J. Pharm. Pharmacol. 1991, 43: 636-639
Received December 18 1990

The Synthesis, and Structure-activity Relationships of some Long Chain Acyl Carnitine Esters on the Coronary Circulation of the Rat Isolated Heart

D. N. CRIDDLE, G. H. DEWAR, M. RADNIKNAM, W. B. WATHEY AND B. WOODWARD

Pharmacology Group, University of Bath, Bath, Avon BA2 7AY, UK

Abstract—The synthesis of the isopropyl ester of carnitine and a series of fatty acid derivatives with fatty acid lengths C8-C30 is described. Bolus doses of these compounds (0·03–300 nmol) showed coronary vasodilator activity in the rat isolated heart. Increasing fatty acid chain length from C8 to C16 resulted in an increased vasodilator potency. Longer lasting vasodilation was observed with the C20 compound. Increasing fatty acid chain length to C30 was associated with a small dilator response preceded by vasoconstriction.

Amphiphilic acyl carnitines accumulate in the ischaemic myocardium (Liedtke et al 1978) and are thought to contribute to ischaemia-induced myocardial damage, either as a consequence of their amphiphilic properties (Corr et al 1984), or indirectly via an action on α-adrenergic receptors (Heathers et al 1987; Allely & Brown 1988). However there are reports of acyl carnitines having a protective effect on the heart (Hulsmann et al 1985). In contrast to the coronary constriction produced by naturally occurring acyl carnitines, we have shown that a number of esters of palmitoyl carnitine have a vasodilator action in the rat isolated heart (Criddle et al 1990). In our initial studies we showed that the isopropyl, ethyl and methyl esters of palmitoyl carnitine possessed coronary dilator properties. In the present paper we describe the synthesis of the isopropyl ester of palmitoyl carnitine and a series of isopropyl ester derivatives of acyl carnitine with differing fatty acid chain lengths (C8-C30). The structure activity relationships of these agents on the coronary circulation of the rat are also described.

Materials and Methods

Pharmacology

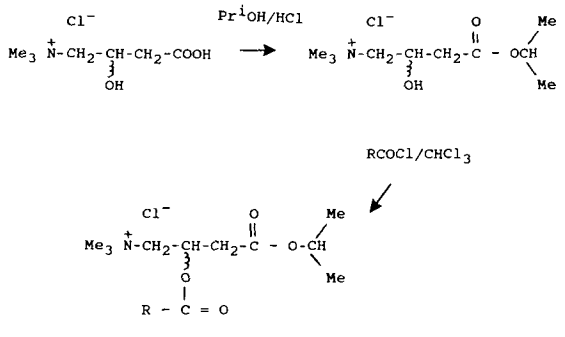
Hearts from male Wistar rats, 250–350 g, were perfused by the Langendorff technique as previously described (Criddle et al 1990). Briefly, hearts were perfused retrogradely via the aorta at a constant flow of 10 mL min^{-1}. Perfusion pressure changes were monitored to give an index of coronary tone. Developed tension at a resting tension of 2 g was measured via an isometric transducer attached to the apex of the heart; this was also used to trigger an instantaneous heart rate meter. Initially hearts were perfused for 10 min with a modified Krebs-Henseleit solution containing (mM), NaCl 118, $CaCl_2$ 1·2, $NaHCO_3$ 25, $MgSO_4$ 1·2, KH_2PO_4 1·2, KCl 4·7, gassed with 95% O_2 5% CO_2. After 10 min the total potassium concentration was reduced to 3·2 mM by reducing the concentration of KCl to 2·0 mM. This small change in extracellular potassium raises the perfusion pressure in this preparation thus allowing vasodilator actions to be seen more easily. Stock solutions of the compounds were made up in distilled water. Drugs were administered as bolus injections in volumes not exceeding 30 μL into the perfusion flow 3 cm above the aortic valves. Changes in perfusion pressure were measured as percent fall in pressure from the resting value. In the various groups of hearts the initial perfusion pressures in the presence of 3·2 mM KCl were within the range 90·3 ± 4·3 to 106 ± 10·6 mmHg. All values quoted are mean ± s.e.m.

Chemistry

Acyl isopropylcarnitines were synthesized by a modification of procedures previously reported (Ziegler et al 1967; Cavazza 1980). Racemic carnitine hydrochloride (Scheme 1) was converted to its corresponding isopropyl ester, and acylation by standard methods yielded the range of agents for study (1–12; see Table 1 for structural details). The isopropyl esters of palmitoyl D- and L-carnitine (6 and 7, respectively; Table 1) were similarly prepared.

Synthetic methods

^1H NMR spectra were recorded on a Jeol GX270 spectrometer. Unless stated otherwise, tetramethylsilane was employed as internal standard, and dimethylsulphoxide (DMSO d_6) as solvent. The NMR data for two representative



Scheme 1.

Correspondence: B. Woodward, Pharmacology Group, University of Bath, Bath, Avon BA2 7AY, UK.

compounds are quoted. This data is consistent with structure in all cases and may be obtained from the authors on request. Abbreviations for data actually quoted are: s, singlet; bs, broad singlet; d, doublet; t, triplet; q, quartet; m, multiplet, plus combinations of dt, doublet of triplets.

Infra-red spectra, recorded for liquids as films and for solids as KBr discs, were obtained using a Unicam SP1020 spectrometer. Optical rotation readings were recorded on an Optical Activity Ltd AA-10 Polarimeter at the sodium D line. Elemental analyses were performed by the Chemistry Department, University of Bath. Melting points (mp) are uncorrected.

Acid chlorides not commercially available were obtained by reacting the appropriate fatty acid with excess thionyl chloride under reflux overnight, and evaporation of excess thionyl chloride in-vacuo. The acid chloride was adequately pure for subsequent use.

Preparation of DL-*carnitine isopropyl ester.* A fast stream of dry HCl gas was passed through a suspension of DL-carnitine hydrochloride (Scheme 1; 40 g; 0·17 M) in dry isopropanol (HPLC grade; 300 mL) at room temperature (22°C). The solution reached reflux without external heating, and bubbling of HCl was continued for 3 h. The isopropanol was removed in-vacuo and the remaining oil dried in a desiccator over P_2O_5/conc. H_2SO_4 for 12 h. The solid produced was suspended in acetone, filtered, and washed with several portions of acetone. Crystallization was effected by dissolution in chloroform and adding acetone to the point of inducing turbidity. The crystallized material (40 g; 83%) had a mp of 139–140°C (Cavazza 1980; mp 145–150°C).

IR: 3200, 1745 cm^{-1}.

NMR (δ): 6·15 (1H, d, O*H*), 4·95 (1H, m, C*H*-OH), 4·45 (1H, m, OC*H*), 3·45 (2H, d, $\overset{+}{N}$-CH_2), 3·25 (9H, s, $\overset{+}{N}$ (CH_3)$_3$, 2·45 (2H, m, CH_2CO), 1·20 (6H, d, OCH (CH_3)$_2$).

Preparation of acyl DL-*carnitine isopropyl esters.* The acyl carnitine derivatives shown in Table 1 were prepared by the following general method described for octanoyl DL-carnitine isopropyl ester (1):

DL-Carnitine isopropyl ester (Scheme 1; 1 g; $4{\cdot}17 \times 10^{-3}$ M) in Analar, dry chloroform (EtOH, amylene-free; 25 mL) was gently heated, and redistilled octanoyl chloride (1·36 g; $8{\cdot}34 \times 10^{-3}$ M) added. The solution was refluxed for 4 h, and the chloroform was removed in-vacuo. Purification was effected by column chromatography (25 × 2·5 cm column; Silica Gel 60, 230–400 mesh (7 g) made up in Et_2O); the sample was applied using the minimum quantity of EtOH and eluted with: 1. Et_2O, until fatty acid and/or chloride was removed. 2. $CHCl_3$ to remove traces of starting ester. 3. EtOH to secure the desired compound. The required compound was obtained as an oil (1·1 g; 70%).

IR: 1750 cm^{-1}

NMR (δ): 5·50 (1H, q, OC*H*), 4·90 (1H, m, OC*H* (CH_3)$_2$, 3·80 (2H, q, $\overset{+}{N}$ CH_2, 3·20 (9H, s, $\overset{+}{N}$ (CH_3)$_3$) 2·75 (2H, m, CH_2CO), 2·30 (2H, t, CH_2CO, fatty acid chain), 1·50 (2H, t, CH_2 CH_2CO), 1·20 (14H, bs, 4 × CH_2 + CH (CH_3)$_2$) 0·85 (3H, t, CH_2 CH_3).

The tetraphenylboron salt of 1 (Table 1), prepared by adding a saturated aqueous solution of sodium tetraphenylborate to a saturated aqueous solution of the above quaternary chloride, was crystallized from MeOH and had a mp of 118–120°C.

Microanalysis for borate salt:
Found: C, 75·5; H, 8·42; N, 2·14%
$C_{42}H_{56}NO_4$ B.1H_2O requires: C, 75·6; H, 8·69; N, 2·10%

The compounds listed in Table 1 were prepared by the above method. With the exceptions shown, the products were oils whose NMR spectra were consistent with structure, and of adequate purity for pharmacological evaluation. Yields for quaternary chlorides, and mp values of tetraphenylboron salts (prepared in most cases) are presented in the table.

Results

All of the isopropyl esters of the acyl carnitines examined exerted a vasodilator action on the coronary circulation of the rat heart, although there were marked differences in the potency of these compounds (Fig. 1). It is clear that the highest potency was seen with the palmitoyl (C16) substituent (compound 5 in Table 1) while increasing or decreasing the fatty acid chain length was associated with a reduction in vasodilator activity. Therefore there was a bell-shaped relationship when comparing fatty acid chain length and vasodilator activity. A maximal dilator response to the octanoyl (C8) derivative (compound 1 in Table 1) could not be obtained because of solubility limitations. The lauroyl (C12), myristoyl (C14) and palmitoyl (C16) fatty acid substituents (compounds 3,4,5 in Table 1) all produced

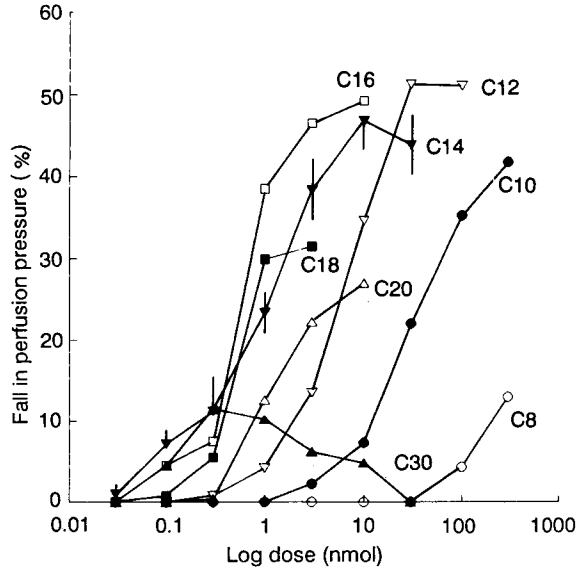
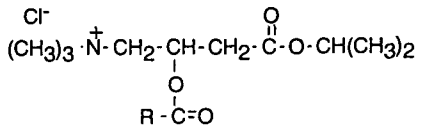


FIG. 1. Dose response relationships of vasodilator activity of acyl derivatives of isopropylcarnitine in the rat isolated heart. C8, C10, C12, C14, C16, C18, C20, C30 correspond to compounds 1, 2, 3, 4, 5, 9, 11, 12 in Table 1. S.e.m. is omitted for sake of clarity. n = 4-6.

Table 1. Data for some acyl carnitine esters (all compounds are DL-carnitines except 6, 7).



R	Compound number	% yield*	mp of tetraphenylboron salt
$(CH_2)_6CH_3$	1	70	118–120
$(CH_2)_8CH_3$	2	73	—
$(CH_2)_{10}CH_3$	3	74	110–113
$(CH_2)_{12}CH_3$	4	75	120–121
$(CH_2)_{14}CH_3$	5	75	—
$(CH_2)_{14}CH_3$	6[d]	75	115–117
$(CH_2)_{14}CH_3$	7[e]	75	—
$(CH_2)_{15}CH_3$	8	75[a]	—
$(CH_2)_{16}CH_3$	9	75	99–100
$(CH_2)_7CHCH(CH_2)_7CH_3$	10	75	—
$(CH_2)_{18}CH_3$	11	83[b]	91–93
$(CH_2)_{28}CH_3$	12	59[c]	—

[a] Solid, mp 142–143°C (acetone; preliminary softening at 58°C).
[b] Solid, mp 148°C (dry acetone; preliminary softening at 65°C).
[c] Solid, mp 154–156°C (dry acetone).
[d] L-Isomer, derived from L-carnitine. Relevant data: (i) yield of isopropyl ester from L-carnitine was 83%, (ii) $[\alpha]_D^{22}$ for the isopropyl ester was −26 (C=0·5; MeOH). $[\alpha]_D^{24}$ for the corresponding palmitoyl ester (6)) was −22·5 (c=0·53; MeOH).
[e] D-Isomer, derived from D-carnitine. Relevant data: (i) yield of isopropyl ester from D-carnitine was 95%, (ii) $[\alpha]_D^{24}$ for the isopropyl ester was +22·9 (C=0·61; MeOH). $[\alpha]_D^{24}$ for the corresponding palmitoyl ester (7) was +21 (C=0·52; MeOH). *All yields quoted are from carnitine isopropyl ester.

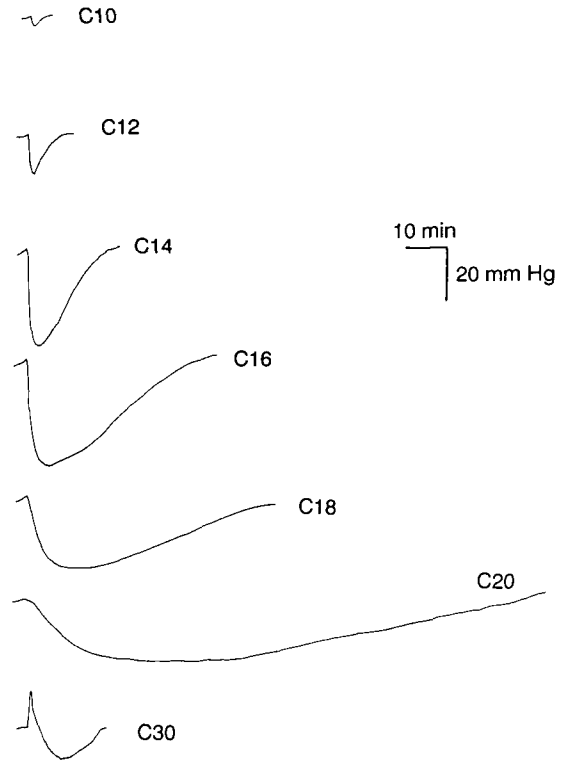


FIG. 2. Typical vasodilator responses to 3 nmol of acyl derivatives of isopropylcarnitine in the rat isolated heart. In all examples starting perfusion pressures were in the range 90·3–106 mmHg (see methods).

similar maximal vasodilator responses of about 50 mmHg. Fig. 2 shows representative traces of the vasodilation produced by a single dose (3 nmol) of the more active compounds and it is clear that the rate of vasodilation is also dependent on fatty acid chain length. The maximal rate of vasodilation occurred with the palmitoyl (C16) derivative while increasing or decreasing fatty acid chain length from this value resulted in a reduced rate of action. The odd numbered fatty acid substituent margaroyl (C17) (compound 8 in Table 1), which does not occur naturally in biological systems, and the introduction of an unsaturated double bond with a chain length of C18 (oleoyl, compound 10 in Table 1) also fitted into this general pattern of structure activity relationships (data not presented).

The duration of the vasodilator responses also showed marked differences between the various compounds (Figs 2, 3). The duration of the vasodilation increased with increasing fatty acid chain length. Depending on the dose used maximal duration of vasodilation was seen with the stearoyl (C18) or eicosanoyl (C20) derivatives (compounds 9 & 11 in Table 1). Increasing chain length to C30 (compound 12 in Table 1) resulted in a marked reduction in vasodilator duration. This latter response may, however, have been offset by an initial vasoconstriction (Fig. 2).

Of the compounds tested, the palmitoyl derivative of the isopropyl ester of carnitine had the most potent vasodilator activity. As this was synthesized as a racemic mixture from DL-carnitine there was the possibility that the two isomers might have differing actions. However, when the individual isomers were synthesized using D- and L-carnitine as the starting material it was found that both compounds had similar vasodilator activity (data not shown).

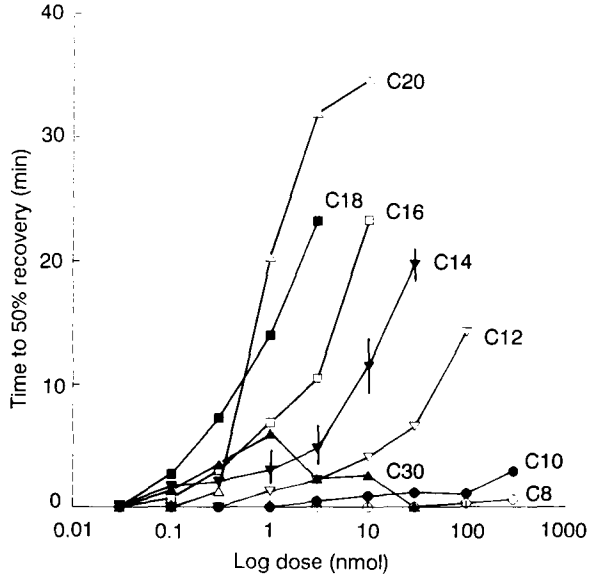


FIG. 3. Dose response relationships to show how fatty acid chain length affects the duration of the vasodilator response as measured by the time taken for perfusion pressure to return to 50% of its initial value. Abbreviations as in Fig. 1. n=4-6.

The effects of these agents on cardiac tension were minor. In all cases there was a gradual reduction in developed

tension throughout the experiment. This was more pronounced with the compounds which had a longer duration of action and was simply a reflection of the time taken for the experiment to be completed. None of the compounds had any significant effect on heart rate.

Discussion

The results of this study confirm and extend our earlier observations that ester derivatives of acyl carnitines possess coronary vasodilator properties. This contrasts with their native acyl carnitine parent compounds which have vasoconstrictor activity. Fatty acid chain length clearly has a marked effect on the vasodilator properties of these agents but there is no simple correlation between chain length and vasodilator activity. Optimal vasodilation occurred with the palmitoyl derivative while the C30 substituent possessed both vasoconstrictor and vasodilator activities.

It is not clear how these agents reduce coronary vascular tone. One possibility is an alteration in the ionic permeability of coronary artery cells. Spedding & Mir (1987) suggested that acyl carnitines could activate L-type calcium channels, and more recently the same group has proposed that acyl carnitines enhance the contractility of isolated cardiac myocytes by an action on calcium release from the sarcoplasmic reticulum (Clarke et al 1990). It is interesting that the positive inotropic action of the acyl carnitines reported by this group was also dependent on fatty acid chain length, such that the C16 acyl carnitine was the most potent of the series they examined. It is possible that the ester derivatives we have studied are having the opposite action to these acyl carnitines. This would lead to a decrease in calcium influx into coronary smooth muscle cells via L-type calcium channels or a reduced calcium release from the sarcoplasmic reticulum; either action would cause vasodilation.

However, it is unlikely that these compounds are simply acting as antagonists at L-type calcium channels, as they did not depress myocardial contractility to any significant extent. The classical calcium antagonist verapamil causes vasodilation and depression of myocardial contractility in the rat isolated heart (Daugherty & Woodward 1981; Baydoun et al 1989). However, if these drugs have a high affinity for incorporation into lipid bilayers, it is possible that by being administered via the coronary circulation they may not reach the cardiac myocytes, and this could account for their lack of affect on cardiac contractility. This possibility is further complicated by the presence of the endothelial cells through which the drugs may or may not pass. It should be noted that the vasodilator action of these compounds is not blocked by 100 μM L-nitroarginine (unpublished data) which suggests that they are not acting via the release of the endothelial derived relaxing factor, nitric oxide (Moore et al 1990).

As palmitoyl carnitine produces coronary vasoconstriction in the rat isolated heart (Criddle et al 1990), while the ester derivative of this and other acyl carnitines possesses vasodilator activity, it is possible that the difference in their actions could be associated with differences in the net charge on these molecules. Both types of compound have a positive charge conferred by the quaternary nitrogen of carnitine, while palmitoyl carnitine also has a negatively charged domain resulting from ionization of the carboxylic group at the other end of the carnitine sub-unit allowing it to behave as an inner salt or zwitterion. The free carboxylic group is not present in the ester derivatives as this is the site of esterification. It is therefore feasible that the net positive charge on the compounds we have investigated contributes to their vasodilator activity. Phillipson et al (1985) demonstrated that cationic amphiphiles can depress the contractility of rabbit papillary muscles possibly via an action on Na/Ca exchange. Thus there is the possibility that the acylcarnitine derivatives we have synthesized are acting in a similar manner, or act by altering the surface charge on the coronary artery cells.

Acknowledgement

This work was supported by a grant from The British Heart Foundation.

References

Allely, M. C., Brown, C. M. (1988) The effects of POCA and TDGA on the ischaemia-induced increase in alpha-1 adrenoceptor density in the rat left ventricle. Br. J. Pharmacol. 95: 705P

Baydoun, A. R., Peers, S. H., Cirino, G., Woodward, B. (1989) Effects of endothelin-1 on the rat isolated heart. J. Cardiovasc. Pharmacol. 13 (Suppl. 5): S193–S196

Cavazza, D. (1980) Esters of acyl-carnitines. UK Patent Application 2, 051. 779A. 1–9

Clarke, B., O'Connor, J., Duncan, G. P., Patmore, L., Spedding, M. (1990) Effects of acyl carnitines on cardiac muscle. J. Mol. Cell. Cardiol. 22 (Suppl. III): PS72

Corr, P. B., Gross, R. W., Sobel, B. E. (1984) Amphipathic metabolites and membrane dysfunction in ischaemic myocardium. Circ. Res. 55: 135–154

Criddle, D. N., Dewar, G. H., Wathey, W. B., Woodward, B. (1990) The effects of novel vasodilator long chain acyl carnitine esters in the isolated perfused heart of the rat. Br. J. Pharmcol. 99: 477–480

Daugherty, A., Woodward, B. (1981) Calcium and calcium slow channel antagonists on cyclic nucleotide levels in the isolated rat heart. J. Mol. Cell. Cardiol. 13: 843–854

Heathers, G. P., Yamada, K. A., Kanter, E. M., Corr, P. B. (1987) Long chain acylcarnitines mediate the hypoxia induced increase in alpha-1 adrenergic receptors on adult canine myocytes. Circ. Res. 61: 735–746

Hulsmann, W. C., Dubelaar, M. L., Lamers, J. M. J., Maccari, F. (1985) Protection by acyl-carnitines and phenylmethylsulfonyl fluoride of rat heart subjected to ischemia and reperfusion. Biochim. Biophys. Acta. 847: 62–66

Liedtke, A. J., Nellis, G. H., Neely, J. R. (1978) Effects of free fatty acids on mechanical and metablic function in normal and ischemic myocardium in swine. Circ. Res. 43: 652–661

Moore, P. K., Al-Swayeh, O. A., Chong, N. W. S., Evans, R. A., Gibson, A. (1990) *L*-NG-nitro arginine (*L*-NOARG), a novel *L*-arginine-reversible inhibitor of endothelium-dependent vasodilatation in vitro. Br. J. Pharmacol. 99: 408–412

Phillipson, K. D., Langer, G. A., Rich, T. L. (1985) Charged amphiphiles regulate heart contractility and sarcolemma-Ca^{2+} interactions. Am. J. Physiol. 248: H147–H150

Spedding, M., Mir, A. (1987) Direct activation of Ca^{2+} channels by palmitoyl carnitine, a putative endogenous ligand. Br. J. Pharmacol. 92: 457–468

Ziegler, H. J., Bruckner, P., Binon, F. (1967) O-Acylation of DL-carnitine chloride. J. Org. Chem. 32: 3989–3991