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Palmitoyl-DL-carnitine has calcium-dependent effects on cultured neurones from rat dorsal root ganglia

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1 The effects of palmitoyl-DL-carnitine (0.01 to 1 mM) on whole cell voltage-activated calcium channel currents carried by calcium or barium and Ca^{2+}-activated chloride currents were studied in cultured neurones from rat dorsal root ganglia.

2 Palmitoyl-DL-carnitine applied to the extracellular environment or intracellularly via the patch solution reduced Ca^{2+} currents activated over a wide voltage range from a holding potential of −90 mV. Inhibition of high voltage activated Ca^{2+} channel currents was dependent on intracellular Ca^{2+} buffering and was reduced by increasing the EGTA concentration from 2 to 10 mM in the patch solution. Barium currents were significantly less sensitive to palmitoyl-DL-carnitine than Ca^{2+} currents.

3 The amplitude of Ca^{2+}-activated Cl^- tail currents was reduced by palmitoyl-DL-carnitine. However, the duration of these Cl^- currents was greatly prolonged by palmitoyl-DL-carnitine, suggesting slower removal of free Ca^{2+} from the cytoplasm following Ca^{2+} entry through voltage-activated channels.

4 Palmitoyl-DL-carnitine evoked Ca^{2+}-dependent inward currents which could be promoted by activation of the residual voltage-activated Ca^{2+} currents and attenuated by intracellular application of EGTA.

5 We conclude that palmitoyl-DL-carnitine reduced the efficiency of intracellular Ca^{2+} handling in cultured dorsal root ganglion neurones and resulted in enhancement of Ca^{2+}-dependent events including inactivation of voltage-activated Ca^{2+} currents. The activation of inward currents by palmitoyl-DL-carnitine may involve Ca^{2+}-induced Ca^{2+} release from intracellular stores, or direct interaction of palmitoyl-DL-carnitine with Ca^{2+} stores.

Keywords: Voltage-activated calcium currents; chloride currents; lipid metabolites; intracellular calcium

Introduction

The loss of the ability of certain cells to maintain low intracellular calcium ion concentrations ($[Ca^{2+}]_i$) while still allowing Ca^{2+} influx for the activation of essential cellular processes may underlie a mechanism of neuronal damage produced by hypoxic or ischaemic insults. Alterations in Ca^{2+} entry through voltage-activated Ca^{2+} channels is one potential mechanism whereby such deleterious changes in Ca^{2+} homeostasis may occur during hypoxia or ischaemia (Choi, 1988).

Cellular metabolites produced in excess during ischaemia (Liedtke *et al.*, 1978) may alter calcium homeostasis by interaction with mechanisms of calcium regulation within the cell or its membranes. These mechanisms include Na^+/Ca^{2+} exchange, ATP-dependent Ca^{2+} pumps, Ca^{2+} binding proteins and intracellular Ca^{2+} storage organelles (McBurney & Neering, 1987). Such metabolites include the long chain acyl carnitines, intermediaries in the transport of fatty acids into mitochondria prior to β-oxidation. During ischaemia, mitochondrial β-oxidation is blocked with the subsequent accumulation of cytosolic fatty acyl carnitines including the palmitoyl derivative. As a result of myocardial ischaemia, levels of palmitoyl carnitine in the sarcolemma may rise 70 fold. This is associated with impaired inotropic function of the heart and the production of dysrhythmias (Knabb *et al.*, 1986). Palmitoyl carnitine itself has been demonstrated to inhibit the Na^+/K^+-ATPase and reduce [^3H]-ouabain binding in canine ventricular muscle (Adams *et al.*, 1979). The Ca^{2+}-ATPase and Ca^{2+} binding to sarcolemmal membranes are also inhibited by palmitoyl carnitine at 50–200 μM concentrations (Pitts *et al.*, 1978; Adams *et al.*, 1979). Further, Inoue & Pappano (1983) demonstrated the similarity of the effects of palmitoyl carnitine and those of elevated extracellular Ca^{2+} concentration in chick ventricular muscle in increasing the maximal amplitude and prolonging the Ca^{2+}-dependent action potential. Palmitoyl carnitine produced during ischaemia may act as an endogenous voltage-activated Ca^{2+} channel activator thus mediating some of the Ca^{2+} overload associated with myocardial ischaemia (Spedding & Mir, 1987; Patmore *et al.*, 1989). The effects of palmitoyl carnitine were shown not to be restricted to smooth muscle and cardiac myocyte preparations; selective interactions with brain cortical membranes have also been investigated. Palmitoyl carnitine inhibited binding of the Ca^{2+} channel ligands nitrendipine, verapamil and diltiazem to rat brain cortical membranes suggesting an interaction with the neuronal voltage-activated Ca^{2+} channels (Spedding & Mir, 1987). Although the metabolism of fatty acids is not a major energy source in the normal brain, (Bird *et al.*, 1985) abnormalities of lipid metabolism do occur during severe ischaemia (Gardiner *et al.*, 1981). Furthermore palmitoyl carnitine derived from non-neuronal ischaemic tissues may affect neuronal activity.

Inhibition of neuronal voltage-activated Ca^{2+} channels may have a role in offering some protection against the damage caused by cerebral ischaemia. We have studied the mechanism of action of palmitoyl carnitine on voltage-activated Ca^{2+} channel currents and Ca^{2+}-activated currents in cultured rat dorsal root ganglion neurones to gain an understanding of the possible modes of action of the lipid and its potential role in mediating neuronal damage. A preliminary account of this work has previously been communicated (Scott *et al.*, 1992a).

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Methods

Primary cell cultures

Dorsal root ganglion (DRG) neurones were obtained from 2 day old Wistar rats and following dissociation the cells were plated on laminin-polyornithine coated cover-slips and maintained in culture for 2–3 weeks at 37°C in humidified air containing 5% CO_2. F14 culture medium (Imperial Laboratories), supplemented with 10% heat-inactivated horse serum (GIBCO), nerve growth factor (Sigma), penicillin and streptomycin (Flow Laboratories), was used.

Electrophysiology

Voltage-activated Ca^{2+} currents and Ca^{2+}-activated currents were studied by the whole-cell variant of the patch-clamp technique (Hamill *et al.*, 1981). Cells were voltage-clamped by use of an Axoclamp-2A switching amplifier, sampling at 28–35 kHz, or an Axopatch 1D, with 70–80% series resistance compensation. Low resistance (3–7 MΩ) borosilicate glass micropipettes were used in this study. Recording medium contained (in mM): choline chloride 130, KCl 3.0, $MgCl_2$ 0.6, $NaHCO_3$ 1.0, HEPES 10, glucose 4.0, tetraethylammonium (TEA) bromide 25, tetrodotoxin (TTX, Sigma) 0.0025 and $CaCl_2$ or $BaCl_2$ 2. The pH and osmolarity were adjusted to 7.4 and 320 mOsM with NaOH and sucrose respectively. Choline chloride based recording medium was used to prevent any contribution from TTX-insensitive sodium currents. Patch pipettes were filled with a solution containing (in mM): CsCl or Cs acetate 140, $MgCl_2$ 2.0, Na-ATP 2.0, HEPES 10, $CaCl_2$ 0.1 and EGTA 2 or 10 to give $[Ca^{2+}]_i$ of 8.4 or 1.6 nM respectively. The pH was adjusted with Tris to 7.2 and the osmolarity to 310 mOsM with sucrose. Stock solutions of 10 mM to 1 mM palmitoyl-DL-carnitine (PC) (Sigma) were prepared freshly each day by dissolving in recording medium or patch solution. The pH was subsequently adjusted with NaOH to 7.2 or 7.4 accordingly and to assure complete dissolution. When appropriate, dilution of the stock solution was carried out as soon as the PC was completely dissolved. Palmitoyl-DL-carnitine was applied extracellularly by low pressure ejection from a pipette (tip diameter approx. 10 μm) placed within 100 μm of the cell being recorded. Palmitoyl-DL-carnitine was applied to the intracellular environment by inclusion in the patch pipette solution.

All electrophysiological recordings were stored on a digital audio tape recorder (Biologic) and analysed with Cambridge Electronic Design computer software. Voltage-activated Ca^{2+} and Ba^{2+} currents were obtained after scaled linear subtraction of leakage and capacitance currents. All data are given as mean ± standard error of mean (s.e.mean). Statistical significance was assessed by use of a 'two-way' Student's *t* test; paired or unpaired when appropriate.

Results

Actions of palmitoyl-DL-carnitine on voltage-activated Ca^{2+} channel currents

Extracellular application of 1 mM PC inhibited voltage-activated Ca^{2+} currents (I_{Ca}) activated over a wide voltage-range; − 30 mV to + 100 mV (Figure 1). The maximum inward high voltage-activated I_{Ca} evoked by depolarizing voltage step commands from − 90 mV to 0 mV were inhibited by extracellular application of PC (1 mM); only modest recovery, usually less than 25% was observed 5 to 10 min after removal of the pressure ejection pipette containing the lipid. The mean peak amplitude of the control high voltage-activated I_{Ca} was − 1.63 ± 0.15 nA and this current inactivated to − 0.91 ± 0.13 nA ($n = 9$) by the end of a 100 ms voltage step command. After 3 to 5 min application of 1 mM PC, the mean peak amplitude of I_{Ca} was significantly reduced to − 1.12 ± 0.12 nA and this current inactivated to − 0.33 ± 0.15 nA ($n = 9$; $P < 0.01$ and $P < 0.001$ for inhibition of peak and end current respectively). The I_{Ca} measured at the end of the voltage step command was inhibited to a greater extent by PC (1 mM) compared with the peak I_{Ca} measured at the beginning of the voltage step. This is reflected by the increased inactivation of I_{Ca} which was 43 ± 7% under control conditions and 71 ± 10% ($n = 9$) in the presence of PC (1 mM). Outward Ca^{2+} channel currents carried by monovalent ions and activated by voltage step commands to potentials positive to the null potential were inhibited by 1 mM PC (Figure 1). Additionally the low threshold T-type I_{Ca} was also attenuated by 1 mM PC (Figure 2). Extracellular application of 100 μM PC for 5 min was less effective than 1 mM PC and inhibited the peak I_{Ca} and end I_{Ca} by 23 ± 11% and 23 ± 10% respectively. Lower concentrations of PC (1 μM and 10 μM) applied extracellularly had no significant action on I_{Ca} ($n = 2$ and 4 respectively). In 2 cases PC (10 μM and 100 μM) caused transient increases in high voltage-activated I_{Ca} in the first minute of PC application; however, this observation was not found consistently.

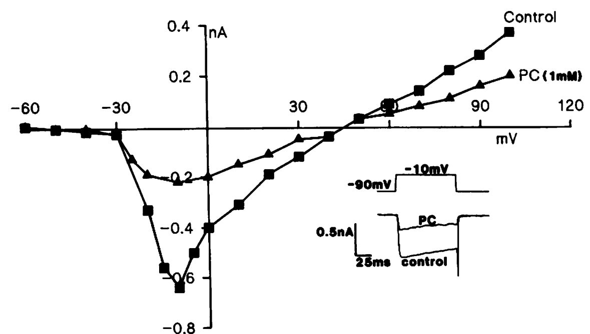

Figure 1 Current-voltage relationship showing that palmitoyl-DL-carnitine (PC, 1 mM) reduced Ca^{2+} currents activated over a wide voltage range, in a cell voltage-clamped at a holding potential of − 90 mV. Both inward Ca^{2+} currents activated at clamp potentials between − 30 mV and + 40 mV, (this cell had no low threshold T-type current) and outward Ca^{2+} channel currents activated at potentials positive to the null potential of + 45 mV were inhibited. PC (1 mM) had no effect on the null potential, (the voltage at which no net current flows through Ca^{2+} channels). The graphs show control data (■) and data generated after 3 to 5 min application of 1 mM PC (▲). The inset traces show the inhibition of the maximum inward Ca^{2+} current activated by 100 ms voltage step commands to − 10 mV.

The inhibitory actions of PC (1 mM) applied extracellularly on high voltage-activated Ca^{2+} channel currents were dependent on intracellular Ca^{2+} buffering and divalent cation charge carrier. Increasing the concentration of the Ca^{2+} chelator, EGTA, in the patch solution from 2 mM to 10 mM reduced the free Ca^{2+} concentration from 8.4 nM to 1.6 nM and increased the Ca^{2+} buffering capacity of the solution. Palmitoyl carnitine (1 mM) was significantly less effective when applied to cells which were recorded from using a patch solution with 10 mM EGTA and with 2 mM Ca^{2+} in the extracellular environment. Similarly, changing extracellular Ca^{2+} for 2 mM Ba^{2+} also reduced the inhibitory actions of PC (1 mM) (Table 1, Figure 2).

Studies were also carried out on the effects of intracellular application of PC (10 μM and 100 μM) which was applied via the patch solution. Palmitoyl carnitine (10 μM) had no significant effect on high voltage-activated inward I_{Ca}. In contrast, 100 μM PC reduced peak and end I_{Ca} over 6 min (Table 2). However, data were gathered for only 6 min because intracellular PC (100 μM) gave rise to unstable recordings (see below). Control data showed that I_{Ca} did not significantly run down during 20 min of recording ($n = 5$).

Actions of palmitoyl-DL-carnitine on Ca^{2+}-activated Cl^- tail currents

Tail currents which were predominantly due to Ca^{2+}-activated Cl^- currents were observed as slowly decaying inward currents in a proportion of DRG neurones loaded with CsCl patch solution (Currie & Scott, 1992). In 5 cells, extracellular application of 1 mM PC in addition to reducing Ca^{2+} influx through voltage-activated Ca^{2+} channels, also reduced in all cases the amplitude of the accompanying Ca^{2+}-activated Cl^- tail currents measured 20 ms after the end of the voltage step command by 29 ± 5%. The mean amplitude of the Cl^- tail current measured 20 ms after the end of the voltage step command was reduced from − 0.98 ± 0.27 nA to − 0.69 ± 0.20 nA, (n = 5) by PC (1 mM). However in 4 out of 5 cells the decay of the Cl^- tail currents were greatly slowed by extracellular application of PC (1 mM) (Figure 3). The mean time for the Cl^- tail currents to decay by 63% of the maximum current was increased from 1390 ± 580 ms to approximately 3600 ms (n = 5) after 3 min application of PC (1 mM). The time courses of the very prolonged Cl^- tail currents were difficult to measure accurately thus limiting quantification, although they did decay completely (Scott et al., 1992a).

Similar observations were made when comparing Cl^- tail currents activated in cells with 10 μM PC applied via the patch solution to the intracellular environment. Intracellular

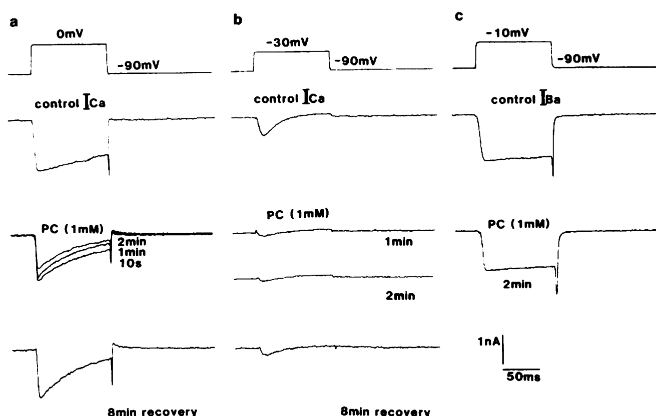

Figure 2 Palmitoyl-DL-carnitine (PC, 1 mM) inhibits voltage-activated Ca^{2+} channel currents carried by Ca^{2+} more effectively than currents carried by Ba^{2+}. (a) Traces showing inhibition of the high voltage-activated Ca^{2+} current during 10 s, 1 and 2 min application of 1 mM PC. Note the accelerated inactivation of the Ca^{2+} current in the presence of PC. (b) Traces showing inhibition of low voltage-activated T-type Ca^{2+} current by 1 and 2 min application of 1 mM PC. The predominant action is inhibition of the peak T-type Ca^{2+} current. Traces in (a) and (b) are from the same cell. Modest recovery of high voltage-activated and T-type Ca^{2+} current 8 min after removing the pipette containing PC is also illustrated. (c) Traces showing the modest inhibition of the high voltage-activated Ba^{2+} current by 2 min application of PC (1 mM). Note that PC (1 mM) produced no change in Ba^{2+} current inactivation.

Table 1 Percentage inhibition of high voltage-activated Ca^{2+} channel currents by extracellular palmitoyl-DL-carnitine (PC, 1 mM)

	2 mM$[Ca^{2+}]_o$ 2 mM$[EGTA]_i$	2 mM$[Ca^{2+}]_o$ 10 mM$[EGTA]_i$	2 mM$[Ba^{2+}]_o$ 2 mM$[EGTA]_i$
Peak I_{Ca}	34 ± 7%	14 ± 5% NS	18 ± 11% NS
End I_{Ca}	62 ± 9%	32 ± 8% †	27 ± 13% *
n	9	7	5

NS: not significant.
*$P<0.05$; †$P<0.03$ comparing Ba^{2+} and high EGTA data with Ca^{2+} and low EGTA data. Values are given as the mean percentages ± s.e.mean.
PC was applied for 3 to 5 min until steady state was achieved and peak and end I_{Ca} were measured at the beginning and end of 100 ms voltage step commands.

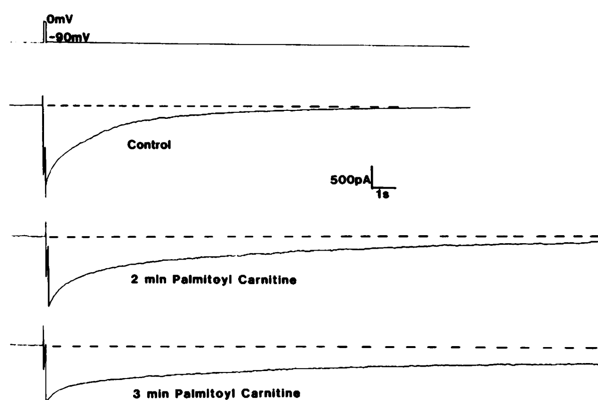

Figure 3 Palmitoyl-DL-carnitine (PC, 1 mM) prolongs Ca^{2+}-activated Cl^- tail currents. Traces show high voltage activated I_{Ca} activated by depolarizing step commands from − 90 mV to 0 mV. Ca^{2+} currents are accompanied by slowly deactivating inward Cl^- tail currents. Currents were activated under control conditions and 2 and 3 min after continuous application of 1 mM PC. Palmitoyl-DL-carnitine (1 mM) inhibited I_{Ca} and reduced the amplitude of the Ca^{2+}-activated Cl^- tail currents measured 20 ms after the end of the voltage step command. However PC greatly slowed the deactivation of the Ca^{2+}-activated Cl^- tail currents. The time course of decay of the Cl^- tail currents recorded in the presence of PC appears incomplete because the time base has been expanded to show I_{Ca}. However after more than 20 s the Ca^{2+}-activated Cl^- tail currents deactivated completely, in the presence of PC.

Table 2 Palmitoyl-DL-carnitine (PC, 100 μM) applied to the intracellular environment inhibits high voltage-activated I_{Ca} recorded from cultured DRG neurones

	10 μM PC		*100 μM PC*	
Time (min)	*Peak* I_{Ca} (nA)	*End* I_{Ca} (nA)	*Peak* I_{Ca} (nA)	*End* I_{Ca} (nA)
0.5	− 2.25 ± 0.21	− 0.97 ± 0.28	− 1.63 ± 0.43	− 0.68 ± 0.31
3.0	− 2.24 ± 0.30	− 1.34 ± 0.26	− 1.25 ± 0.32	− 0.78 ± 0.21
6.0	− 1.90 ± 0.34	− 1.24 ± 0.30	− 0.82 ± 0.25	− 0.49 ± 0.16
	NS	NS	*	NS

n = 6 for both 10 μM and 100 μM PC. Time is the duration of recording after entering the whole cell recording configuration. Peak I_{Ca} and end I_{Ca} are the mean amplitudes of I_{Ca} measured at the beginning and end of 100 ms voltage step command.
NS: not significant. *$P<0.01$ comparing currents activated at 0.5 min with those activated after 6 min. Peak I_{Ca} and End I_{Ca} measured in the presence of 100 μM PC were significantly smaller than currents measured in the presence of 10 μM PC, ($P<0.03$ and $P<0.05$ respectively) throughout.

PC (10 μM) did not significantly affect I_{Ca} (Table 1) or the amplitude of Ca^{2+}-activated Cl^- tail currents. However the Cl^- tail currents were greatly prolonged when comparing currents activated 30 s and 3 min after entering the whole cell configuration. After 30 s recording the Cl^- tail currents decayed by 63% of the maximum current in 1500 ± 200 ms ($n = 3$); after 3 min recording the value was > 3000 ms in all cases.

Palmitoyl-DL-carnitine activates Ca^{2+}-dependent currents

Extracellular application of PC (1 mM) evoked inward currents after a delay of approximately 30 s in cells voltage clamped at − 90 mV. These PC activated currents were usually transient in nature, but repeated oscillating currents were observed even after PC was no longer being applied (Figure 4a,b,c). It is difficult to quantify these responses to 1 mM PC, however, the mean maximum transient inward current amplitude was 1280 ± 80 pA ($n = 6$). After the PC-induced currents were activated the cells usually stabilized; however, if a train of 20 voltage step commands was applied to activate the residual I_{Ca} after a delay the activity was restarted ($n = 3$) (Figure 5a). These responses were not observed in control cells ($n = 5$) and repeated activation of larger control I_{Ca} did not induce oscillating inward currents. Palmitoyl-DL-carnitine (1 mM) could also produce damage to some of the cultured DRG neurones ($n = 3$). Cell damage was identified by sustained increases in inward current (Figure 4a,b) and in some cases the current records became very noisy (Figure 4a). Additionally cell swelling was observed and developed with the sustained current. The cells being recorded from by the whole cell technique were particularly vulnerable to PC-induced damage; neighbouring cells from which recordings were not made but which were exposed to PC did not undergo physical changes such as swelling. This may be a consequence of loss of cytoplasmic constituents involved in Ca^{2+} and/or cell volume regulation, with the whole cell recording technique.

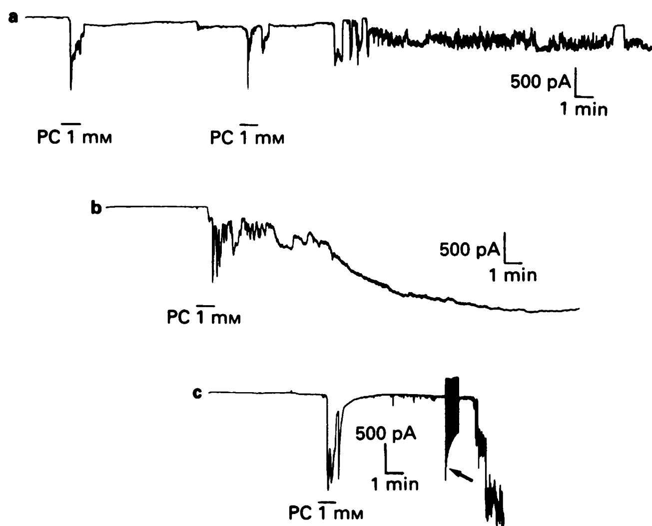

Figure 4 Palmitoyl-DL-carnitine (PC, 1 mM) evoked Ca^{2+}-dependent currents in cultured DRG neurones. (a) Trace showing transient responses to two periods of application of 1 mM PC followed by sustained noisy inward current which was associated with cell swelling. (b) Trace showing a series of inward current oscillations in response to 1 mM PC. The oscillating currents were followed by a large slowly developing sustained inward current and cell swelling. (c) PC (1 mM) activated several transient inward currents. Once the cell stabilized twenty 100 ms voltage step commands were applied at a frequency of 0.33 Hz to activate repeatedly the residual I_{Ca} (arrow) and load the cell with Ca^{2+}. Note the rapid inactivation of I_{Ca} with repeated activation. After I_{Ca} was activated at a clamp potential of 0 mV there was a short delay and then a rapid but large stepwise increase in inward current which was accompanied by cell swelling. Cells in (a), (b) and (c) were voltage-clamped at a holding potential of − 90 mV.

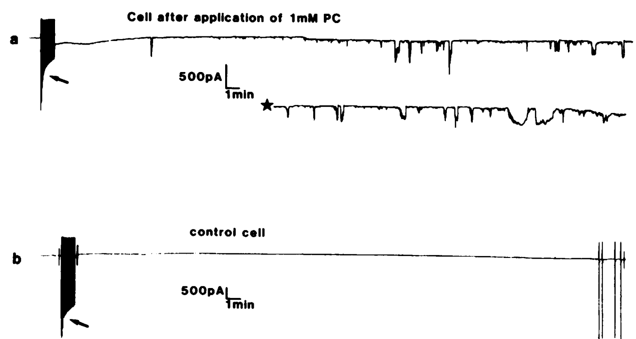

Figure 5 Repeated activation of high voltage-activated Ca^{2+} currents after application of palmitoyl-DL-carnitine (PC) triggers repeated spontaneous inward currents. (a) After 2 min application of 1 mM PC a single 1 nA transient current was observed and the cell stabilized (not illustrated). Five min after application of PC the cell was loaded with Ca^{2+} by activating I_{Ca} with twenty 100 ms voltage step commands to 0 mV at a frequency of 0.33 Hz (arrow). After a delay, transient oscillating inward currents were observed. The current record continues at (★). (b) Record from a control cell where repeated activation of the high voltage activated I_{Ca} (arrow) using the same protocol as described in (a), failed to evoke oscillating inward currents. At the end of the record, four Ca^{2+} currents and one leak current were activated to show that I_{Ca} was still present and that no run down had occurred. Both cells in (a) and (b) were voltage-calmped at a holding potential of − 90 mV. The amplification in each trace is different (note the scale bars). The high voltage-activated Ca^{2+} currents were much smaller when PC (1 mM) had been applied (a) and inactivation of I_{Ca} during the twenty voltage step commands was much more apparent compared with the control cell (b).

The PC-activated currents were dependent on intracellular $[Ca^{2+}]_i$. Increasing the EGTA concentration in the patch solution from 2 mM to 10 mM increased intracellular Ca^{2+} buffering and greatly attenuated PC-activated currents; delaying onset, reducing occurrence and decreasing current amplitude. Of 6 cells, PC (1 mM) activated transient inward currents in only 1 cell (Figure 6a). The onset of this activity was delayed for 8 min and the maximum current amplitude observed was only 700 pA. Palmitoyl carnitine-induced repeated spontaneous inward currents could not be triggered by 20 voltage step commands to activate I_{Ca} repeatedly in cells containing patch solution with 10 mM EGTA ($n = 3$). Intracellular application via the patch solution of 100 μM PC also activated transient inward currents in 2 out of 6 cells.

The ionic nature of the repeated spontaneous inward currents activated by PC was not investigated in detail; however, these currents were still observed when Cs acetate based patch solution was used. In all 6 cells studied PC-activated currents were seen. The mean maximum transient inward current observed was 920 ± 130 pA ($n = 6$). Many of the events seen in the presence of Cs acetate patch solution decayed more slowly than the fast transient events observed when the cells were loaded with CsCl (Figure 6b). This anion effect is similar to that previously reported when caffeine was used to release Ca^{2+} from stores (Currie & Scott, 1992).

Palmitoyl-DL-carnitine-activated currents are attenuated by caffeine pretreatment

We have investigated the possibility that PC mobilizes Ca^{2+} from a caffeine-sensitive intracellular store. Dorsal root ganglion cells were pretreated with 1 mM caffeine in Ba^{2+} rather than Ca^{2+} containing recording medium for two 7.5 min periods. The cells were then washed three times with Ba^{2+} containing recording medium. Following caffeine pretreat-

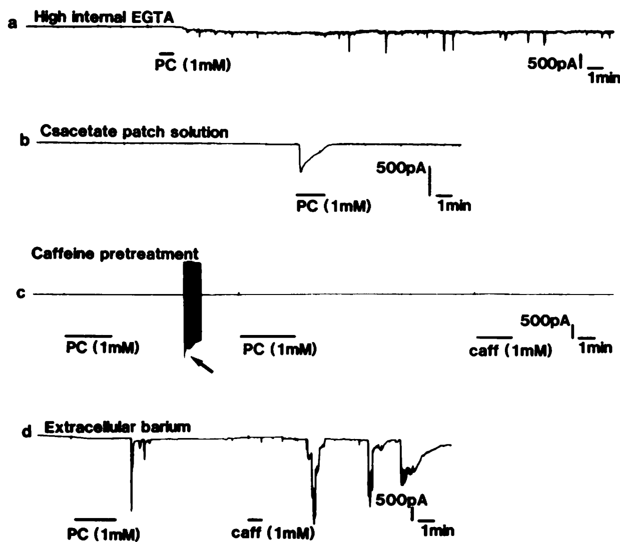

Figure 6 Ca^{2+}-dependence of palmitoyl-DL-carnitine (PC)-activated spontaneous transient inward currents recorded from DRG neurones at a holding potential of − 90 mV. (a) Trace shows PC-activated currents delayed and attenuated by intracellular EGTA (10 mM), recorded from a cell with extracellular Ca^{2+} and CsCl based patch solution. (b) Inward current activated by PC (1 mM) recorded with Cs acetate based patch solution. (c) Current trace recorded from a DRG neurone pretreated with caffeine (Caff, 1 mM) in the presence of Ba^{2+} containing recording medium. Application of PC (1 mM) and caffeine (1 mM) failed to elicit spontaneous transient inward currents. Twenty 100 ms voltage step commands to 0 mV applied at a frequency of 0.33 Hz to activate barium currents (see arrow), again failed to elicit these inward transients or to alter the sensitivity of the cell to either PC or caffeine. (d) PC (1 mM) and caffeine (1 mM) activate spontaneous transient inward currents in a cell bathed in medium containing Ba^{2+} and not pretreated with caffeine. Compare with (c).

ment and in the presence of extracellular Ba^{2+} rather than Ca^{2+}, PC (1 mM) did not evoke repeated spontaneous inward currents in 5 cells. Under these conditions caffeine responses were also abolished ($n = 5$). Repeated activation of Ba^{2+} currents by 100 ms voltage step commands failed to sensitize the cells to PC or caffeine (Figure 6c).

With extracellular Ba^{2+} but without caffeine pretreatment, both PC (1 mM) and caffeine (1 mM) responses could still be elicited (Figure 6d).

Discussion

Both extracellular and intracellular application of PC reduced voltage-activated Ca^{2+} currents recorded from cultured DRG neurones. The actions of PC on high voltage-activated Ca^{2+} currents were at least in part Ca^{2+}-dependent which raises the possibility that the reductions in current observed were due to enhanced Ca^{2+}-dependent inactivation. Calcium-dependent processes play an important role in the inactivation of high voltage-activated Ca^{2+} currents (Chad & Eckert, 1986). Intracellular EGTA (Eckert & Tillotson, 1981) and substitution of extracellular Ca^{2+} by Ba^{2+} (Tillotson, 1979) reduced current inactivation. In this study, PC was less effective at reducing high voltage-activated Ca^{2+} channel currents carried by Ba^{2+} and I_{Ca} recorded from cells with 10 mM intracellular EGTA. Since the action of PC on high voltage-activated I_{Ca} was quite rapid in onset (10 s), the effect of PC was not likely to be rate-limited by the Ca^{2+}-induced inactivation process which is fast, occurring with a time constant < 10 ms (Morad *et al.*, 1988).

The role of Ca^{2+} in mediating the action of PC was supported by the finding that PC slowed deactivation of Ca^{2+}-activated Cl^- tail currents. The deactivation of Ca^{2+}-activated Cl^- tail currents is slowed by both increased Ca^{2+} entry through voltage-activated Ca^{2+} channels (Mayer, 1985; Currie & Scott, 1992) and by impairing $[Ca^{2+}]_i$ homeostatic mechanisms; for example with cyanide (Duchen, 1990) or caffeine (Scott *et al.*, 1992b). The maximal amplitude of the Ca^{2+}-activated Cl^- tail currents was reduced by PC. This was probably due to the decrease in I_{Ca}, resulting in less Ca^{2+} being available to activate the tail current.

Palmitoyl-DL-carnitine also activated inward currents which were similar to those observed following caffeine-induced Ca^{2+} release from intracellular stores (Currie & Scott, 1992). Like these caffeine-induced responses, PC-induced currents were attenuated by intracellular EGTA, suggesting that the currents were Ca^{2+}-dependent. These inward currents activated by PC were predominantly due to cation influx, however, we cannot exclude the possibility that when using CsCl based patch solution there is not also a component due to Cl^- efflux.

Several mechanisms may be associated with the PC action: (1) PC may reduce the efficiency of Ca^{2+} homeostatic mechanisms and allow a build up of free cytoplasmic Ca^{2+} with subsequent Ca^{2+}-induced Ca^{2+} release from intracellular stores. Support for this hypothesis comes from the finding that PC inhibits Ca^{2+}-ATPase and Ca^{2+} transport (Pitts *et al.*, 1978; Adams *et al.*, 1979). ATP-dependent Ca^{2+} transport plays an important role in regulation of $[Ca^{2+}]_i$ in DRG neurones (Thayer & Miller, 1990). Additionally, inhibition of Ca^{2+}-ATPase has been found to slow recovery from a Ca^{2+} load produced by voltage-activation of Ca^{2+} currents (Benham *et al.*, 1989). (2) PC may enter DRG neurones and itself trigger Ca^{2+} release from an intracellular store. Spedding (Zernig, 1991) has recently reported that acylcarnitines cause cell damage in part by activation of a ryanodine-sensitive Ca^{2+} release process associated with sarcoplasmic reticulum.

The use of Ba^{2+} as the charge carrier limits activation of Ca^{2+}-dependent processes including filling and release from caffeine-sensitive stores and activation of Ca^{2+}-dependent currents. However PC and caffeine-induced release of Ca^{2+} from intracellular stores still occurs with Ba^{2+} in the extracellular environment. Evidence from this study where caffeine pretreatment blocks PC responses suggests that in part, PC activates spontaneous transient inward currents by either direct or indirect Ca^{2+} release from a caffeine-sensitive store. L-Palmitoyl carnitine responses in single ventricular myocytes have previously been shown to be blocked by ryanodine, also suggesting a role for intracellular Ca^{2+} stores in PC responses (Mészàros & Pappano, 1990). PC has a lipophilic tail and was active whether applied intra- or extracellularly, so an intracellular site of action cannot be excluded. Although significant effects of PC were only observed with concentrations of 0.1 and 1 mM applied extracellularly, intracellular application of PC (0.1 mM) was more effective at reducing I_{Ca}. The same concentrations of PC as used in this study have previously been found to interact with Ca^{2+} channel ligand binding (Spedding & Mir, 1987).

Following the initiation of PC-induced Ca^{2+}-dependent inward currents, cell swelling and sustained increases in inward current were sometimes observed. These phenomena were not studied in detail but may reflect current generated by stretch-activated ion channels or activity of other channels associated with volume regulation. These effects may have developed as a result of disruption of cation gradients and osmotic balance after PC application or as a result of mobilization of intracellular Ca^{2+} and activation of inward currents. These effects of PC on $[Ca^{2+}]_i$ homeostasis may underlie an important mechanism of cell damage produced by ischaemic or hypoxic insults.

Not all the actions of PC can be accounted for by altered handling of intracellular free Ca^{2+}. The reduction of T-type low voltage-activated I_{Ca} probably involves a different mechanism because this current does not show marked Ca^{2+}-dependent inactivation (Bossu & Feltz, 1986; Carbone &

Lux, 1987). Furthermore, T-type I_{Ca} can be isolated from high voltage-activated I_{Ca} because it is much less sensitive to raised $[Ca^{2+}]_i$ (Carbone & Lux, 1987). T-type I_{Ca} is very sensitive to agents such as 1-octanol (Llinás, 1988) and an arginine polyamine (Scott *et al.*, 1992c) which interact with lipid components of cell membranes. The action of PC on T-type I_{Ca} may involve modification of the lipid micro-environment of the Ca^{2+} channels rather than direct interaction.

We have not investigated the actions of other long chain acyl carnitines on our preparation. However, altering the fatty acid chain length has been shown to attenuate fatty acid responses in other systems (Criddle *et al.*, 1990). Fatty acids are known to have regulatory actions on a variety of ion channels (Ordway *et al.*, 1991). This may be due to direct interaction with channels or indirect effects via second messenger formation or on the lipid microenvironment. Actions on Na^+ and Ca^{2+} currents have been described in neuroblastoma cells where fatty acids appear to attenuate these currents by a protein kinase C-mediated mechanism (Linden & Routtenberg, 1989).

At present we cannot exclude the possibility that PC has some inhibitory properties which involve direct interactions with voltage-activated Ca^{2+} channels. We have not observed any consistent increase in I_{Ca} produced by PC in this functional assay system. However the dominant action of PC to reduce I_{Ca} observed in this study may have masked neuronal Ca^{2+} channel activator properties previously reported (Spedding & Mir, 1987).

In conclusion we have observed actions of PC on DRG neurones which are consistent with reduced cellular homeostatic control of $[Ca^{2+}]_i$.

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