote from the active site, probably at a "regulatory site" on the cytoplasmic side of the outer mitochondrial membrane [17]. Moreover, drugs such as HPG (the active metabolite of oxfenicine) appear to bind to the same cytoplasmic site on hepatic CPT-1 as malonyl-CoA [32].

From the current experiments, perhexiline appears to act at a mitochondrial site that is protected from the action of protease treatment. In this regard, it differs from both HPG and malonyl-CoA, whose inhibitory effects on cardiac CPT-1 [17] and hepatic CPT-1 [17, 33] are reduced after protease treatment of the mitochondria. Nevertheless, our dual inhibitor studies indicated that perhexiline interacts in some way with the malonyl-CoA binding site in that its inhibition is less than additive with both HPG and malonyl-CoA on cardiac CPT-1. The inhibition of perhexiline and malonyl-CoA with cardiac CPT-1 was more complicated than that of perhexiline with HPG, in that coincubation with malonyl-CoA, and perhexiline did not lead to greater inhibition than that due to perhexiline alone. At present we have no explanation for the difference between the interaction of perhexiline with HPG and that with malonyl-CoA. Although the binding site for perhexiline on CPT-1 is protected from protease treatment in a way similar to that for active-site directed agents like Co-A [33], the non-competitive interaction of perhexiline with Co-A indicates that they inhibit the enzyme at different sites. HPG and malonyl-CoA both show similar non-competitive interactions with Co-A on hepatic CPT-1 [33]. Hence, perhexiline appears to be unique among CPT-1 inhibitors described thus far. Perhexiline appears to interact with the same site on CPT-1 as HPG and malonyl-CoA, but unlike the latter two agents it does not appear to require a protein facing the cytoplasmic aspect of the mitochondrion for inhibitory activity. Hence, the inhibitory site for perhexiline on CPT-1 is not identical to that of any previously described endogenous or exogenous inhibitor.

Other authors have suggested that the cationic amphiphilic nature of perhexiline and amiodarone, favouring the formation of ion pairs with polar lipids [34], may facilitate their cellular actions [9, 16]. Whether this property contributes to their inhibitory action on CPT-1 is unknown at present. However, it is possible that they could interact with phospholipid components of the mitochondrial membrane containing CPT-1, as well as to some component of CPT-1.

Malonyl-CoA-sensitive CPT has been identified recently in liver peroxisomes [35] and in cardiac sarcoplasmic reticulum [36]. In the present experiments, the contribution of extramitochondrial CPT has not been determined. However, the ability of perhexiline, like malonyl-CoA, to cause approximately 90% inhibition of CPT activity in the cardiac homogenate preparations may indicate that any extramitochondrial sources of the enzyme present in these preparations are sensitive to inhibition by perhexiline.

The curvilinear Dixon plot for inhibition of the cardiac enzyme by perhexiline may be due to positive cooperativity in binding of the inhibitor to the enzyme, or to binding of the inhibitor to multiple sites. Hence, any K_i estimated by extrapolation of the Dixon plots is only an "apparent K_i." Nevertheless, these apparent K_i values from heart and liver

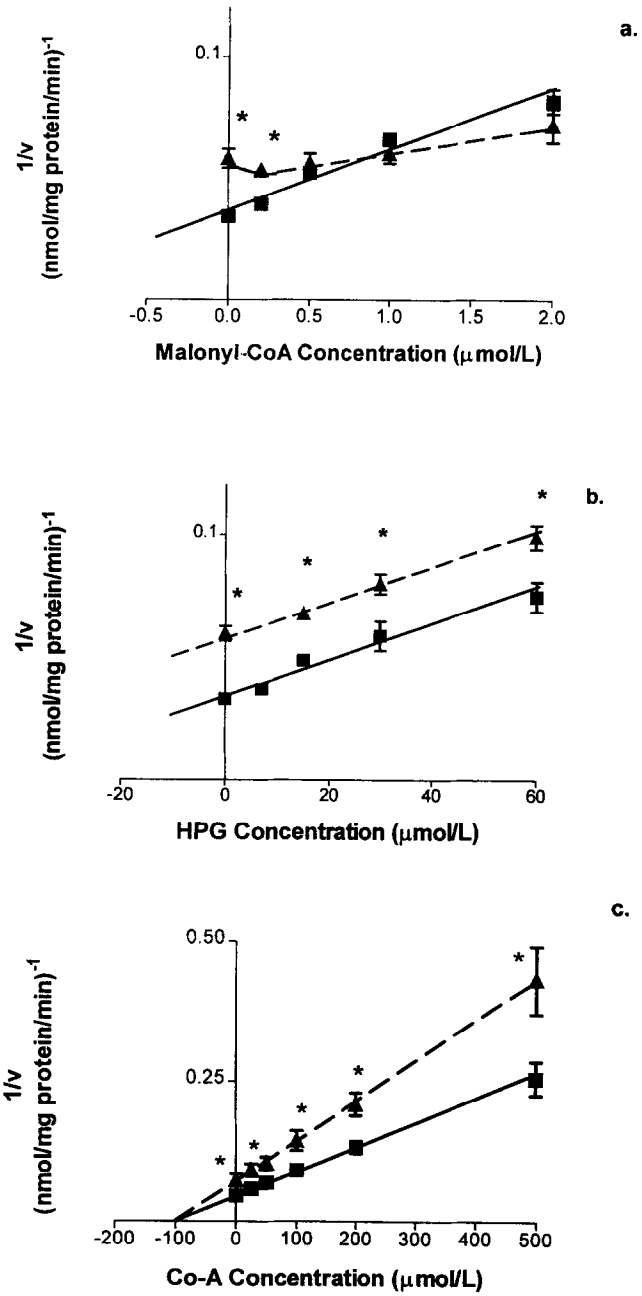


FIG. 5. Yonetani–Theorell plot of the inhibition of cardiac mitochondrial CPT-1 by (a) malonyl-CoA, (b) HPG, and (c) Co-A, in the presence (▲) and in the absence (■) of perhexiline (60 μmol/L). Enzyme preparations were preincubated with the inhibitors for 15 min, and then CPT-1 activity was estimated by incubating with palmitoyl-CoA (50 μmol/L) and carnitine (400 μmol/L) for 4 min. Values are means ± SEM, N = 5. Statistical analysis: (*) $P < 0.05$, compared with no perhexiline, paired *t*-test.

support a greater sensitivity of cardiac CPT-1 to perhexiline compared with hepatic CPT-1. This difference may relate to the fact that cardiac CPT-1 exists as two isoforms only one of which is similar to the hepatic isoenzyme [37], a characteristic that may also explain the greater sensitivity of the cardiac enzyme to malonyl-CoA [38].

The finding that perhexiline and amiodarone are CPT-1 inhibitors is consistent with the previously postulated "metabolic" mechanism of action of perhexiline [6] and with the common mechanisms of potential long-term toxicity of both agents. Previous studies have linked the possible occurrence of phospholipidosis, which predisposes towards hepatotoxicity and peripheral neuropathy seen with both agents, with a number of amphiphilic compounds [34], rather than with CPT-1 inhibition. However, potential accumulation of phospholipids as a consequence of prolonged inhibition of long chain fatty acid metabolism may be a predictable adverse effect of CPT-1 inhibitors. It remains to be determined whether regional heterogeneity of drug affinity for CPT-1 and/or differences in tissue concentration of CPT-1 inhibitors may account for variation in predominant sites of phospholipidosis with differing agents.

In conclusion, perhexiline and amiodarone are inhibitors of CPT-1 in cardiac and liver mitochondria. As such, they would be expected to inhibit the access of long chain fatty acids to the mitochondrial matrix and thereby inhibit long chain fatty acid oxidation. This mechanism would account for their anti-anginal properties. In addition, the inhibition of long chain acyl carnitine formation may contribute towards an anti-arrhythmic effect in the presence of myocardial ischaemia, since other inhibitors of CPT-1 have been shown to delay the cellular uncoupling effects of ischaemia in cardiac tissue [23].

Although CPT-1 inhibitors have been shown to have anti-ischaemic actions in *in vitro* animal models [22, 39], this is the first report of inhibition of CPT-1 by clinically useful anti-anginal agents. As such, a previously defined biochemical class of drugs (CPT-1 inhibitors) now accords with a previously defined, therapeutically validated group of "metabolic" anti-anginal agents. Appreciation that this biochemical mechanism of action may correlate with marked suppression of myocardial ischaemia may facilitate the development of future anti-anginal agents, as well as improving understanding of the actions and potential toxicity of perhexiline and amiodarone.

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