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Inhibition of long-chain fatty acid metabolism does not affect platelet aggregation responses

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Abstract

A number of anti-anginal agents (perhexiline, amiodarone, trimetazidine) have been shown to inhibit myocardial carnitine palmitoyltransferase-1, which controls access of long-chain fatty acids to mitochondrial sites of β-oxidation. In view of clinical data suggesting that perhexiline improves symptomatic status in unstable angina pectoris, and the known role of mitochondrial β-oxidation in platelet metabolism, we compared the platelet carnitine palmitoyltransferase-1 inhibitory and putative anti-aggregatory effects of perhexiline, amiodarone and trimetazidine with those of specific carnitine palmitoyltransferase-1 inhibitors: etomoxir and hydroxyphenylglyoxylate in both normal subjects and patients with stable angina. All of the compounds examined inhibited platelet carnitine palmitoyltransferase-1 activity; rank order of potency etomoxir > malonyl-CoA > hydroxyphenylglyoxylate > amiodarone ≥ perhexiline > trimetazidine. However, only perhexiline, amiodarone and trimetazidine inhibited platelet aggregation. We conclude that (a) the carnitine palmitoyltransferase-1 inhibitors perhexiline, amiodarone and trimetazidine exert significant anti-aggregatory effects which may be therapeutically relevant and, (b) these effects are independent of carnitine palmitoyltransferase-1 inhibition.

Keywords: Perhexiline; Amiodarone; Trimetazidine; Platelet aggregation; Carnitine palmitoyltransferase-1; Etomoxir

1. Introduction

Energy production in platelets has been shown to be dependent on oxidation of fatty acids, in addition to glycolysis and the oxidation of carbohydrates (Donabedian and Nemerson, 1971; Iida et al., 1991). Carnitine palmitoyltransferase-1, which is located in the outer mitochondrial membrane, is the key enzyme for oxidation of long-chain fatty acids since it controls their access to the site of β-oxidation in the mitochondrial matrix. Recently, Iida et al. (1991) have characterised carnitine palmitoyltransferase-1 in saponin-permeabilized rat platelets, demonstrating that the activity of the enzyme is increased by streptozotocin-induced diabetes and decreased by insulin in vitro. Moreover, increased carnitine palmitoyltransferase-1 activity in diabetic rat platelets is associated with increased oxygen consumption and increased ADP- and thrombin-induced aggregation in washed platelets (Iida et al., 1993), suggesting that carnitine palmitoyltransferase-1 may be involved in the control of platelet function. In support of this possibility, Ishikura et al. (1992) demonstrated that in vivo treatment of rats with the irreversible carnitine palmitoyltransferase-1 inhibitor, 2-tetradecylglycidic acid, reduced the ex vivo oxygen consumption, ATP concentration, ATP/ADP ratio and maximum aggregation rate of platelets in response to ADP, thrombin and Ca^{2+} ionophore, A23187. Thus, there is some evidence to suggest that carnitine palmitoyltransferase-1 activity may modulate platelet aggregability. On the other hand, it is possible that this is not a specific cause and effect phenomenon: fatty acid oxidation is stimulated after the addition of thrombin to human platelets in vitro (Donabedian and Nemerson, 1971).

It has been shown recently that perhexiline, amiodarone and trimetazidine, all anti-anginal agents modifying my-

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ocardial metabolism, inhibit β-oxidation of long-chain fatty acids in liver cells (Fromenty et al., 1990; Deschamps et al., 1994) and cardiac mitochondria (Fantini et al., 1994); this effect may reflect inhibition of carnitine palmitoyltransferase-1 activity, which has also been demonstrated in rat cardiac mitochondria (Kennedy et al., 1996; Kennedy and Horowitz, 1998). Perhexiline has previously been shown to inhibit aggregation induced by ADP, adrenaline and collagen in human platelet-rich plasma (Ono and Kimura, 1981), an effect which did not correlate in potency with the Ca^{2+} channel blocking effect of perhexiline when compared with the anti-aggregatory effect of other agents such as verapamil. Since there is evidence that perhexiline is effective in suppressing symptoms in patients with unstable angina (Stewart et al., 1996), a syndrome associated with activation of platelet aggregation, this suggests that perhexiline may have a clinically relevant anti-aggregatory effect. The aim of the present study was to compare the effects of perhexiline, amiodarone and trimetazidine on carnitine palmitoyltransferase-1 activity and aggregation in human platelets in vitro in order to determine whether the two effects were associated. A comparison was made with the well-characterised carnitine palmitoyltransferase-1 inhibitors, hydroxyphenylglyoxylate and etomoxir.

2. Materials and methods

2.1. Subjects / patients

Subjects studied included normal volunteers (9 men and 19 women aged 44 ± 12.4 (S.D.) years, range 24–75), and patients undergoing routine diagnostic coronary angiography for investigation of stable angina pectoris (53 men and 21 women aged 61 ± 8.9 (S.D.) years, range 25–80). A total of 45 patients were receiving either low dose aspirin, nitrates or verapamil; no other patient had taken any medication known to affect platelet function during 2 weeks prior to study. Of the 102 subjects, only 10 had both carnitine palmitoyltransferase-1 inhibition and platelet aggregation studies. The protocol was approved by the Ethics of Research Committee of the Queen Elizabeth Hospital and informed consent was obtained prior to study entry.

2.2. Blood sampling

Blood samples for platelets from normal volunteers were drawn from the antecubital vein, while blood samples from patients were withdrawn through a femoral arterial sheath during cardiac catheterization. In all cases, blood was collected in plastic tubes containing 1:10 volume of citric acid–sodium citrate anticoagulant (2 parts of 0.1 M citric acid to 3 parts of 0.1 M trisodium citrate, pH 5). Acidified citrate was utilised in order to minimise deterioration of platelet function during experiments (Kinlough-Rathbone et al., 1983). The time interval between collection of blood samples and platelet aggregation studies was 10–15 min.

2.3. Platelet carnitine palmitoyltransferase-1 activity

Platelets were prepared essentially as described by Iida et al. (1991). Briefly, platelet-rich plasma was prepared by centrifugation of blood at $130 \times g$ for 7 min at room temperature, followed by two washes (and centrifugation at $800 \times g$, 15 min, room temperature) with a solution containing 36 mM citric acid, 5 mM glucose, 5 mM KCl, 90 mM NaCl, 1 μM prostaglandin E_1, pH 6.5. The platelet pellet was then resuspended in modified Tyrode's solution as described by Iida et al. (1991) at a final concentration of 10^9 platelets/ml. Carnitine palmitoyltransferase-1 activity was estimated in saponin-permeabilized platelets as the formation of palmitoyl-[^{3}H]carnitine from palmitoyl-CoA and [^{3}H]L-carnitine, essentially as described by McGarry et al. (1978). Platelets were permeabilized by incubating with 60 μg/ml saponin for 5 min at 37°C. The disruption of the plasma membrane by saponin, without significant effect on the mitochondrial membrane, was verified by the release of the cytoplasmic enzyme, lactate dehydrogenase (EC 1.1.1.27), into the medium without any appreciable release of the mitochondrial matrix enzyme, glutamate dehydrogenase (EC 1.4.1.3). Lactate dehydrogenase release was 61% and glutamate dehydrogenase 1% of total releasable enzyme (estimated by incubation with Triton X-100, 0.1% w/v). A total of 2.5×10^8 platelets were added to the incubation medium (final volume 1 ml) containing 50 mM mannitol, 25 mM HEPES, 0.2 mM EGTA, 75 mM KCl, pH 7.0, 5 mM dithiothreitol, 2 mM KCN, 0.25 mM NaH_2PO_4, 2.6 mg fatty acid-free bovine serum albumin, 60 μM palmitoyl-CoA and 0.4 mM L-carnitine. The platelets were preincubated for 15 min at 37°C with inhibitors, or control vehicle, with the exception of malonyl-CoA (or its control) which was preincubated with the cells for 5 min only. The reaction was started by the addition of saponin and continued for 5 min, after which the reaction was stopped by the addition of 0.1 ml of concentrated HCl and tubes placed on ice. Blanks were identical except that concentrated HCl was added at the start of incubation. Preliminary experiments indicated that the reaction was linear up to (1) platelet concentrations of 5×10^8 platelets/ml, (2) a palmitoyl-CoA concentration of 100 μM, and (3) 15 min incubation. At the end of incubation, samples were diluted from 1 ml to 4 ml with distilled water and the product, [^{3}H]palmitoylcarnitine, was extracted with 2 ml *n*-butanol as described by Kiorpes et al. (1984).

2.4. Platelet aggregation studies

Platelet aggregation in whole blood was examined utilizing a dual channel whole blood aggregometer (Model

560, Chrono-Log, Haverstown, PA, USA). After a two-fold dilution with physiological saline, blood was placed in plastic cuvettes, (final volume 1 ml) and warmed in a heating block at 37°C. Siliconized stir bars were used to stir the blood at a rate of 1000 rpm. Aggregation experiments were monitored continually for 7 min and responses were recorded (Rikadenki chart recorder, Rikadenki Kogyo, Tokyo, Japan) for electrical impedance, in Ohms. Each test was performed in triplicate and from these average values were calculated. Stability of platelets' responsiveness to ADP 1 μM was tested every 30 min over periods up to 2 h after blood collection, with coefficient of variation $< 10\%$ in all cases. Unless otherwise stated, blood samples were preincubated with either perhexiline, amiodarone, trimetazidine, hydroxyphenylglyoxylate or etomoxir for 5 min prior to induction of aggregation.

2.5. Multiple agonist studies

These studies were performed in the case of perhexiline only, in order to determine whether anti-aggregatory effects were accentuated in a more 'physiological' form of aggregation (Willoughby et al., 1996). Multiple agonist methodology is described in full elsewhere (Willoughby et al., 1996). Briefly, when ADP was used as a single pro-aggregant, ADP was utilised in concentrations (1–2 μM) causing approximately a 7 Ω response. For multiple agonist studies, ADP concentrations (0.1–0.5 μM) were chosen on the basis of a response of approximately 2 Ω with ADP alone. Platelet responses towards adrenaline, serotonin and thrombin were determined and subthreshold concentrations of these three pro-aggregants (adrenaline 1 nM, serotonin 1 nM and thrombin 0.005 U/ml) were utilised in multiple agonist studies. Adrenaline, serotonin and thrombin used individually or in combination (Fig. 1) at these concentrations induced no detectable aggregation. However, when all four agonists were added simultaneously the resultant aggregation produced approximately a 7 Ω response.

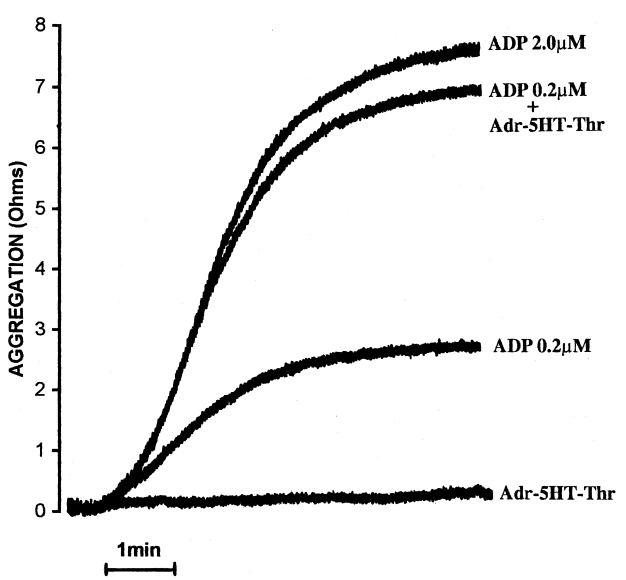

Fig. 1. Typical aggregograms for induction of platelet aggregation in whole blood by ADP alone and ADP in combination with adrenaline (1 nM), serotonin (1 nM) and thrombin (0.005 U/ml) [Adr–5HT–Thr]. Concentrations of ADP shown adjacent to appropriate aggregograms.

2.6. Determination of intraplatelet cGMP and cAMP content

This procedure was performed as described in previous publications (Chirkov et al., 1993, 1995). Briefly, platelet-rich plasma (0.5 ml) was incubated with perhexiline for 5 min at 37°C. After incubation, plasma was filtered through GF/C Glass Microfibre Filters (Whatman, UK) utilising plastic filter holders Swinnex-25 (Millipore, USA) for harvesting the platelets. Filters were rinsed with 0.5 ml of physiological saline, and placed into 0.5 ml of 4 mmol/l EDTA for further extraction of cAMP and cGMP in a boiling water bath for 5 min. After centrifugation of samples at $3000 \times g$ for 10 min, cAMP and cGMP concentrations in supernatant were assayed using cAMP ^{125}I and cGMP ^{125}I assay systems (Amersham, UK).

2.7. Data Analysis

The data are presented as mean ± S.E.M. unless otherwise indicated. Inhibition of aggregation by perhexiline, amiodarone, trimetazidine, hydroxyphenylglyoxylate and etomoxir were evaluated as a percentage of maximal aggregation achieved in the absence of either agent. IC_{50} was defined as the concentration of drug inhibiting carnitine palmitoyltransferase-1 activity and platelet aggregation by 50%, while IC_{30} was defined as the concentration of drug inhibiting carnitine palmitoyltransferase-1 activity by 30%. The IC_{50} and IC_{30} data are presented as geometric means with 95% confidence limits. The data were analysed by non-paired *t*-test, and a critical value of $P = 0.05$ was used throughout.

2.8. Chemicals

ADP disodium salt, amiodarone–HCl, L-carnitine, dithiothreitol, fatty acid-free bovine serum albumin, malonyl-CoA lithium salt, oxfenicine, palmitoyl-CoA free acid, perhexiline–HCl, and saponin were obtained from Sigma (St. Louis, MO, USA). [^{3}H]L-carnitine (specific activity 77 Ci/mmol) was obtained from Amersham International. Hydroxyphenylglyoxylate was a gift from Hoffmann-La Roche (USA) and trimetazidine–HCl was a gift from Servier (France). Stock solutions of trimetazidine, hydroxyphenylglyoxylate and etomoxir were made in physiological saline and subsequent dilutions were added to blood samples. Perhexiline was diluted from an initial ethanol solution using physiological saline while amiodarone was diluted from an initial methanol solution.

Control studies with matched concentrations of ethanol and methanol showed no effect on platelet aggregation.

3. Results

3.1. Platelet carnitine palmitoyltransferase-1 activity

The endogenous carnitine palmitoyltransferase-1 inhibitor, malonyl-CoA, produced 91 ± 2% ($n = 6$) reduction of palmitoylcarnitine formation in permeabilized, washed human platelets, indicating that the majority of the activity measured was due to carnitine palmitoyltransferase-1. Perhexiline ($n = 7$), amiodarone ($n = 4$) and trimetazidine ($n = 4$) inhibited platelet carnitine palmitoyltransferase-1 in a concentration-dependent manner (Fig. 2). A full concentration–response curve could not be obtained for amiodarone due to difficulties in solubilizing the drug at high concentrations in the incubation medium. However, the 45 ± 2% inhibition of carnitine palmitoyltransferase-1 at 200 μM amiodarone suggests that it has a slightly higher potency than perhexiline which had a IC_{50} of 300 μM (Table 1). Trimetazidine was the least potent of these agents as a platelet carnitine palmitoyltransferase-1 inhibitor. Oxfenicine ($n = 5$) produced no inhibition of platelet carnitine palmitoyltransferase-1 up to a concentration of 2 mM, but its active metabolite, hydroxyphenylglyoxylate ($n = 11$), inhibited carnitine palmitoyltransferase-1 in a concentration-dependent manner. Etomoxir ($n = 5$) was the most potent of these agents with an IC_{50} of 0.11 μM. Thus, the rank order of potency for carnitine palmitoyltransferase-1 inhibition in platelets was etomoxir > malonyl-CoA > hydroxyphenylglyoxylate > amiodarone ≥ perhexiline > trimetazidine (Table 1).

Potential variability in potency of carnitine palmitoyltransferase-1 inhibitors between normal subjects and patients with angina pectoris was investigated for perhexiline and etomoxir. In the case of perhexiline potency could be compared only in terms of IC_{30}, which was slightly but significantly ($P = 0.047$) less for patients [117 μM (72–191 μM)] than for normal volunteers [200 μM (140–280 μM)]. While with etomoxir IC_{50} values in normals [0.11 μM (0.05–0.21 μM)] were significantly ($P = 0.04$) lower than in patients with angina [0.36 μM (0.16–0.83 μM)] (Fig. 3).

Table 1
IC_{50} values for platelet aggregation and carnitine palmitoyltransferase-1 activity

Agent	Platelet aggregation	CPT-1 activity
	IC_{50} (μM)	
Perhexiline	21(18–26)	300(70–1400)
Amiodarone	62(44–87)	[a]
Trimetazidine	614(396–954)	6500(5212–8375)
Hydroxyphenylglyoxylate	[b]	45(30–67)
Etomoxir	[b]	0.11(0.05–0.21)
Malonyl-CoA	[c]	3.6(1.7–7.6)

IC_{50} values are expressed as mean ± 95% confidence limits.
[a] In washed platelets, amiodarone (200 μM, $n = 4$) inhibit carnitine palmitoyltransferase-1 activity by 45 ± 2%.
Amiodarone was insoluble in the assay medium above 200 μM.
[b] Hydroxyphenylglyoxylate and etomoxir did not inhibit platelet aggregation at any concentration tested (Fig. 4).
[c] Malonyl-CoA was not utilised in whole blood experiments.

3.2. Platelet aggregation

Perhexiline, amiodarone and trimetazidine added to blood samples 5 min prior to induction of aggregation by ADP (1 μM), produced inhibition of aggregation in a concentration-dependent manner (Fig. 4). Threshold concentrations for inhibition of aggregation by both perhexiline ($n = 16$) and amiodarone ($n = 10$) were approximately 1 μM, while for trimetazidine ($n = 5$) threshold

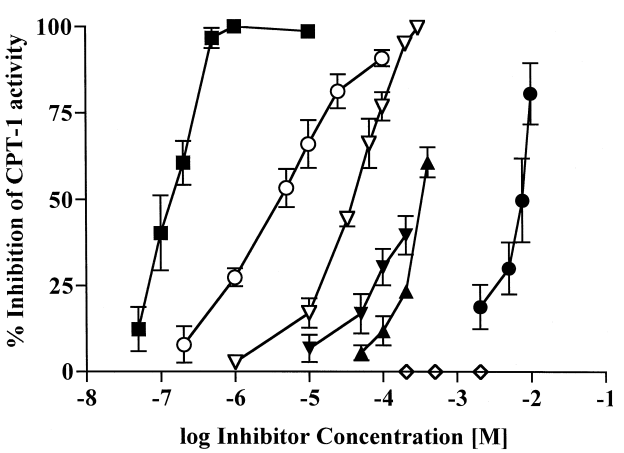

Fig. 2. Inhibition of carnitine palmitoyltransferase-1 in human platelets by etomoxir (■), malonyl-CoA (○), hydroxyphenylglyoxylate (▽), oxfenicine (◇), amiodarone (▼), perhexiline (▲) and trimetazidine (●).

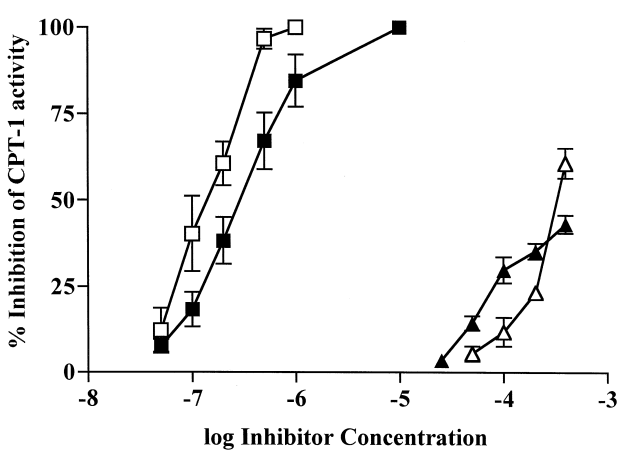

Fig. 3. Inhibition of carnitine palmitoyltransferase-1 in human platelets from normal volunteers (open symbols) and patients (closed symbols), by etomoxir (■) and perhexiline (▲).

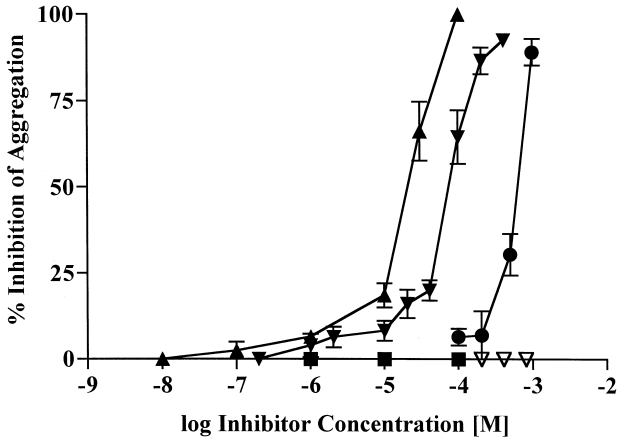

Fig. 4. Inhibition by perhexiline (▲), amiodarone (▼) and trimetazidine (●) of ADP-induced platelet aggregation in whole blood taken from patients with stable angina. Hydroxyphenylglyoxylate (▽) and etomoxir (■) did not inhibit aggregation.

was 100 μM. Perhexiline was the most potent inhibitor of platelet aggregation on a molar basis (Table 1). The inhibitory effects of perhexiline, amiodarone and trimetazidine were unaffected in patients on either aspirin, nitrates or verapamil. The well-characterised carnitine palmitoyltransferase-1 inhibitors hydroxyphenylglyoxylate ($n = 6$) and etomoxir ($n = 5$) did not inhibit ADP-induced aggregation in concentrations up to 800 μM and 100 μM respectively, which were in excess of those which produced inhibition of platelet carnitine palmitoyltransferase-1 activity (Fig. 2). More prolonged pre-incubation of platelets (30 min) with etomoxir or hydroxyphenylglyoxylate did not produce inhibition of aggregation. Therefore, the rank order of potency of inhibition of ADP-induced aggregation was perhexiline > amiodarone > trimetazidine. There was no difference in regards anti-aggregatory effects of perhexiline between normals and patients.

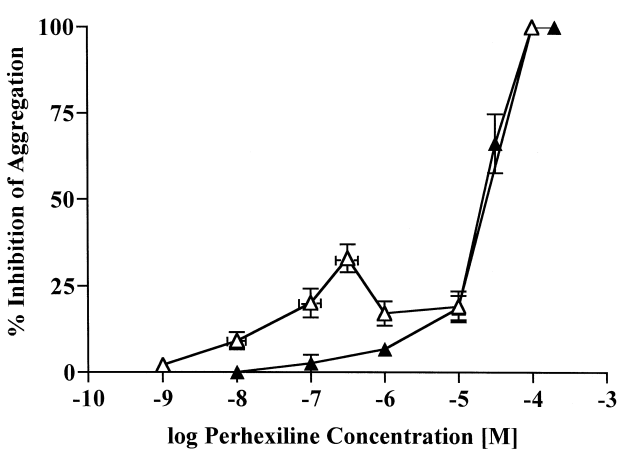

Fig. 5. Inhibition of ADP-alone (▲) and multiple agonist-induced (△) aggregation by perhexiline.

3.3. Multiple agonist potentiation of aggregation

When perhexiline was added to blood samples prior to induction of aggregation by multiple agonists the concentration–response curve for perhexiline was no longer sigmoidal as it was for ADP-alone but triphasic, with an average local maximum occurring at 0.3 μM (Fig. 5). Significant suppression of multiple agonist-induced aggregation was observed with perhexiline concentrations > 1 nM). The perhexiline concentration associated with total inhibition of aggregation was 100 μM, thus corresponding to the responsiveness with aggregation induced by ADP alone.

3.4. Intraplatelet cGMP and cAMP

The effect of perhexiline (1 μM) on intraplatelet cGMP and cAMP was examined in 18 patients. No consistent changes in either intraplatelet cGMP (106 ± 7% of control) or cAMP (103 ± 8% of control) content were detected.

4. Discussion

Platelets, like other mammalian cells take up long-chain fatty acids and metabolise them to either CO_2 (via mitochondrial β-oxidation) or to lipid esters, a process primarily controlled by carnitine palmitoyltransferase-1. The carnitine palmitoyltransferase-1 enzyme has previously been shown to exist in rat platelet mitochondria (Iida et al., 1991), while palmitoylcarnitine formation has been demonstrated in human platelet mitochondria (Vollset and Farstad, 1979) suggesting the involvement of carnitine palmitoyltransferase-1. However, the importance of carnitine palmitoyltransferase-1 to platelet energy metabolism is unknown. The IC_{50} for malonyl-CoA obtained for human platelets in the present study was of the same order of magnitude as that obtained by Iida et al. (1991) for rat platelets (3.6 μM compared with 0.9 μM, respectively) using similar incubation conditions. Oxfenicine, an inhibitor of carnitine palmitoyltransferase-1 in other tissues (Higgins et al., 1981; Bielefeld et al., 1985) produced no inhibition of human platelet carnitine palmitoyltransferase-1 up to a concentration of 2 mM, but its active metabolite, hydroxyphenylglyoxylate, was inhibitory with a IC_{50} of 45 μM (Table 1). These data suggest that human platelets do not metabolise oxfenicine to hydroxyphenylglyoxylate as readily as does cardiac tissue (Stephens et al., 1985). Thus, as previously demonstrated in rat myocardium (Kennedy et al., 1996), those inhibitors like perhexiline, amiodarone and trimetazidine whose carnitine palmitoyltransferase-1 binding site is destroyed by nagarse treatment are less potent carnitine palmitoyltransferase-1 inhibitors than the nagarse-sensitive agents hydroxyphenylglyoxylate and etomoxir in human platelets.

Despite the marked inhibition of human platelet carnitine palmitoyltransferase-1 by the irreversible inhibitor, etomoxir, and by the reversible inhibitor, hydroxyphenylglyoxylate, neither agent inhibited ADP-induced aggregation in whole blood. Hence, the present study has failed to provide evidence for a functional role of carnitine palmitoyltransferase-1 in platelet aggregation in vitro. The reason for the discrepancy between the present data and the inhibitory effect of tetradecylglycidic acid on rat platelet aggregability observed by Ishikura et al. (1992) is not clear from these results. However, Ishikura et al. (1992) treated rats in vivo with tetradecylglycidic acid: it is possible that the metabolic consequences of carnitine palmitoyltransferase-1 inhibition in circulating platelets are more significant than those of in vitro application of carnitine palmitoyltransferase-1 inhibitors, this possibility could not be examined in the current study.

The finding that perhexiline inhibits aggregation of human platelets in whole blood extends the previous observations of Ono and Kimura (1981) in platelet-rich plasma. Intriguingly a recent study by Stewart et al. (1996) suggested that perhexiline was effective in suppressing symptoms in patients with otherwise refractory unstable angina, an observation consistent with the observed anti-aggregatory effect of perhexiline (Fig. 5). In the present study the threshold concentrations for anti-aggregatory effects of perhexiline (1 μM) correspond approximately to its therapeutic plasma concentrations (Stewart et al., 1996; Unger et al., 1997) when aggregation is induced by ADP-alone. However, when aggregation was induced by the multiple agonist model threshold perhexiline effects decreased to 1 nM. Therefore, significant anti-platelet effects were detected at concentrations achievable therapeutically. Perhexiline produced no consistent changes in intraplatelet cGMP and cAMP content. Therefore the anti-aggregatory effect of perhexiline are not mediated by the cGMP and cAMP pathways. The response of perhexiline is triphasic in the multiple agonist aggregation model suggests that there are multiple mechanisms of interaction between perhexiline and intraplatelet systems controlling the process of aggregation.

Amiodarone inhibits in vitro platelet aggregation in whole blood taken from patients with angina pectoris (Fig. 4). Significant suppression of platelet aggregation was produced by concentrations greater than 20 μM. Amiodarone was less potent as an inhibitor of platelet aggregation than perhexiline on a molar basis. Trimetazidine has previously been shown to inhibit human platelet aggregation in vitro (Devynck et al., 1993; Astarie-Dequeker et al., 1994). Threshold effects in the present study were seen at 100 μM, this concentration is consistent with that obtained by Devynck et al. (1993). The concentrations of trimetazidine utilised in vitro are considerably greater than those occurring in vivo in humans (0.1–1 μM) and therefore the relevance of the observed inhibition of aggregation to the in vivo effects of trimetazidine might be questioned (Devynck et al., 1993). However, trimetazidine has previously been shown to reduce ex vivo platelet aggregation in patients with ischaemic heart disease (Higuchi et al., 1981) and to decrease the cyclic flow variation (platelet-rich thrombus formation) in stenosed canine coronary arteries (Belcher et al., 1993). Both these observations suggest that trimetazidine has a clinically relevant anti-aggregatory effect and raise the possibility that the anti-aggregatory effects of trimetazidine are enhanced in vivo.

The finding that carnitine palmitoyltransferase-1 inhibition per se (by hydroxyphenylglyoxylate or etomoxir) is not associated with detectable effects on platelet aggregability has only been explored at this stage in normoxic platelets. It is unknown whether during or after hypoxia in vivo, effects on relative glucose vs. long chain fatty acid metabolism may have greater consequences. The therapeutic efficacy of perhexiline and potentially of other carnitine palmitoyltransferase-1 inhibitors in patients with severe exertional angina pectoris and aortic stenosis (Unger et al., 1997) may be ascribed to the 'oxygen-sparing' effects of carnitine palmitoyltransferase-1 inhibition (Vaughan Williams, 1980; Jeffrey et al., 1995). However, the results of the present study suggest that perhexiline, amiodarone and trimetazidine have anti-aggregatory effects reflecting a different, as yet unidentified, pharmacological effect. In view of the recent observations suggesting a clinical role for perhexiline in unstable angina pectoris (Stewart et al., 1996) this additional effect is likely to be of therapeutic significance.

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