Interaction of palmitoyl carnitine with calcium antagonists in myocytes

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1 Beating of aggregates of embryonic chick myocytes, in primary culture, was quantified by use of a motion-detector and video-recorder technique. Interactions of palmitoyl carnitine, a putative endogenous ligand at Ca^{2+} channels, with calcium antagonists were investigated.

2 Bay K 8644 (1–100 nM) and palmitoyl carnitine (0.2–30 μM) increased edge movement of the aggregates; beats fused so that there was an increase in baseline 'tone'. The concentrations required to produce a 50% increase in edge movement were 2.5 nM for Bay K 8644 and 2 μM for palmitoyl carnitine. Higher concentrations (20–30 μM) of palmitoyl carnitine caused tachycardia of abrupt onset but resulted in cessation of beating. The effects of palmitoyl carnitine were not stereo-selective in that the (+)- and (–)-isomers were equieffective. Lysophosphatidyl choline (LPC) had no effect in concentrations up to 10 μM but higher concentrations caused tachycardia followed by cessation of beating. High concentrations of both palmitoyl carnitine and LPC (100 μM) caused break-up of the aggregates, presumably as a result of detergent effects.

3 Palmitoyl carnitine (1–100 μM) reversed the inhibitory effects of nisoldipine (0.3 μM), diltiazem (10 μM) and verapamil (1 μM). Ouabain was ineffective in reversing the effects of nisoldipine, differentiating the effects of palmitoyl carnitine from those of Na^+/K^+ ATPase inhibition. In contrast, palmitoyl carnitine did not reverse the inhibitory effects of pimozone (2 μM) or lidoflazine (7 μM); palmitoyl carnitine showed a similar profile to Bay K 8644 in this respect.

4 These findings indicate that the effects of palmitoyl carnitine closely resemble those of Bay K 8644 and can be differentiated from those of lysophospholipids. As palmitoyl carnitine accumulates in the sarcolemma during myocardial ischaemia, the mode of action in the Ca^{2+} channel may have clinical relevance for the use of calcium antagonists in ischaemia.

Introduction

Although there are several high affinity binding sites for exogenous ligands (dihydropyridines, verapamil, diltiazem) on voltage-dependent Ca^{2+} channels (VOCs), there are no endogenous ligands which have high affinity for these sites and modulation of VOC activity appears to be indirect, as a result of changes in membrane potential (Reuter, 1967), channel phosphorylation (Tsien *et al.*, 1972; Sperlakakis & Schnieder, 1976) or interactions with G proteins (Holz *et al.*, 1986).

However, palmitoyl carnitine, a lipid metabolite which accumulates in the cytosol and sarcolemma during ischaemia (Idell-Wenger *et al.*, 1978; Liedtke *et al.*, 1978; Neely & Feuvray, 1981; Corr *et al.*, 1984), has been shown to resemble Bay K 8644, a dihydropyridine Ca^{2+} channel activator (Schramm

et al., 1983), in K^+ -depolarized smooth muscle (Mir & Spedding, 1986; Spedding & Mir, 1987). As palmitoyl carnitine has selective interactions with tritiated calcium-antagonists ($[^3\text{H}]$ -nitrendipine, $[^3\text{H}]$ -verapamil and $[^3\text{H}]$ -diltiazem) in rat cerebral cortex membranes at concentrations which may be achieved during ischaemia, it has been suggested that acylcarnitines may be endogenous modulators of Ca^{2+} channel function, at least during ischaemic conditions (Mir & Spedding, 1986; Spedding & Mir, 1987).

We have therefore investigated whether the effects of palmitoyl carnitine mimic those of Bay K 8644 in beating aggregates of chick myocytes, maintained in primary cell culture. Beating was quantified with a motion detector video system which was developed to investigate the effects of Ca^{2+} channel activators on myocardial cells (Patmore & Whiting 1985;

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Patmore & Duncan 1988). Calcium-antagonists may act at various sites in VOCs to block the channels (Spedding, 1985b) and very distinct interactions have been shown to occur between the various subgroups of antagonists and dihydropyridine Ca^{2+} channel activators both in smooth muscle (Schramm & Towart, 1984; Su *et al.*, 1984; Spedding & Berg, 1984; Spedding, 1985a) and in heart (Boddeke *et al.*, 1988; Patmore & Duncan, 1988).

Thus, Bay K 8644 has been shown to reverse competitively the effects of the dihydropyridine calcium-antagonists, to reverse non-competitively the effects of verapamil and diltiazem, but not to reverse the inhibitory effects of lipophilic 'class III' (Spedding, 1985b) calcium antagonists, such as flunarizine, pimozone and lidoflazine. These interactions have also been defined in chick myocytes (Patmore & Duncan, 1988). The aims of the present study were to assess whether the interactions between Bay K 8644 and the different groups of calcium-antagonists in myocardium were similar to those between palmitoyl carnitine and the calcium antagonists. Such an approach would indicate whether palmitoyl carnitine had a site of action in the myocardial Ca^{2+} channel and could be considered as an endogenous activator. As palmitoyl carnitine has detergent effects in high concentrations, we have also compared its effects with those of the lysophospholipids which also accumulate during ischaemia; much of this accumulation will be consequent to inhibition of lysophospholipase and lysophospholipase transacylase by acylcarnitines (Gross *et al.*, 1982; Gross & Sobel, 1983). Some of this work has been presented to the British Pharmacological Society (Duncan *et al.*, 1986; 1987).

Methods

Cell cultures

Eleven day old Leghorn K chicken embryos were killed by decapitation and the hearts removed under aseptic conditions. Ventricles were chopped into fragments and were washed in Hanks Ca^{2+} - and Mg^{2+} -free balanced salt solution (Gibco) and then agitated at 37°C in the presence of 0.05% trypsin as described by Clusin (1981). Trypsinisation was then repeated 3–4 times and the dispersed cells were pooled, centrifuged and resuspended in culture media containing 20% nutrient media M-199 (Gibco), 6% heat-inactivated foetal calf serum, 1% penicillin/streptomycin solution and 73% low potassium salt solution the composition of which was (mM): NaCl 116, NaHCO_3 26.2, MgSO_4 0.8, NaH_2PO_4 0.9, CaCl_2 1.8 and dextrose 5.5, adjusted to pH 7.3. The cells were seeded at a density of 3×10^5 cells ml^{-1} into 96 well trays. The bottom of each well was coated with a layer of Sylgard 184 (Dow Corning) silicon elastomer resin. This process inhibits the formation of monolayers and over a period of 3 days in culture the cells reaggregate to form roughly spherical clusters of 100–200 μm diameter.

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Assessment of beating and experimental procedures

Aggregates normally display automaticity and the edge movement associated with contractions could be detected in each aggregate on observation under low power ($40\times$) microscopy. Around 15 aggregates formed in each culture well and contractility was assessed by scoring the % of aggregates in each well which were beating. This was done on an all-or-nothing basis and 10 randomly identified aggregates were scored. Control beating was normally 100%. The salt solution was designed to resemble the ionic composition of the culture media and contained (mM): NaCl 110, KCl 1.07, CaCl_2 1.7, MgSO_4 0.73, HEPES 19.3, NaHCO_3 0.83 and dextrose 5.5. Following drug application cultures were incubated for 30 min at 37°C and then identified wells scored again for % of aggregates beating. Only 40 of the 96 wells were used per tray and different treatments (drugs or concentrations) were applied to each of the 40 wells. Replicate tests for each concentration of each drug was made on 3 further trays. On each tray a series of wells were treated as 'sham' controls where salt solution containing no drug was added. This normally caused no inhibition of beating. Concentration-response curves were calculated for drugs by assessing the % of aggregates in which beating was completely inhibited.

In experiments where the interaction between palmitoyl carnitine and antagonists was investigated, concentrations of antagonists which caused approximately 85% inhibition of beating were added simultaneously with varying concentrations of palmitoyl carnitine. The scoring protocol was the same as that described for antagonist experiments and the data were again expressed as % of the sham-treated control aggregate beating. Data were fitted to a sigmoidal function between 15 and 100% of control and EC_{50} values (actually taken at 57.5% of control) calculated to express the potency of the palmitoyl carnitine reversal of antagonist-induced inhibition of beating.

Measurements of contractility

Contractility of individual spontaneously beating aggregates was estimated by measuring edge movement of individual aggregates. This technique has

been described briefly elsewhere (Patmore & Whiting, 1985). Aggregates grown on sylgard elastomer treated dishes were transferred to individual wells from Lux select-a-well culture plates. Over a period of 15–20 min very firm contacts were usually made with the substrate and the well was transferred to an inverted microscope stage and aggregates viewed at $400\times$ magnification. An aggregate was selected which displayed substantial edge movement, the area occupied by the aggregate changing on contraction. A video camera was attached to the microscope and on the video image a detection window was superimposed on the area of aggregate showing most edge movement. The window was composed of 20–30 raster lines and the point at which the image changed from light to dark (background to aggregate) along each line was compared with the length of the line. This was repeated for each line, the data integrated to calculate the area of aggregate occupying the window and this value was updated every video scan (20 ms). On contraction the area of aggregate occupying the detection window changes and an analogue output of area describing the waveform of contraction was obtained. This is essentially a measure of edge displacement and would typically be of the order of $5\text{ }\mu\text{m}$. Edge movement has been previously shown to be a function of the force of contraction (Clusin, 1981) and in this study edge movement has been used synonymously with contraction. Aggregates were continually superfused with culture media substitute salt solution as described earlier prewarmed to 37°C . Drugs were added to the superfusate in the presence of half the normal level of calcium to allow observation of positive inotropism.

Drugs

The following substances were used: palmitoyl (–) and (±)-carnitine, lysophosphatidyl choline (grade 1) (Sigma); (±)-verapamil (Sigma); pimozone, lidoflazine (Janssen); Bay K 8644 (methyl 1,4-dihydro-2,6-methyl-3-nitro-4-(2-trifluoromethylphenyl)-pyridine-5-carboxylate) and nisoldipine (Bayer). Palmitoyl (+)-carnitine was synthesized by Dr G. Ramirez, Institute of Organic Chemistry, Palo Alto, Ca, U.S.A. Throughout the text, the racemic form is referred to as palmitoyl carnitine.

Results

Aggregates of myocytes beat spontaneously with a rate of $107 \pm 4\text{ beats min}^{-1}$ ($n = 20$) at 37°C . The extent of edge movement of each aggregate, which was taken as being equivalent to an inotropic effect

Figure 1 Effects of palmitoyl carnitine ($5\text{ }\mu\text{M}$) on edge movement of aggregates of myocytes from chick embryos.

was dependent on the Ca^{2+} concentration of the medium; 1 mM Ca^{2+} resulted in half maximal contractility with a near maximal effect occurring with 2 mM Ca^{2+} (Figures 1 and 2; Patmore & Duncan, 1988). Increasing the Ca^{2+} concentration caused the beats to fuse partially so that the base-line increased; presumably relaxant processes were insufficient to account for Ca^{2+} entry so that cytosolic Ca^{2+} increased during diastole. Baseline changes reflected edge movement in all the experiments.

The protocol for investigation of the effects of palmitoyl carnitine is depicted in Figure 1. Preparations were set up in high Ca^{2+} (2 mM), allowing an estimate of near-maximal inotropic effects followed by a 10 min incubation in 1 mM Ca^{2+} ; edge movement after 10 min was taken as 100%. Palmitoyl carnitine ($0.5\text{--}30\text{ }\mu\text{M}$) caused a concentration-dependent increase in contractility, with a maximum effect similar to doubling the Ca^{2+} concentration. The concentration which caused a 50% increase in edge movement was $2\text{ }\mu\text{M}$ (Figure 3). However, prolonged incubation ($>10\text{ min}$ at $10\text{ }\mu\text{M}$) or shorter incubation with high concentrations ($>5\text{ min}$ with $20\text{--}30\text{ }\mu\text{M}$) resulted in a short period (5–360 s) of tachycardia followed by cessation of beating (Figures 2a and 4a). The inotropic effects of palmitoyl carnitine were not stereo-selective, because concentration-response curves to the racemate, (–)-isomer (concentration for 50% increase in edge movement: $3.5\text{ }\mu\text{M}$) and (+)-isomer (concentration for 50% increase in edge movement: $2.5\text{ }\mu\text{M}$) were superimposable in >4 experiments.

Bay K 8644 ($1\text{--}100\text{ nM}$) augmented edge movement in a concentration-dependent manner and also increased the baseline (Figures 2b and 3); rate of beating was not affected. The augmented contractility was maintained for $>30\text{ min}$.

Lysophosphatidyl choline (LPC; $1\text{--}10\text{ }\mu\text{M}$) did not increase edge movement (Figure 3), although higher concentrations ($30\text{--}50\text{ }\mu\text{M}$; Figure 4b) increased edge movement and rate of beating transiently; these concentrations were associated with break-up of the edge so that tracking was no longer possible. The aggregates broke up at higher concentrations and in four experiments at $100\text{ }\mu\text{M}$ break-up occurred within 2 min without marked inotropic effects. Palmitoyl

Figure 2 Effects of lowering the Ca²⁺ concentration from 2 mM to 1 mM on edge movement in chick myocytes and of perfusing for 10 min with palmitoyl carnitine (a, 1 μM then 10 min at 20 μM) or with Bay K 8644 (b, 10 nM).

carnitine caused edge break-up at high concentrations (> 100 μM).

Acyl carnitines inhibit the degradation of LPC in cell membranes, so the effects of a threshold concentration of LPC (10 μM) in the presence of palmitoyl carnitine (1 μM) were determined. In four experiments, LPC (10 μM) caused a similar threshold effect ($14.5 \pm 22\%$ augmentation, $P > 0.1$) to that obtained previously (Figure 3). However, after incubation with palmitoyl carnitine, LPC caused a rapid tachycardia

Figure 3 Concentration-response curves for the increase in edge movement caused by Bay K 8644 (●, $n = 5$), palmitoyl carnitine (■, $n = 5-18$) or lysophosphatidyl choline (◆, $n = 5-6$). Edge movement in the presence of 1 mM Ca²⁺ was taken as 100%. Edge movement in the presence of 2 mM Ca²⁺ was augmented to 190% ($n = 9$). Vertical bars represent s.e.mean.

Figure 4 Effects of palmitoyl carnitine (PC) (a, 30 μM) and lysophosphatidyl choline (LPC) (b, 30 μM) on edge movement in chick myocytes.

in all 17 preparations tested and within 10 min beating ceased in 9 of the preparations; the maximal increase in edge movement observed was $19.0 \pm 17\%$ above that caused by palmitoyl carnitine (11% augmentation, $n = 17$). Thus it would appear that although palmitoyl carnitine may increase the susceptibility of tissues to the well-known arrhythmic properties of LPC, pre-exposure to acyl carnitines did not reveal marked inotropic effects.

Calcium-antagonists

The inhibitory effects of calcium-antagonists on cell automaticity were quantified by an all or none observational technique whereby groups of 10 aggregates are scored for evidence of edge movement; incubation with increasing concentrations of calcium antagonists results in a concentration-dependent inhibition of the number of aggregates beating. Preliminary experiments established concentrations of the antagonists (nisoldipine 0.3 μM, diltiazem 10 μM, verapamil 1 μM, pimozide 2 μM, lidoflazine 7 μM) which were equieffective in causing approximately 85% inhibition of beating. Increasing the concentration of Bay K 8644 completely counteracted the

Table 1 Comparison of the potencies of Bay K 8644 and palmitoyl carnitine in augmenting edge movement and reversing the effects of calcium-antagonists in chick myocytes and in augmenting contractility in K^+ -depolarized smooth muscle preparations (taenia caeci from the guinea-pig) or in displacing tritiated ligands from the Ca^{2+} channels in rat cerebral cortex

	Palmitoyl carnitine (A)	Bay K 8644 (B)	A/B
<i>Myocytes:</i>			
Inotropy	5.7	8.6	954
<i>Reversal:</i>			
Nisoldipine	4.1	7.1'	1000
Verapamil	3.9	7.1'	3010
Diltiazem	4.4	6.9'	700
Pimozide	NE	NE	—
Lidoflazine	NE	NE	—
<i>Smooth muscle:*</i>			
Augmentation	4.1	7.9	6310
<i>Cerebral cortex:*</i>			
[3H]-nitrendipine	3.9	7.7	6310
[3H]-verapamil	4.0	8.1	12590
[3H]-diltiazem	4.0	7.9	7940

Data are expressed as pEC_{50} s or pIC_{50} s, except for the ratio.

* Data from Spedding & Mir, 1987; Patmore & Duncan, 1988.

NE – not effective.

Figure 5 Reversal of the effects of nisoldipine ($0.3 \mu M$, ▲), verapamil ($1 \mu M$, ■), diltiazem ($10 \mu M$, ▼) but not lidoflazine ($7 \mu M$, ●) or pimozide ($2 \mu M$, ◆) by palmitoyl carnitine. The number of aggregates beating in a culture plate was quantified, using 10 plates per observation. Vertical bars represent s.e.mean.

inhibitory effects of nisoldipine ($0.3 \mu M$) with a pEC_{50} of 7.1 (Table 1).

Palmitoyl carnitine (1 – $200 \mu M$) reversed the inhibitory effects of nisoldipine ($0.3 \mu M$) with a pEC_{50} of 4.1 (Figure 5; Table 1). However the reversal was not complete and at higher concentrations of palmitoyl carnitine (0.5 and $1 mM$) inhibitory effects were observed, presumably due to antagonist effects or to the detergent properties of the substance. Palmitoyl carnitine reversed the inhibitory effects of verapamil and diltiazem with pEC_{50} s of 3.9 and 4.4 respectively (Figure 5; Table 1). As was the case with Bay K 8644, palmitoyl carnitine did not reverse the inhibitory effects of pimozide and lidoflazine (Figure 5).

Unlike palmitoyl carnitine, ouabain ($10 \mu M$) did not reverse the inhibitory effects of nisoldipine in this model ($n = 4$), indicating that the reversal of the effects of nisoldipine by palmitoyl carnitine were not mediated via inhibition of Na^+/K^+ ATPase.

Discussion

This study shows that palmitoyl carnitine (0.5 – $30 \mu M$) increased edge movement in myocytes, and the effects are similar to those of Bay K 8644 and of increasing the Ca^{2+} concentration. This is consistent with the findings of Inoue & Pappano (1983), who reported that higher concentrations (30 – $300 \mu M$) increased Ca^{2+} -dependent action potentials in avian ventricular muscle, and of Spedding & Mir (1987) who found that palmitoyl carnitine (10 – $100 \mu M$) resembled Bay K 8644 in K^+ -depolarized smooth muscle. However, the concentrations required to increase edge movement in the myocytes were much lower than in the previous studies, although the concentration in the sarcolemma is not known. Thus the myocytes appeared particularly susceptible, due either to a greater accumulation of acyl carnitines than in the other preparations, which would be facilitated by easier access to these small preparations, or perhaps because of a greater susceptibility of the Ca^{2+} channels in these cells. The low concentrations are relevant because Knabb *et al.* (1986) have shown, in cultured rat myocytes, that sarcolemmal acylcarnitines increase 70 fold during hypoxia and after 60 min of hypoxia constituted approximately 3.5% of total sarcolemmal phospholipid. It is therefore likely that such an accumulation would interact with Ca^{2+} channels, as seen in our study. Piper *et al.* (1984) found that the concentrations of acyl carnitines present during ischaemia were insufficient to cause mitochondrial damage directly, to affect Na^+/Ca^{2+} exchange or Ca^{2+} pumping activity in the sarcoplasmic reticulum. However, these workers did not consider the possi-

bility of effects on Ca^{2+} mobilisation. Increased Ca^{2+} mobilisation during hypoxia or ischaemia (see Allen & Orchard, 1987; Barry *et al.*, 1987) via Ca^{2+} channels would be deleterious to cellular function and would be a major contributor to cell death following ischaemia and is discussed later.

What evidence is there that palmitoyl carnitine interacts with Ca^{2+} channels or tightly associated proteins? The effects of palmitoyl carnitine mimic those of the selective Ca^{2+} channel activator Bay K 8644 and although palmitoyl carnitine is approximately 1000 fold less potent than the dihydropyridine there is a good parallelism between the concentrations required to augment contractility and to counteract the effects of the calcium-antagonists (Table 1). Bay K 8644 is a powerful tool for defining the site of action of agents at the Ca^{2+} channel (Su *et al.*, 1984; Spedding & Berg, 1984; Spedding, 1985a,b). Using the present experimental protocol, Patmore & Duncan (1988) have shown that Bay K 8644 counteracts the effects of nisoldipine at non-molar concentrations (Table 1), which is compatible with the competitive interaction between dihydropyridines observed in smooth muscle. Bay K 8644 also counteracted the effects of verapamil and diltiazem; the binding sites for these drugs are linked by an allosteric mechanism within the Ca^{2+} channel (Galizzi *et al.*, 1985; Glossmann *et al.*, 1985; Mir & Spedding, 1987). However, Bay K 8644 did not reverse the inhibitory effects of pimozone or lidoflazine, lipophilic class III calcium antagonists (Spedding, 1985b). Bay K 8644 does not reverse the effects of these agents in smooth muscle (Spedding & Berg, 1984) nor in heart (Boddeke *et al.*, 1988; Patmore & Duncan, 1988) implying that these drugs either do not act at the Ca^{2+} channel (Spedding, 1985b; Grima *et al.*, 1986) or act at a distinct site in the channel, perhaps trapping it in a state which has low affinity for dihydropyridines (Spedding, 1985b).

Thus, palmitoyl carnitine shows the same selective interactions between the different classes of calcium-antagonists as does Bay K 8644. Furthermore, the pIC_{50} s for palmitoyl carnitine to reverse nisoldipine, diltiazem and verapamil in the heart (Table 1) are very similar to the pIC_{50} s for inhibition of [^3H]-nitrendipine, [^3H]-diltiazem and [^3H]-verapamil binding (Table 1) pointing to a direct interaction with the Ca^{2+} channel. However, the lack of stereoselectivity indicates that the interaction may be consequent to some form of selective accumulation and increased Ca^{2+} availability, perhaps consequent to a surface charge effect (Inoue & Papanno, 1983; Spedding, 1984). Electrophysiological studies are underway to define the mode of action.

Acyl carnitines and lysophospholipids have marked detergent properties and Adams *et al.* (1979) considered these substances to be 'naturally

occurring detergents', although Spedding & Mir (1987) found that a wide range of surface-active molecules did not mimic the effects of palmitoyl carnitine in smooth muscle. Nevertheless the detergent effects of lysophospholipids have been implicated as causative factors of arrhythmogenesis during ischaemia (Corr *et al.*, 1981; 1982; 1984; 1987) by affecting a wide variety of membrane-linked processes, particularly by inhibiting the conductance of the inward rectifier K^+ channel (Clarkson & Ten Eick, 1983; Kiyosue & Arita, 1986). The threshold concentration for affecting K^+ conductance in guinea-pig myocytes was found to be $10\text{ }\mu\text{M}$ (Kiyosue & Arita, 1986), which was the threshold concentration for the effects of LPC in the present study. Tachycardia followed by asystole would be expected from an agent affecting K^+ conductance and this parallels the excitability changes seen in ischaemic tissue (Corr *et al.*, 1987). A full electrophysiological analysis in myocytes will be required to ascertain whether the tachycardia followed by asystole caused by LPC and palmitoyl carnitine, at high concentrations ($20\text{ }\mu\text{M}$) is due to this phenomenon.

Nevertheless although high concentrations of LPC and palmitoyl carnitine have similar detergent properties (see Spedding & Mir, 1987), LPC tends to inhibit rather than augment Ca^{2+} current (Clarkson & Ten Eick, 1983; Corr *et al.*, 1987) and in the present study was effectively devoid of positive inotropic effects. The positive inotropic effects of palmitoyl carnitine can therefore be clearly differentiated from those of LPC. Will an effect of palmitoyl carnitine on Ca^{2+} availability be of relevance during ischaemia?

Knabb *et al.* (1986) have shown a 70 fold accumulation of acylcarnitines in the sarcolemma of rat myocytes during hypoxia and that inhibition of this accumulation with an acylcarnitine transferase inhibitor prevented the electrophysiological changes and depolarization which accompanied hypoxia, indicating that acylcarnitines are major determinants of ischaemic processes. Acyl carnitines would also be pivotal in controlling the lysophospholipid content of the sarcolemma. It has previously been assumed by many workers that Ca^{2+} overload following reperfusion which leads to cell death was the result of non-specific membrane disruption. However, Crake & Poole-Wilson (1986) have shown that the massive Ca^{2+} influx following ischaemia and reoxygenation in rabbit isolated heart is not accompanied by non-specific membrane disruption and we consider that it may be via modified calcium channels, especially as the number of channels available to tritiated calcium antagonists is modified in myocardial ischaemia (Dillon & Nayler, 1987; Matucci *et al.*, 1987); such data are consistent with a pool of channels being affected by acyl carnitines.

In conclusion, acyl carnitines have inotropic effects in myocytes and selective interactions with calcium-antagonists which closely resemble those of Ca^{2+} channel activators. Such findings support

further work investigating the hypothesis that Ca^{2+} overload following ischaemia and reperfusion may be via channels modified by acyl carnitines.

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