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Structure–activity Relationships of Some Novel Coronary Dilator Derivatives of Palmitoyl Carnitine in the Rat Isolated Heart

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Abstract

The structure–activity relationships of some novel coronary dilator derivatives of palmitoyl carnitine in the rat isolated perfused heart are described.

It has been shown previously that esterification of palmitoyl carnitine changes the activity of the compound from a coronary constrictor to a coronary dilator. In this study, it was found that the ester group is not a necessary requirement for coronary dilator activity, but only the absence of the negatively charged carboxylic acid group of palmitoyl carnitine, as compounds containing an ethyl group in place of the ester group were also active coronary dilators. Furthermore, substituting the methyl groups attached to the nitrogen atom of the molecule profoundly altered coronary dilator activity. A quaternary ammonium group was a necessary requirement for potent coronary dilator activity.

We have previously shown that palmitoyl carnitine can produce a coronary constriction in the rat isolated heart, whilst a number of ester derivatives of palmitoyl and other acyl carnitines show coronary dilator activity (Criddle et al 1990, 1991). These novel coronary dilator derivatives show no concomitant effect on heart rate or developed tension. Initial structure–activity relationship studies have also shown that the dilator activity of these acyl carnitine derivatives is highly dependent on the length of the fatty acid (acyl) chain (Criddle et al 1991). The relationship between acyl chain length and coronary dilator potency for the isopropyl ester derivatives of acyl carnitines shows a bell-shaped curve, with a 16-carbon fatty acid chain length, palmitoyl, having the greatest coronary dilator potency. Altering the size of the ester grouping has a less profound effect on coronary dilator activity. However, the most potent compound was found to be the isopropyl ester of palmitoyl carnitine (Criddle et al 1990).

Conversion of palmitoyl carnitine to an ester derivative converts the compound from a zwitterion, containing positively charged (quaternary ammonium) and negatively charged (carboxylic acid) groups, to a molecule with a single positive charge (quaternary ammonium group; see Table 1); the current study investigates the importance of the quaternary ammonium group of the molecule in conferring coronary dilator activity, using various structural derivatives of palmitoyl carnitine, in the rat isolated perfused heart. The effect of replacing the ester group with an ethyl group on coronary dilator potency was also investigated, to establish whether the ester group itself or simply the absence of the negative (carboxylic acid) group of the parent molecule palmitoyl carnitine is important for coronary dilator activity. In addition, studies were carried out using compounds with different substituents attached to the nitrogen atom of the molecule to establish the importance of the quaternary nitrogen group on coronary dilator activity.

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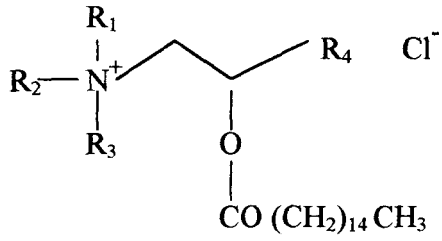
Materials and Methods

The rat isolated perfused heart (Langendorff) technique

Male Wistar rats (270–320 g) were anaesthetized with sodium pentobarbitone (60 mg kg^{-1}, i.p.) and the hearts excised and perfused at 10 mL min^{-1}, 37°C, using a modified Langendorff technique. The Krebs–Henseleit solution was of the following composition (mM): NaCl 118, $NaHCO_3$ 25, KCl 4·7, KH_2PO_4 1·2, $MgSO_4$ 1·2, L-glucose 11·1, $CaCl_2$ 2·4, gassed with 95% O_2-5% CO_2, pH 7·4. Hearts were perfused via the coronary vessels, following retrograde perfusion of the aorta.

After a 20-min perfusion, the perfusate was switched to one containing 2 mM KCl, i.e. the total K^+ concentration was reduced from 5·9 to 3·2 mM. This has the effect of raising the coronary perfusion pressure, thus allowing greater scope for coronary dilator responses to be seen. Following perfusion pressure stabilization, a dose–response curve to bolus doses of the compound under investigation was carried out, allowing perfusion pressure to return to basal levels before each subsequent dose. Coronary perfusion pressure was measured using a Gould pressure transducer attached to a side-arm on the aortic canula, developed tension was recorded under a resting tension of 2 g via a hook placed in the apex of the heart, connected to a Devices isometric transducer. The tension recording triggered a Gould rate meter to give a concurrent heart rate record. All recordings were made on Gould 3400/2400 recorders and drugs were added in volumes of no more than 30 μL via an injection port on the aortic canula.

Table 1. Structures of the compounds tested in this study, showing the ED50 values, maximum dilation produced, and the dose producing maximum dilation for each of the compounds tested.



Compound	R_1	R_2	R_3	R_4	Basal perfusion pressure (mmHg)	ED50 (nmol)	Maximum dilation (% relaxation)	Dose for optimum dilation (nmol)
Palmitoyl carnitine	CH_3	CH_3	CH_3	O=C-OH	Coronary constrictor (Criddle et al 1990)			
PlPi	CH_3	CH_3	CH_3	$O=C\text{-}OCH(CH_3)_2$	114 ± 3·1	0·44 ± 0·1	56 ± 6·3†	3
A	CH_3	CH_3	CH_3	CH_2CH_3	101 ± 4·1	1·1 ± 0·1	36 ± 1·7	10
B	H	CH_3	CH_3	CH_2CH_3	78 ± 8·8	13 ± 2·5‡	15 ± 1·4†	30
C	CH_2CH_3	CH_2CH_3	CH_2CH_3	CH_2CH_3	83 ± 10·6	0·26 ± 0·1‡	35 ± 7·9	1
D	$CH_2CH_2CH_3$	$CH_2CH_2CH_3$	$CH_2CH_2CH_3$	CH_2CH_3	117 ± 6·6	0·60 ± 0·1	36 ± 3·7	10
E	H	$(CH_2CH_2)_2O$		CH_2CH_3	109 ± 8·3	–	10 ± 3·3‡	100

*An ED50 value was not obtainable for compound E, due to the low coronary dilator activity of this compound. Maximum dilation = % relaxation from basal perfusion pressure at the optimum dose. For ED50 and maximum dilation, †$P < 0.05$; ‡$P < 0.01$, compared with compound A, one-way analysis of variance. n = 4.

Synthesis of novel compounds

All novel compounds were synthesized by M. Rad-Niknam and G. H. Dewar in the School of Pharmacy and Pharmacology laboratories, University of Bath. Compounds were dissolved and made up as stock solutions in saline.

Statistical analysis

All data are expressed as mean ± s.e.m. ED50 values and maximum dilator responses were compared using one way analysis of variance. A P value of less than 0·05 was considered significant.

Results

In this study, perfusion pressure in the presence of 5·9 mM K^+ Krebs–Henseleit was 73 ± 4·9 mmHg. On switching to modified Krebs–Henseleit containing 3·2 mM K^+, the perfusion pressure increased to a mean of 102 ± 3·9 mmHg, n = 30, i.e. an average increase in perfusion pressure of 29 mmHg. This allowed a greater scope for coronary dilator responses to be seen.

Effect of removal of the ester group on coronary dilator activity

The coronary dilator palmitoyl carnitine isopropyl ester (PlPi) differs from the parent compound palmitoyl carnitine by the presence of an isopropyl ester group in place of the carboxylic acid group (Table 1). Whereas palmitoyl carnitine is a potent constrictor in the coronary vasculature (Criddle et al 1990), it can be seen from Fig. 1 that PlPi is a potent vasodilator. Fig. 1 shows representative traces comparing the effects of PlPi and compound A on perfusion pressure in the isolated perfused heart preparation in the presence of 3·2 mM K^+. Bolus doses of PlPi produced a long-lasting coronary dilator effect, the maximum dilation being produced at 3 nmol. There was no concomitant effect on developed tension or heart rate (data not shown). Compound A differs from PlPi by the presence of an ethyl group in place of the isopropyl ester group (Table 1). It can be seen that this compound is also a coronary dilator in the isolated perfused heart, but the maximum dilation achieved (% relaxation from basal perfusion pressure) was significantly ($P < 0.05$) less than that of PlPi (Table 1, Fig. 2). The duration of the coronary dilator effect of compound A was also less than that of PlPi (Fig. 1). In addition, this compound produced a transient variable coronary constriction preceding the coronary dilator effect, an effect less marked in the case of PlPi.

Effect of different substitutions of the three methyl groups attached to the nitrogen atom of the molecule on coronary dilator activity

Table 1 and Fig. 3 compare the potencies of compounds all containing an ethyl group in place of the carboxylic acid group of palmitoyl carnitine but with different substituents attached to the nitrogen atom of the molecule (compounds A–E).

With regard to the quaternary ammonium compounds tested (compounds A, C and D), compound C, with three ethyl substituents on the nitrogen atom was significantly more potent, i.e. had a significantly lower ED50 value than compound A ($P < 0.01$), containing three methyl groups. Conversely, compound D with even larger alkyl groups attached to the nitrogen, i.e. three (straight chain) propyl

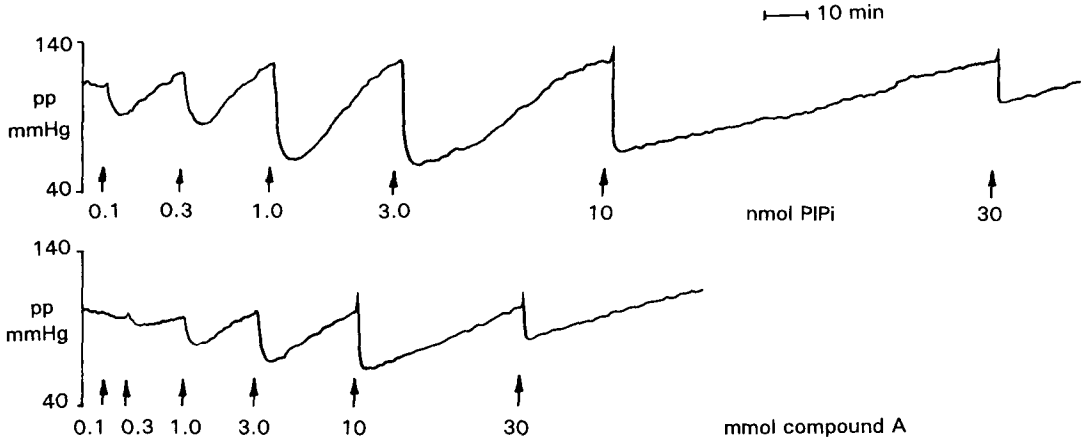


FIG. 1. Representative traces showing dose–response curves to PIPi and compound A in the coronary circulation of the rat isolated perfused heart.

groups, was not significantly different from compound A in terms of potency. It can be seen from Fig. 3 and Table 1 that compounds A, C and D were equi-effective in terms of the degree of coronary dilation achieved, i.e. % relaxation from basal perfusion pressure at the optimum dose.

Non-quaternary ammonium compounds tested, i.e. the tertiary amine salt, compound B and the morpholin-1-yl compound, compound E, were significantly less potent and less effective, in terms of degree of coronary dilation achieved, than compound A. An ED50 was not obtainable for compound E due to the lack of a dose-related vasodilation.

All the compounds tested showed a transient coronary constrictor response preceding dilation, similar to that shown for compound A in Fig. 1, although this was less marked in the case of the ester derivative, PIPi. In addition, none of the compounds tested showed any effect on developed tension or heart rate at the doses used.

Discussion

Palmitoyl carnitine has previously been shown to be a potent coronary constrictor in the rat isolated perfused heart (Criddle et al 1990) and the conversion of this zwitterionic amphiphile to a cationic ester derivative by the action of an alcohol (see Table 1 for structures) has been shown to convert the molecule from a coronary constrictor to a coronary dilator (Criddle et al 1990). The present study shows that the presence of an ester group is not necessary for the coronary dilator response. Compound A differs from the parent compound, palmitoyl carnitine, in the presence of an ethyl group in place of the carboxylic acid group (see Table 1). Like palmitoyl carnitine isopropyl ester (PIPi), compound A was found to be an active dilator in the coronary circulation. This suggests that the absence of a negatively charged carboxylic acid group, producing a molecule with an overall positive charge, is the necessary requirement in the conversion of the molecule from a coronary constrictor to a coronary dilator. However, PIPi was a significantly more effective coronary dilator than compound A, which may be related to the presence of a transient coronary constrictor component of the response to compound A preceding dilation, which was less marked in the case of PIPi.

Keeping an ethyl group in place of the carboxylic acid group of palmitoyl carnitine, but altering the quaternary

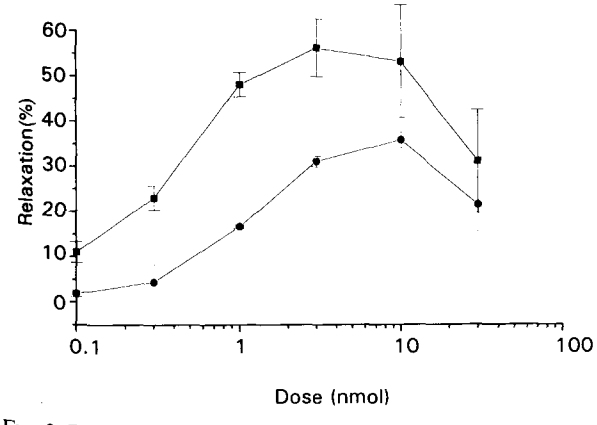


FIG. 2. Dose–response curves to PIPi (■) and compound A (●) in the rat isolated heart. Data shown as % relaxation from the basal perfusion pressure. n = 4.

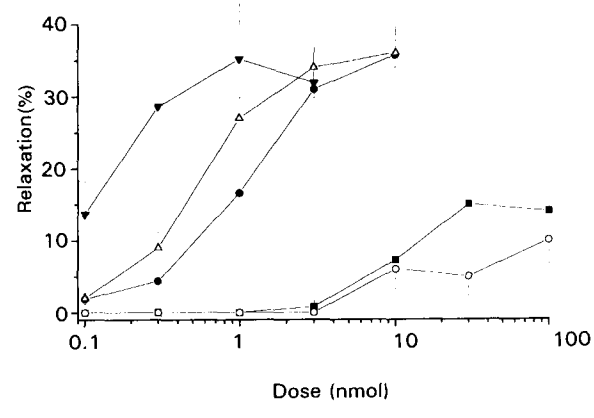


FIG. 3. Dose–response curves to compounds A (●), B (■), C (▼), D (△) and E (○). Data shown as % relaxation from the basal perfusion pressure. n = 4.

nitrogen group of the molecule, considerably affected coronary dilator potency. In the case of compounds which are ammonium salts, i.e. compounds A, C and D, the results obtained show that there is no simple relationship between the size of the alkyl substituents and coronary dilator activity. The potency order was compound C > compound A > compound D, i.e. three ethyl groups produced a compound more potent, and three propyl groups produced a compound less potent than three methyl groups. All these compounds are 100% positively charged at pH 7·4. The non-quaternary ammonium compounds B and E were less potent and effective vasodilators than the quaternary ammonium compounds tested. Compound B is a tertiary amine salt and compound E a morpholinyl compound (Table 1), and therefore as well as being in the charged form (protonated), these compounds may also exist in the uncharged form at pH 7·4. Thus it appears that the conformation and charge on the nitrogen is essential for the dilator activity of these compounds, and a quaternary ammonium group is necessary to produce a potent vasodilator.

As small changes to the palmitoyl carnitine molecule have such a profound effect on coronary dilator activity, it seems likely that the mechanism of action is due to a specific interaction at an active site rather than a non-specific detergent effect. Previous studies showing fatty acid chain length to be important for coronary dilator potency suggests that these compounds may incorporate into the lipid bilayer, by means of their fatty acid chains. The fact that a positive charge is essential for coronary dilator activity suggests that the mechanism may involve alteration of the surface charge on the membrane of the smooth muscle cells. As positively charged compounds do not readily pass through the lipid bilayer of cells, it is likely that these compounds are having an effect on the outer surface of the smooth muscle cell membrane. Criddle et al (1994) showed that in the potassium-depolarized mesenteric vascular bed, the isopropyl ester of palmitoyl carnitine (PlPi) was able to inhibit calcium-induced constrictions, and therefore this suggests that PlPi, and hence the other coronary dilator palmitoyl carnitine derivatives tested in this study, are able to inhibit calcium entry through voltage-gated calcium channels in smooth muscle. The fact that PlPi also suppresses coronary constrictions mediated by the dihydropyridine calcium-channel activator BAY K 8644 is further evidence for this effect (Reeves et al 1995). An increase in positive charge in the region of ion channels on the cell surface is known to alter ion-channel gating (Green & Andersen 1991; Latorre et al 1992), and therefore these compounds may be affecting this gating by means of their positive charge following incorporation of the fatty acid (palmitoyl) chain close to the ion channel. This effect has been shown for other cationic amphiphiles using isolated cardiac myocytes. For example, Burt et al (1983) found a decrease in ion fluxes in response to the cationic amphiphile polymyxin B, and Post et al (1991) reported a reduced current through L-type calcium channels in response to another cationic amphiphile, dodecyltrimethylammonium chloride (DDTMA). The cationic amphiphilic derivatives of palmitoyl carnitine used in our studies could be producing a similar decrease in calcium current within vascular smooth muscle cells. The transient coronary constrictor response preceding the coronary dilator response to most of these compounds could indicate a biphasic effect on calcium entry.

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