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The effects of novel vasodilator long chain acyl carnitine esters in the isolated perfused heart of the rat

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1 The effects of palmitoyl carnitine (PC) and novel derivatives were examined on the isolated Langendorff perfused heart of the rat.

2 Bolus injections of PC (1–300 nmol) produced coronary constriction accompanied by a cumulative irreversible depression of contractility.

3 Prior storage of PC in chloroform containing 2% ethanol in heat-sealed ampoules resulted in production of the ethyl ester of the compound (PCE). This compound was isolated and also synthesized (P1E). In contrast to PC, both PCE and P1E exhibited potent vasodilator activity.

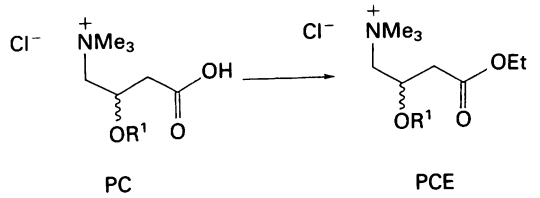
4 Increasing the fatty acid chain length from palmitoyl to stearoyl resulted in a significant reduction in coronary dilator activity of the ester compound, whereas different ester groups did not affect the vasodilator action appreciably. Complete removal of the fatty acid chain abolished all vascular effects at the doses used.

5 The vasodilatation produced by these acyl carnitine esters was comparable to that produced by several known vasodilator drugs including verapamil, cromakalim, amyl nitrate and iloprost; however, the duration of the vasodilator response was more prolonged with the carnitate derivatives.

Introduction

Accumulation of long chain acyl carnitines occurs in the ischaemic myocardium (Liedtke *et al.*, 1978; Corr *et al.*, 1984) and these fatty acid metabolites exert detrimental electrophysiological, biochemical and mechanical effects on the heart (Adams *et al.*, 1979; Knabb *et al.*, 1988; Nakaya & Tohse, 1986). They have also been reported to be calcium channel activators (Spedding & Mir, 1987) and are thought to be the mediators of the increase in α-adrenoceptor numbers seen in the ischaemic myocardium (Corr *et al.*, 1981; Heathers *et al.*, 1987; Allely & Brown, 1988). Much of the existing work has been carried out using Purkinje fibres, myocytes or subcellular fractions whilst there have been few studies on perfused hearts or blood vessels. There is a report on the interaction between palmitoyl carnitine and α-adrenoceptor-mediated responses in the rat caudal artery (Ugwu *et al.*, 1987) and we have previously shown that palmitoyl carnitine exerts both constrictor and dilator actions on the coronary circulation of the rat (Criddle *et al.*, 1987). However, we now attribute most of this activity to a derivative of the parent compound.

In our previous experiments palmitoyl carnitine had been stored for convenience in chloroform, on the assumption of it being an inert solvent, in heat-sealed ampoules. However, chloroform contains 2% ethanol included as stabilizer to prevent phosgene formation. We have subsequently established that the ethanol reacts with palmitoyl carnitine to produce the ethyl ester of the compound (Figure 1). The structure has now been confirmed by chemical and spectroscopic analysis and unambiguous synthesis of the compound. Following this serendipitous discovery, further pharmacological investigations of this novel vasodilator agent and its derivatives have been carried out on the Langendorff isolated perfused heart of the rat.



PC PCE

Figure 1 Conversion of palmitoyl carnitine (PC) to its ethyl ester (PCE) on storage in $CHCl_3$ (2% ethanol) in heat-sealed ampoules (R^1 = palmitoyl).

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Methods

Hearts from male Wistar rats (University of Bath strain) were perfused by the Langendorff technique at a constant flow of 10 ml min^{-1} at 37°C with a modified Krebs-Henseleit solution containing 5.9 mM K^+, gassed with 95% O_2, 5% CO_2. Following a 15 min stabilization period, the perfusate was changed to one containing 3.2 mM K^+ which raises coronary tone. Perfusion pressure changes were monitored with a Bell and Howell pressure transducer connected to a side arm. Developed tension under a resting tension of 2 g was recorded with a Devices isometric transducer attached to the apex of the heart; the signal from this transducer was also used to trigger an instantaneous rate meter. All recordings were made on a Devices M19 recorder. Drug injections were made in volumes of less than 100 μl via a side arm situated 3 cm from the aortic valves.

The following drugs were used; (±)-palmitoyl carnitine, sodium palmitate, atropine sulphate (Sigma), amyl nitrate (BDH), cromakalim (Beechams Pharmaceuticals), verapamil hydrochloride (Abbott) and iloprost (Schering). All novel compounds were synthesized in our laboratories by W.B.W. (University of Bath). Drugs were prepared in 0.9% saline, except cromakalim and sodium palmitate which were first dissolved in ethanol and then diluted in saline; at the final concentration used the ethanol was without effect on the coronary circulation.

Statistical analysis of the differences between means was assessed by a non-paired Student's *t* test.

Results

Palmitoyl carnitine exhibited a dose-related vasoconstrictor action on the coronary vascular bed of the rat (Figure 2). In addition, it caused a cumulative irreversible depression of

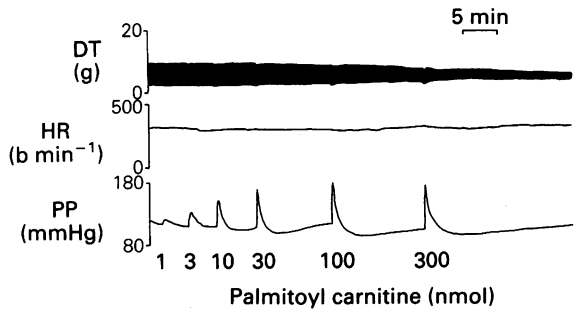


Figure 2 Effects of palmitoyl carnitine (1–300 nmol) on developed tension (DT), heart rate (HR) and perfusion pressure (PP) in the isolated Langendorff perfused heart of the rat.

myocardial contractility with no concomitant effect on heart rate ($n = 4$). This profile of activity was observed for the other acyl carnitines tested, myristoyl(C14) and stearoyl(C18), whilst carnitine (50–250 nmol) had no effect on coronary vascular tone ($n = 4$) (data not shown).

In contrast, the synthetic ethyl ester of palmitoyl carnitine (P1E) exhibited a completely different profile of action from that of the parent compound. We now observed a dose-related vasodilatation that was both rapid in onset and sustained at higher doses ($n = 4$) (Figure 3). A maximal response was produced by 10 nmol P1E; higher doses produced a smaller fall in perfusion pressure preceded in several cases by a transient vasoconstriction possibly reflecting a non-specific effect of the drug. The decreases in perfusion pressure elicited by P1E were dependent on the basal tone of the preparation i.e. the greater the resting perfusion pressure the larger the vasodilator response. Small rises in resting tension were observed on addition of bolus injections of P1E and at no time was any effect on heart rate observed. The vasodilator activities of the synthetic ester P1E and the 'chloroform-produced' equivalent PCE were compared (Figure 4) providing pharmacological confirmation of previous chemical and spectroscopic analyses suggesting that palmitoyl carnitine was being esterified to P1E while undergoing storage in chloroform in heat-sealed ampoules.

Having established the identity of the novel vasodilator compound, structural variations of the molecule were investigated for pharmacological activity, alterations of chemical groups R^1 and R^2 being made (Table 1). Figures 5 and 6 show the effect of increasing the fatty acid chain length by 2 carbon atoms from palmitoyl to stearoyl on the vasodilator activity. Both the maximal dilatation elicited, and the rate at which the

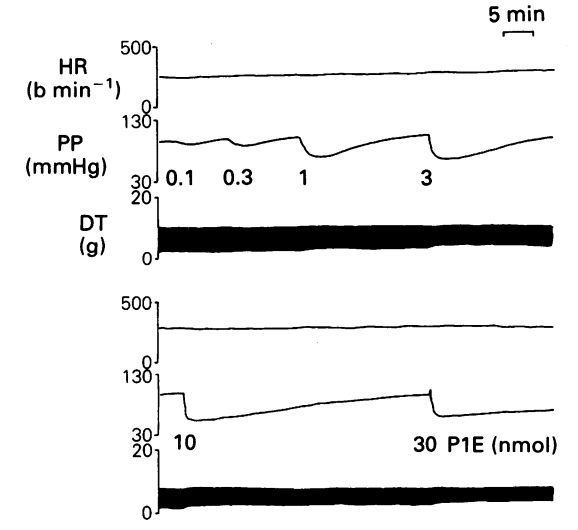


Figure 3 Effect of P1E (0.1–30 nmol) on heart rate (HR), perfusion pressure (PP) and developed tension (DT) in the isolated Langendorff perfused heart of the rat.

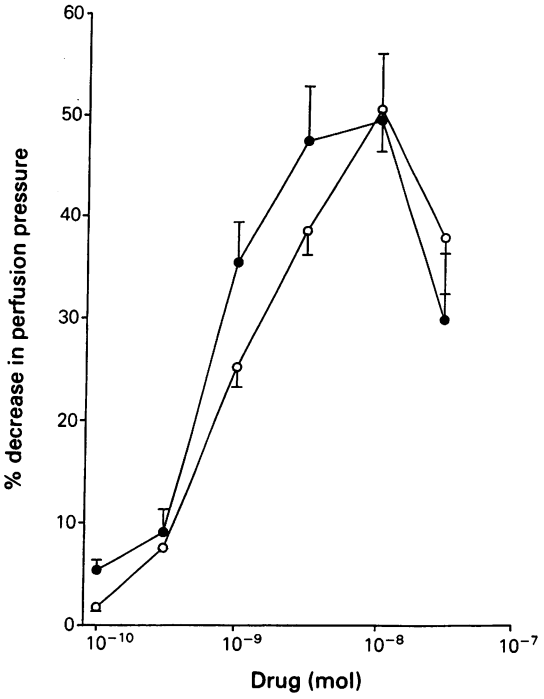


Figure 4 A comparison of the effects of the 'chloroform-produced' ethyl ester of palmitoyl carnitine (PCE, ○), [100% = 102.5 ± 5.7 mmHg, $n = 4$] and the synthetic ester (P1E, ●), [100% = 95.8 ± 3.5 mmHg, $n = 4$] on perfusion pressure in the isolated Langendorff perfused heart of the rat, expressed as % decrease from basal values. Vertical bars represent s.e.mean.

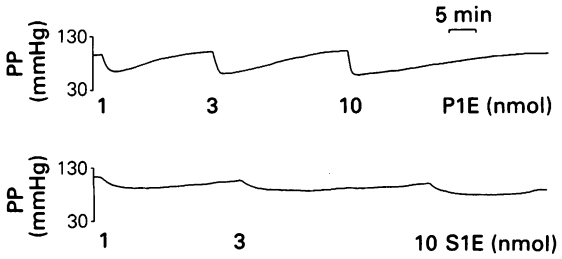
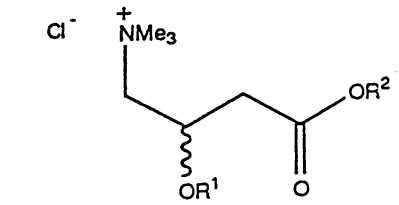


Figure 5 Effects of P1E (1–10 nmol) and S1E (1–10 nmol) on perfusion pressure (PP) in the isolated Langendorff perfused heart of the rat.

Table 1 Guide to the structure and coding of synthetic compounds



Code	R^1	R^2
P1M	$CO(CH_2)_{14}Me$	Me
P1E	$CO(CH_2)_{14}Me$	Et
P1P^i	$CO(CH_2)_{14}Me$	Pr^i
S1E	$CO(CH_2)_{16}Me$	Et
1P^i	H	Pr^i

decrease in perfusion pressure occured were smaller with S1E than the P1E (Figure 6). Complete removal of the fatty acid moiety (1P^i) abolished any effect on coronary vascular tone (1–300 nmol, $n = 3$) (data not shown).

Substitution of the ethyl ester group with methyl (P1M) or isopropyl (P1P^i) groups did not alter the vasodilator activity

of the compounds as markedly as altering the fatty acid chain, although a significant reduction in the response elicited by P1M compared to P1E or P1P^i was observed with some doses (Figure 7).

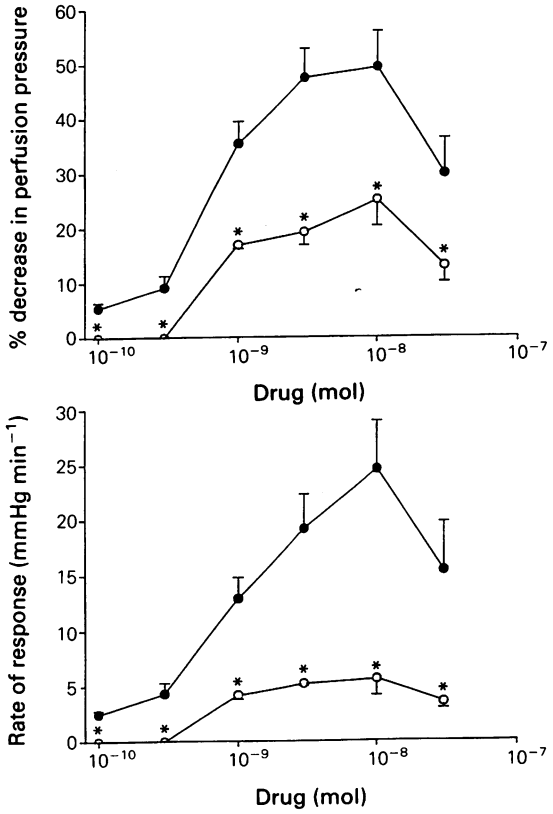


Figure 6 Effects of P1E (●), [100% = 95.8 ± 3.5 mmHg, $n = 4$], and S1E (○), [100% = 86.8 ± 9.0 mmHg, $n = 4$] on perfusion pressure in the rat isolated Langendorff heart, expressed both as % decrease from basal values and as rate of response. Vertical bars represent s.e.mean (*$P < 0.05$).

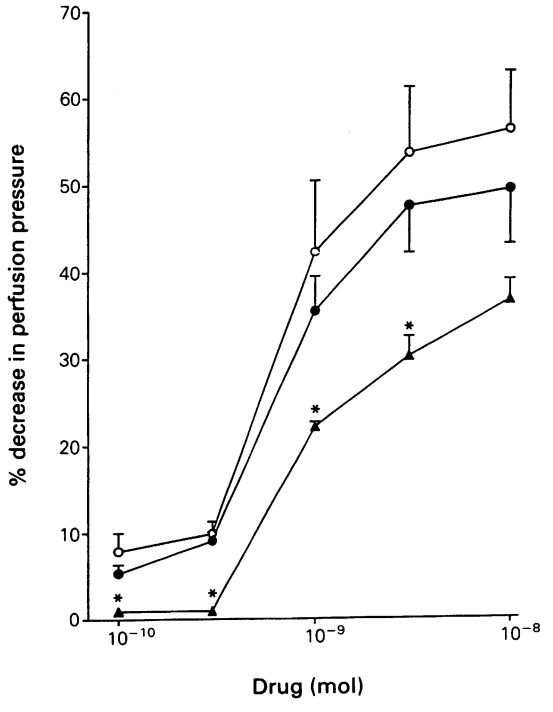


Figure 7 Effects of P1M (▲), [100% = 92.5 ± 6.7 mmHg, $n = 4$], P1E (●), [100% = 95.8 ± 3.5 mmHg, $n = 4$] and P1P^i (○), [100% = 104.0 ± 5.0 mmHg, $n = 4$] on perfusion pressure in the isolated Langendorff perfused heart of the rat, expressed as % decrease from basal values. Vertical bars represent s.e.mean (*$P < 0.05$ compared with P1P^i and P1E).

A comparison of the synthetic carnitates with known vasodilators amyl nitrate, verapamil, cromakalim and iloprost is shown in Figure 8. Vasodilatations of similar magnitude to those elicited by the known agents were produced by the novel compounds at comparable doses; however the duration of responses was greater with the acyl carnitine esters.

High concentrations of palmitic acid relax potassium depolarized guinea-pig taenia-coli preparations (Spedding & Mir, 1987). In order to rule out the possibility that P1P^i was being broken down to release palmitate, and that this was responsible for mediating the coronary relaxation seen with P1P^i, the effects of palmitate were examined in the rat isolated heart. In contrast to its effect in the potassium depolarized taenia preparation, bolus injections of sodium palmitate (1–30 nmol) produced dose-dependent vasoconstrictions in the rat coronary circulation ($n = 4$); at the higher doses the vasoconstrictor response to palmitate was followed by a vasodilator component which was smaller and less well maintained than that seen with smaller doses of P1P^i.

In view of the structural similarities between acetylcholine and the acyl carnitine esters it was possible that these compounds could be acting as muscarinic agonists on endothelial cells to release endothelial derived relaxant factor (Vanhoutte & Miller, 1985). However, in the rat isolated heart the coronary vascular effects of acetylcholine (1–10 nmol) were completely abolished by atropine (5–50 nM) while these concentrations of atropine had no effect on the vasodilator action of P1P^i. Also, the major effect of acetylcholine on the rat coronary circulation is to produce vasoconstriction which is sometimes preceded by a small inconsistent vasodilator response, unlike the long-lasting vasodilatation seen with the acyl carnitine esters.

Discussion

We have shown in the rat isolated heart that bolus injections of palmitoyl carnitine cause constriction of the coronary circulation associated with an irreversible depression of myocardial contractility. Alteration of the carboxyl group of the molecule to an ester group produces compounds with an opposite action on the coronary vasculature.

In common with the acyl carnitines from which they were derived, the novel esters are amphiphiles i.e. they have both hydrophobic and hydrophilic groups. Incorporation of such compounds into membranes alters ionic permeability (Levitsky & Skulachev, 1972; Corr *et al.*, 1984) and this property may underlie their mechanism of action. However, in view of the opposing actions of acyl carnitines and their esters on coronary vascular tone, such a non-specific membrane effect appears an unlikely explanation of their mode of action. It has recently been shown that palmitoyl, myristoyl and lauroyl carnitines caused damage of Langendorff perfused rat hearts as measured by enzyme leakage and that there was a 20 fold difference in potency between palmitoyl and lauroyl car-

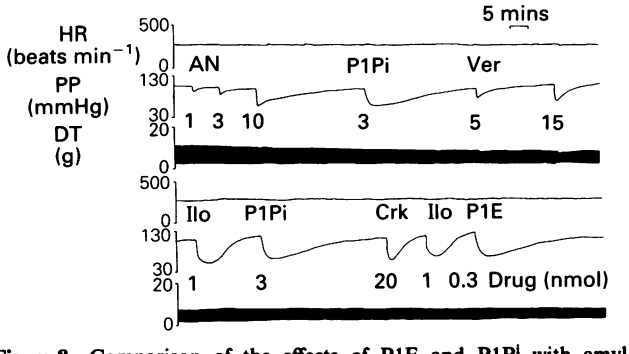


Figure 8 Comparison of the effects of P1E and P1P^i with amyl nitrate (AN), verapamil (Ver), iloprost (Ilo) and cromakalim (Crk) on heart rate (HR), perfusion pressure (PP) and developed tension (DT) in the isolated Langendorf perfused rat heart.

nitines in this respect (Sercu et al., 1986). We have observed differences in the activities of the carnitine esters depending on the functional groups present, most noticeably the saturated fatty acid chain. These structure-activity relationships of P1E and related compounds, may therefore indicate a specific receptor mechanism underlying the effects of acyl carnitines and their esters.

It has been shown that in K^+ depolarized smooth muscle and chick myocytes, palmitoyl carnitine resembles Bay K 8644, the dihydropyridine calcium channel activator (Spedding & Mir, 1987; Patmore et al., 1989). Palmitoyl carnitine also causes displacement of bound tritiated calcium antagonists in rat cortex and it has been suggested that acyl carnitines may be endogenous modulators of calcium channel function (Spedding & Mir, 1987). In addition, effects of palmitoyl carnitine have been reported on mitochondrial calcium handling in rat heart (Baydoun et al., 1988). The possibility arises therefore that carnitine esters may be exerting opposite effects to acyl carnitines regarding calcium modulation in the cell.

The fact that in the rat heart the coronary response to acetylcholine is primarily one of vasoconstriction and the vasodilator action of $P1P^i$ was not inhibited by atropine rules out the possibility that $P1P^i$ was acting on endothelial cell muscarinic receptors to release endothelial derived relaxant factor. It is also unlikely that the vasodilatation seen with these compounds was due to the release of their fatty acid component. Over the dose range 0.1–10 nmol, $P1P^i$, P1M and P1E, all of which are palmitoyl carnitine derivatives, produced vasodilatation (Figure 7), while similar doses of palmitate produced a vasoconstrictor response with a secondary vasodilator component only becoming apparent at doses of 10 nmol or more.

In conclusion, the pharmacological profile of the compounds we have synthesized shows that relatively small changes in the acyl carnitine structure can have a profound effect on the vascular activity, changing them from vasoconstrictors to vasodilators. As yet, the precise mechanism whereby the novel carnitine esters elicit a profound and sustained relaxation of the coronary vasculature is unclear; however, further study of these compounds is continuing and may provide a fuller understanding of the actions and role of acyl carnitines in the regulation of vascular tone.

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