Interactions of palmitoyl carnitine with the endothelium in rat aorta

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1 Palmitoyl carnitine (10–1000 μM) resembled Bay K 8644 (10–1000 nM) in that it directly contracted rat aortic rings which were partially depolarized with K^+ (12 mM). However, the effects of Bay K 8644 were reduced in the presence of endothelium whereas the presence of the endothelium hardly affected the palmitoyl carnitine-induced contractions, which occurred at high concentrations ($> 10 \mu\text{M}$).

2 Lower concentrations of palmitoyl carnitine (0.3–30 μM ; EC_{50} 1.1 μM), but not Bay K 8644, carnitine or palmitic acid, antagonized the relaxant effects of acetylcholine in rat aorta. The antagonism was specific for endothelium-dependent relaxations, in that the relaxations to ATP and the calcium ionophore A23187 were also non-competitively antagonized, albeit at slightly higher concentrations, whereas the direct relaxant effects of sodium nitroprusside were unaffected. Palmitoyl carnitine therefore antagonizes the effects or the release of endothelial-derived relaxant factor (EDRF). The inhibitory effects were reversed on prolonged washout, indicating that the effects were not due to destruction of the endothelial cells.

3 In superfusion experiments, palmitoyl carnitine inhibited the release of EDRF from rat aorta but did not affect the responsiveness to exogenous EDRF, indicating a site of action at the endothelial cell. In superfusion experiments, palmitoyl carnitine, and lysophosphatidyl choline, caused direct relaxations of the aorta, indicating EDRF release, prior to inhibition of release evoked by receptor stimulation. These substances may modulate vascular responsiveness under certain conditions.

Introduction

Acyl carnitines are key intermediates in lipid metabolism, effecting the transfer of lipids into mitochondria. During ischaemic episodes β -oxidation of fatty acids in mitochondria is inhibited, resulting in large increases in the acyl carnitine content of the cytoplasm (Idell-Wenger *et al.*, 1978; Liedtke *et al.*, 1978; Neely & Feuvray, 1981; Corr *et al.*, 1984) and associated cellular membranes (Knabb *et al.*, 1986). The accumulation of acyl carnitines in myocardial sarcolemma may be responsible for some of the electrophysiological aberrations caused by ischaemia (Knabb *et al.*, 1986). Spedding & Mir (1987) have advanced the hypothesis that palmitoyl carnitine may be an endogenous modulator of Ca^{2+} mobilisation, on the basis that the effects of palmitoyl carnitine in K^+ -depolarized smooth muscle resemble those of the dihydropyridine Ca^{2+} channel activator, Bay K 8644. Inoue & Pappano (1983) had previously shown that palmitoyl carnitine increases Ca^{2+} current in avian ventricular muscle. Furthermore, low concentrations (0.5–5 μM) of palmitoyl carnitine cause positive inotropic effects in chick myocytes, and higher concentrations reverse the inhibitory effects of verapamil and nisoldipine (Duncan *et al.*, 1986; 1987; Patmore *et al.*, 1989). However, more recent evidence points to a site of action at the sarcoplasmic reticulum in the heart, tightly linked to the Ca^{2+} channel (Spedding *et al.*, 1989). It is therefore possible that acyl carnitines, produced in high concentrations during ischaemia, might mediate some or all of their deleterious effects via an interaction with the Ca^{2+} channel or a tightly coupled structure associated with it.

Endothelial cells have key interactions with lipids, being involved in the transport of free fatty acids from the circulation to muscle. Endothelial cells also can rapidly metabolise palmitate, which can be used as an energy source; oxidation is increased in the presence of carnitine (Hulsmann *et al.*, 1988), although it is not known to what extent acyl carnitines accumulate in endothelial cells during ischaemia.

Endothelial cells release a relaxant factor (endothelial-derived relaxant factor, EDRF) which may be nitric oxide

(Furchgott & Zawadzki, 1980; Rubanyi & Vanhoutte, 1985; Gryglewski *et al.*, 1986; Palmer *et al.*, 1987; Furchgott, 1988). EDRF may have a critical role in inhibiting vascular spasm during or after ischaemic episodes.

The question whether Ca^{2+} channel activators cause EDRF release is controversial. Rubanyi *et al.* (1985) found that very low concentrations of Bay K 8644 released EDRF in dog femoral arteries and concluded that Ca^{2+} channels were involved. In contrast, Spedding *et al.* (1986) concluded that Bay K 8644 did not release EDRF from rat aorta, but that the contractile effects of Bay K 8644 in smooth muscle could be countered by a tonic liberation of EDRF. A thorough electrophysiological study found no evidence for the presence of L channels in endothelial cells (Takeda *et al.*, 1987). We have tested the effects of the putative Ca^{2+} channel activator palmitoyl carnitine in rat aorta, in the presence and absence of endothelium. A preliminary account of these findings has been published in abstract form (Bigaud & Spedding, 1986; Dainty *et al.*, 1987).

Methods

Rings of rat aorta, taken from male Sprague-Dawley rats (200–340 g), were set up between two horizontally arranged steel wires in 10 ml isolated baths, filled with Krebs solution (composition, mM: NaCl 137, KCl 2.7, MgCl_2 1.1, CaCl_2 1.8, NaHCO_3 11.9, NaH_2PO_4 0.4, glucose 5.5) which was maintained at 35°C and gassed with 95% O_2 :5% CO_2 . Where depolarizing solutions were used, 12 mM K^+ was substituted for the equivalent concentration of Na^+ . In some experiments a slightly different solution was used (composition, mM: NaCl 118.4, KCl 4.7, MgSO_4 1.2, CaCl_2 2.5, KH_2PO_4 1.2, NaHCO_3 25, glucose 11.1; Figure 4) which had no effect on the sensitivity to acetylcholine. Isometric developed tension was recorded after resting tension had been set to 1 g. After a 60 min equilibration period, the rings were contracted with phenylephrine, 1 μM , and then challenged with acetylcholine, 1 μM , to verify the presence or absence of endothelium. Strips which did not relax by more than 40% were not used. Strips which were denuded of endothelium were gently rubbed 4–5

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times over the steel wires. The presence of the endothelium was verified histologically in some preparations by staining with silver nitrate (12.5% for 20 min). More than 90% of the interior surface of the aorta was covered with endothelial cells in control preparations, and <10% was covered in the rubbed preparations.

Superfusion experiments

In experiments where EDRF release from one aorta to a recipient, endothelium-denuded, preparation was assessed, 4 mm lengths of rat aorta were opened by a single lengthways cut, denuded of endothelium, and tensioned between two pins, one of which was connected to an isometric force transducer, in a plastic gutter; the preparations were perfused with Krebs solution (6 ml min^{-1}). Resting tension was adjusted to 1 g. A 4 mm strip of aorta, with endothelium present, was placed transversely over the intimal surface of the endothelium-denuded aorta, which was contracted with phenylephrine ($0.1 \mu\text{M}$). EDRF was liberated from the upper preparation by superfusion with carbachol ($10 \mu\text{M}$). The lower concentration of phenylephrine and the higher concentration of carbachol were used in order to increase the sensitivity of the superfused preparation.

In experiments where palmitoyl carnitine was shown to release EDRF directly, rings of rat aorta were used and the substance injected as a bolus into the perfusate.

Drugs

The following drugs were used: adenosine 5'-triphosphate (Sigma), A23187 (Sigma), acetylcholine hydrochloride (Sigma), Bay K 8644 (methyl-1,4-dihydro-2,6-dimethyl-3-nitro-4-(2-trifluoromethyl-phenyl)pyridine-5-carboxylate, a gift from Dr M. Schramm, Bayer AG), L-carnitine hydrochloride (Sigma), palmitoyl-DL-carnitine (Sigma), sodium nitroprusside (Sigma), palmitic acid (Sigma), lysophosphatidyl choline (Sigma). The drugs were dissolved in distilled water and all solutions prepared fresh daily.

Statistics

Student's *t* test was used for comparison of mean values. Mann-Whitney U-test was used for comparison of % changes. All concentrations refer to final bath concentrations of drugs.

Results

Bay K 8644, in the presence of a threshold depolarization (12 mM K^+), caused a concentration-dependent contraction of the rat aorta. The magnitude of the contractions was greater in preparations which had been rubbed to destroy the endothelial cells (Figure 1; maximum tension rubbed $1.25 \pm 0.15 \text{ g}$, unrubbed 0.75 ± 0.15 , $P < 0.05$). In the presence of 12 mM K^+ , palmitoyl carnitine caused a concentration-dependent con-

Figure 1 Contraction of rat aorta by cumulative addition of (a) Bay K 8644 or (b) palmitoyl carnitine in the presence (●) or absence (○) of endothelium. The preparations were partially depolarized with 12 mM K^+ . Vertical bars represent s.e.mean; $n = 5$.

traction of the rat aorta, but there was no significant difference between the maximum effects of palmitoyl carnitine in the presence or absence of endothelium (Figure 1; rubbed $1.3 \pm 0.3 \text{ g}$, unrubbed $1.1 \pm 0.2 \text{ g}$, $P > 0.1$). In these experiments the drugs were administered cumulatively. Thus, although palmitoyl carnitine resembled Bay K 8644 in its ability to contract the aorta, the effects were unchanged in the presence of the endothelium. Palmitoyl carnitine in concentrations up to 1 mM did not contract the aorta directly in non-depolarizing conditions (data not shown, $n > 12$) and did not significantly modify submaximal contractions to phenylephrine ($n = 6$, Figure 2).

Bay K 8644 ($1 \mu\text{M}$ for 20 min) did not significantly affect the relaxant effects of acetylcholine ($1 \mu\text{M}$) in tissues contracted by phenylephrine ($1 \mu\text{M}$), in that acetylcholine relaxed the tissues by $54 \pm 10\%$ in the presence of Bay K 8644 and by $59 \pm 9\%$ in control conditions ($n = 5$). In contrast, palmitoyl carnitine ($100 \mu\text{M}$ for 20 min) reduced acetylcholine-induced relaxations from $68 \pm 10\%$ to $3 \pm 3\%$ ($n = 5$). Palmitoyl carnitine ($10 \mu\text{M}$) caused an immediate reversal of acetylcholine-induced relaxations (Figure 2), without affecting tone in rubbed preparations. The effects of palmitoyl carnitine did not washout immediately (Figure 2) and were not mimicked by palmitic acid ($10 \mu\text{M}$, Figure 2) or L-carnitine ($10 \mu\text{M}$; data not shown).

Because palmitoyl carnitine has surface-active properties we assessed whether the endothelium was being damaged by incubation with high concentrations of the substance. Prolonged wash out (60–120 min) fully reversed the inhibitory effects of palmitoyl carnitine ($30 \mu\text{M}$) in three experiments, indicating that the endothelial cells were not destroyed by the procedure. This washout period did not significantly modify the relaxant effects of acetylcholine, in that three successive cumulative concentration-response curves were unchanged (concentration ratio < 2 , $n = 28$).

The concentration-dependency of the ability of palmitoyl carnitine to reverse carbachol-induced relaxations is shown in

Figure 2 Effects of acetylcholine (●, ACh, $1 \mu\text{M}$) on phenylephrine (▲, PE, $1 \mu\text{M}$)-induced contractions of rat aorta rings in the absence (two upper preparations) or presence (two lower preparations) of endothelium. There is a 20 min time interval between left and right panels during which period the preparations were washed four times. Palmitoyl carnitine (Δ, PC, $10 \mu\text{M}$) partially reversed the effects of acetylcholine (lower panel) but had no effect in the absence of endothelium. The effects of palmitoyl carnitine were not immediately reversed by washing because acetylcholine-induced relaxation of a subsequent contraction to phenylephrine was also attenuated (lower right panel) although further challenge with acetylcholine ($\pm 10 \mu\text{M}$) relaxed the tissue fully. Palmitic acid (◇, PA, $10 \mu\text{M}$) did not affect acetylcholine-induced relaxations. This experiment is a representative example of 4 similar experiments.

Figure 3 Concentration-dependent reversal by palmitoyl carnitine of the relaxant effects of carbachol ($1 \mu\text{M}$) in rat aorta precontracted by phenylephrine ($1 \mu\text{M}$). Palmitoyl carnitine was added in increasing concentrations at 5 min intervals (●); the spontaneous recontraction in parallel control preparations is also shown (○). The initial phenylephrine contraction was $1.5 \pm 0.3 \text{ g}$. Vertical bars represent s.e.mean, $n = 5-6$.

Figure 3. In these experiments palmitoyl carnitine was added cumulatively to the organ baths when the carbachol-induced relaxation was maximal; the threshold for the effect was $0.3 \mu\text{M}$ and the EC_{50} $1.1 \mu\text{M}$. With a different protocol, the effects of pretreatment with palmitoyl carnitine ($10-100 \mu\text{M}$ for 20 min) on cumulative concentration-response curves to acetylcholine were obtained (Figure 4); the antagonism was non-competitive.

Adenosine 5'-triphosphate (ATP) and the Ca^{2+} ionophore A23187 relax vascular smooth muscle by an endothelium-dependent mechanism. Relaxant responses to ATP and A-23187 were antagonized by palmitoyl carnitine (Figure 4).

Figure 4 (a) The effect of palmitoyl carnitine (Δ , 10 ; $\blacktriangle$, 30 ; $\square$, $100 \mu\text{M}$) on acetylcholine (ACh)-induced relaxations of rat aortic rings precontracted with phenylephrine ($1 \mu\text{M}$). (b) The effect of palmitoyl carnitine ($\bullet$, 3 ; Δ , 10 ; $\blacktriangle$, $30 \mu\text{M}$) on ATP-induced relaxations of rat aortic rings precontracted with phenylephrine ($1 \mu\text{M}$). (c) The effect of palmitoyl carnitine ($\bullet$, 3 ; Δ , 10 ; $\blacktriangle$, $30 \mu\text{M}$) on A23187-induced relaxations of rat aortic rings precontracted with phenylephrine ($1 \mu\text{M}$). (d) The effect of palmitoyl carnitine ($\blacktriangle$, $30 \mu\text{M}$) on sodium nitroprusside-induced relaxations of rat aortic rings precontracted with phenylephrine ($1 \mu\text{M}$). All relaxations (○, control) are expressed as % of the phenylephrine-induced contraction. Vertical bars represent s.e.mean, $n = 4-6$.

In contrast, the relaxant responses to sodium nitroprusside, a vasodilator with direct effects on vascular smooth muscle cells, were unaffected by palmitoyl carnitine ($30 \mu\text{M}$ for 20 min; Figure 4). Thus, although palmitoyl carnitine causes a non-specific antagonism of the effects of a variety of agents which stimulate EDRF release, it does not appear to affect relaxant processes in smooth muscle cells.

Superfusion experiments

A simple bioassay of EDRF release from rat aorta was developed (Figure 5). Rubbed rat aorta preparations were used to bioassay EDRF released from the endothelial cells of an aortic preparation placed above them. The assay preparations, once stabilized, were used to test a series of endothelial preparations; in 20 preparations, from 10 rats, phenylephrine-induced contractions were relaxed by $41.8 \pm 3.7\%$. The relaxation was rapidly reversed by lifting off the 'donor' preparation (Figure 5). There was no relaxation if the 'donor' preparations were rubbed of endothelium (4 preparations from 2 rats). Pretreatment of the 'donor' preparations with palmitoyl carnitine ($30 \mu\text{M}$ for 20 min, 12 preparations from 6 rats) reduced EDRF-induced relaxation to $11.7 \pm 2.9\%$, in comparison with a 35.7 ± 5.3 relaxation observed in parallel control preparations (10 preparations from 6 rats; $P < 0.005$). Thus pretreatment with palmitoyl carnitine inhibited EDRF release. The assay preparations did not lose their sensitivity to EDRF, compared to untreated preparations, if they had previously been perfused with palmitoyl carnitine ($30 \mu\text{M}$ for 20 min; $35.3 \pm 8.7\%$ relaxation; 4 preparations from 2 rats), indicating that the site of action of palmitoyl carnitine was at the endothelial cell.

In static isolated organ bath experiments palmitoyl carnitine occasionally caused relaxations when added directly to preparations with endothelium, but not in preparations devoid of endothelium. However, these effects were not consistently reproducible. We therefore tested the effects of palmitoyl carnitine in superfused ring preparations. The preparations responded with marked relaxations to doses of carbachol added to the superfusate. Palmitoyl carnitine

Figure 5 (a) Diagram of the superfusion apparatus used to ascertain effects on endothelial cells. Contractile responses were obtained from the lower rubbed strip, in the presence or absence of a strip of aorta with the endothelium located over the rubbed intimal surface of the lower preparation. (b) Effects of inclusion of phenylephrine (▲, $0.1 \mu\text{M}$) and carbachol (●, $10 \mu\text{M}$) in the superfusion solution; at (△) the donor strip was lifted from the lower rubbed strip, which subsequently recontracted to the level prior to addition of carbachol.

caused dose-dependent relaxations of phenylephrine-induced contractions under these conditions, although the relaxations were smaller than those induced by carbachol (Figure 6). In these experiments palmitoyl carnitine was added in a single dose and the effect allowed to wane before the next administration of compound. Subsequent carbachol-induced relaxations were attenuated after exposure to the last dose of palmitoyl carnitine, indicating that the compound caused a certain degree of release of EDRF before preventing further release; responses to carbachol did not change with time in 4 control experiments.

Figure 6 Relaxant effects of carbachol (CCh, $0.3 \mu\text{mol}$) and of single doses of palmitoyl carnitine (0.1 , 0.3 , 1 , $3 \mu\text{mol}$) on rat superfused aorta preparations, with intact endothelium. Immediately after the relaxant effects of palmitoyl carnitine had washed out, the preparations were rechallenged with carbachol ($0.3 \mu\text{mol}$), and the relaxant effects were significantly attenuated ($P < 0.005$) compared with the initial response to carbachol. All drugs were added to the superfusate as a bolus. Vertical bars represent s.e.mean of the % maximum relaxation of the phenylephrine-induced contraction, $n = 6$.

Figure 7 Relaxant effects of carbachol (CCh, $0.3 \mu\text{mol}$) and of lysophosphatidyl choline (0.1 , 0.3 , 1 , $3 \mu\text{mol}$) on rat superfused aorta preparations, with intact endothelium. Immediately after the relaxant effects of lysophosphatidyl choline had washed out, the preparations were rechallenged with carbachol ($0.3 \mu\text{mol}$), and the relaxant effects were significantly attenuated ($P < 0.005$) compared with the initial response to carbachol. Drugs were added to the superfusate as a bolus. Vertical bars represent s.e.mean of % maximum relaxation of the phenylephrine-induced contraction, $n = 6$.

Lysophosphatidyl choline, another amphiphile which accumulates during ischaemia, has also been shown to release EDRF and claimed to be an endogenous factor modifying release (Saito *et al.*, 1988). Lysophosphatidyl choline resembled palmitoyl carnitine in its ability to relax phenylephrine-induced contractions and subsequently to attenuate the effects of carbachol (Figure 7).

Discussion

Endothelial cells appear to exert a tonic inhibition of vascular smooth muscle by release of EDRF, which may be nitric oxide (Furchgott & Zawadzki, 1980; Gryglewski *et al.*, 1986; Rubanyi & Vanhoutte, 1986; Palmer *et al.*, 1987). Endothelial cells also metabolise and transport lipids. In addition, endothelial damage may be a key factor in atherogenesis and other circulatory disorders and is regularly seen after ischaemic episodes (Heber *et al.*, 1987). However, most pharmacological experiments investigating the role of EDRF have been conducted in physiological salt solutions without exogenous lipids. The finding that palmitoyl carnitine may inhibit the release of EDRF at concentrations which are similar to those found to occur in ischaemic myocardial cells (Knabb *et al.*, 1986) may therefore be of importance, especially as acyl carnitines will be critical for mitochondrial lipid oxidation in endothelial cells (Hulsmann *et al.*, 1988).

Palmitoyl carnitine resembled Bay K 8644 in causing contractions of the aorta in the presence of a depolarizing stimulus. However, unlike Bay K 8644, the maximal contractile effects of palmitoyl carnitine were not attenuated by the presence of the endothelium. This is presumably because palmitoyl carnitine inhibits EDRF release, thereby inhibiting the influence of the endothelium. In the case of Bay K 8644, baseline levels of guanosine 3':5'-cyclic monophosphate (cyclic GMP) in the aorta are increased by spontaneously released EDRF, which can reduce the contractile effects of Bay K 8644 (Spedding *et al.*, 1986). Only high ($> 10 \mu\text{M}$) concentrations of palmitoyl carnitine caused a direct contraction of the aorta, which was evident only under depolarizing conditions (data not shown). These direct effects on the smooth muscle cells occurred at higher concentrations than those on the endothelium (threshold $0.3 \mu\text{M}$).

The site of action of palmitoyl carnitine was clearly dependent on the endothelial cells, because the superfusion experiments indicated that the responses of smooth muscle to EDRF were unaffected. Furthermore, the effects of sodium

nitroprusside, which, like EDRF, relaxes smooth muscle by a cyclic GMP-dependent mechanism, were not modified by palmitoyl carnitine. This amphiphilic molecule did not cause irreversible endothelial disruption under the present experimental conditions, although such amphiphiles may be expected to modify membrane characteristics, and ultimately membrane permeability, in concentrations similar to those used in this study (Busselen *et al.*, 1988).

Palmitoyl carnitine inhibited EDRF release induced by both muscarinic agonists and ATP, indicating that the site of action was not as a receptor antagonist at the endothelial cell. Thus, responses to the calcium ionophore A23187 were also inhibited, although responses to ATP and A23187 were slightly more resistant to palmitoyl carnitine than were those to acetylcholine. Palmitoyl carnitine, at the concentrations used in the present study, does inhibit receptor activation in gastrointestinal smooth muscle (Bigaud & Spedding, 1986; Spedding *et al.*, unpublished observations) and receptor-mediated EDRF release is associated with increased levels of inositol 1,4,5-trisphosphate (Loeb *et al.*, 1988). The site of action of palmitoyl carnitine remains to be defined.

In this paper we have concentrated on inhibition of release of EDRF. However, Criddle *et al.* (1987) showed that palmitoyl carnitine caused relaxation of rat aorta by an endothelial-dependent mechanism. In our hands, this aspect appeared less marked than the subsequent impairment of endothelium-dependent relaxations. Interestingly, the relaxation observed by Criddle *et al.* (1987) may be due to the formation of an ethyl carnitate ester which is selectively vasodilator (B. Woodward, personal communication). Such an ester may be important if it occurs physiologically. In our experiments, relaxant effects to palmitoyl carnitine occurred only in superfusion

experiments. It is not known whether metabolism of palmitoyl carnitine would be different under the two experimental procedures, but the relaxant effects were only apparent following administration of bolus doses in the superfusate. The final effect of palmitoyl carnitine in a given tissue, i.e. increased or decreased release of EDRF, will presumably be dependent on the tissues involved and the degree of ongoing receptor activation. This is being investigated currently.

Lysophosphatidyl choline, which also accumulates during ischaemia (Corr *et al.*, 1981; 1982; 1984) following activation of phospholipases, resembled palmitoyl carnitine in its ability to relax phenylephrine-induced contractions of rat aorta and to block the subsequent effects of carbachol. This finding indicates that, even if the membrane is not irreversibly disrupted, the effects of both palmitoyl carnitine and lysophosphatidyl choline may be secondary to changes in membrane fluidity, as membrane fluidity increases following incorporation of the amphiphiles in sarcolemmal membranes (Fink & Gross, 1984). Such an effect may explain the rather non-specific inhibition of EDRF release following a number of different stimuli. It remains to be seen whether these agents modulate the inhibition of EDRF release which follows inhibition of oxidative phosphorylation (Griffith *et al.*, 1986), but both palmitoyl carnitine and lysophosphatidyl choline can inhibit EDRF release under our experimental conditions. As endothelial cells metabolise lipids to a considerable extent, then acyl carnitines may be a critical factor in endothelial mitochondrial function. Our data indicate that they may be expected to exert a variety of effects on endothelial function as they accumulate during ischaemia. Further studies are required to define the extent of accumulation and consequent effects in ischaemia.

References

- BIGAUD, M. & SPEDDING, M. (1986). Inhibition of the effects of endothelial-derived relaxant factor (EDRF) in aorta by palmitoyl carnitine. *Br. J. Pharmacol.*, **89**, 540P.
- BUSSELEN, P., SERCU, D. & VERDONCK, F. (1988). Exogenous palmitoyl carnitine and membrane damage in rat hearts. *J. Mol. Cell. Cardiol.*, **20**, 905–916.
- CORR, P.B., SNYDER, D.W., CAIN, M.E., CRAFTFORD JR, W.A., GROSS, R.W. & SOBEL, B.E. (1981). Electrophysiological effects of amphiphiles on canine Purkinje fibers. Implications for dysrhythmia secondary to ischaemia. *Circ. Res.*, **49**, 354–363.
- CORR, P.B., SNYDER, D.W., LEE, B.I., GROSS, R.W., KEIM, C.R. & SOBEL, B.E. (1982). Pathophysiological concentrations of lysophosphatides and the slow response. *Am. J. Physiol.*, **243**, H187–H195.
- CORR, P.B., GROSS, R.W. & SOBEL, B.E. (1984). Amphipathic metabolites and membrane dysfunction in ischemic myocardium. *Circ. Res.*, **55**, 135–154.
- CRIDDLE, D.N., HIGGINS, A.J. & WOODWARD, B. (1987). Effects of palmitoyl carnitine on the isolated rat heart and thoracic aorta. *J. Physiol.*, **391**, 47P.
- DAINTY, I.A., McGRATH, J.C. & SPEDDING, M. (1987). The effect of palmitoyl carnitine on endothelial-dependent relaxations of rat aorta. *Blood Vessels*, **24**, 203.
- DUNCAN, G.P., PATMORE, L. & SPEDDING, M. (1986). Positive inotropic effects of palmitoyl carnitine on embryonic chick heart cell aggregates. *Br. J. Pharmacol.*, **89**, 757P.
- DUNCAN, G.P., PATMORE, L. & SPEDDING, M. (1987). Selective antagonism of the effects of Bay K 8644 and palmitoyl carnitine by calcium-antagonists. *Br. J. Pharmacol.*, **92**, 554P.
- FINK, K.L. & GROSS, R.W. (1984). Modulation of canine myocardial sarcolemmal membrane fluidity by amphiphilic compounds. *Circ. Res.*, **55**, 585–594.
- FURCHGOTT, R.F. (1988). Studies on relaxation of rabbit aorta by sodium nitrite: the basis for the proposal that the acid-activatable inhibitory factor from the bovine retractor penis is inorganic nitrite and the endothelium-derived relaxing factor is nitric oxide. In *Mechanisms of Vasodilatation*. ed. Vanhoutte, P.M. New York: Raven Press.
- FURCHGOTT, R.F. & ZAWADZKI, J.V. (1980). The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. *Nature*, **288**, 373–376.
- GRIFFITH, T.M., EDWARDS, D.H., NEWBY, A.C., LEWIS, M.J. & HENDERSON, A.H. (1986). Production of endothelium derived relaxant factor is dependent on oxidative phosphorylation and extracellular calcium. *Cardiovasc. Res.*, **20**, 7–12.
- GRYGLEWSKI, R.J., PALMER, R.M.J. & MONCADA, S. (1986). Superoxide anion is involved in the breakdown of endothelium-derived relaxant factor. *Nature*, **320**, 454–456.
- HEBER, D., KOZIOL, B.J. & HENSON, L.C. (1987). Low density lipoprotein receptor regulation and the cellular basis of atherosclerosis: implications for nutritional and pharmacologic treatment of hypercholesterolemia. *Am. J. Cardiol.*, **60**, 4G–8G.
- HULSMANN, W.C., DE WIT, L., DUBELAAR, M.-L. & STAM, H. (1988). The myocardial interstitium; drain of metabolic products from the heart. *Basic Res. Cardiol.*, (in press).
- IDELL-WENGER, J.A., GROTYOHANN, L.W. & NEELY, J.R. (1978). Coenzyme A and carnitine distribution in normal and ischemic hearts. *J. Biol. Chem.*, **253**, 4310–4318.
- INOUE, D. & PAPPANO, A.J. (1983). L-Palmitoylcarnitine and calcium ions act similarly on excitatory ionic currents in avian ventricular muscle. *Circ. Res.*, **52**, 625–634.
- KNABB, M.T., SAFFITZ, J.E., CORR, P.B. & SOBEL, B.E. (1986). The dependence of electrophysiological derangements on accumulation of endogenous long-chain acyl carnitine in hypoxic neonatal rat myocytes. *Circ. Res.*, **58**, 230–240.
- LIEDTKE, A.J., NELLIS, S. & NEELY, J.R. (1978). Effects of excess and free fatty acids on mechanical and metabolic function in normal and ischemic myocardium in swine. *Circ. Res.*, **43**, 652–661.
- LOEB, A.I., IZZO JR., N.J., JOHNSON, R.M., GARRISON, J.C. & PEACH, M.J. (1988). Endothelial-derived relaxant factor release associated with increased endothelial cell inositol trisphosphate and intracellular calcium. *Am. J. Cardiol.*, **62**, 36G–40G.
- NEELY, J.R. & FEUVRAY, D. (1981). Metabolic products and myocardial ischemia. *Am. J. Pathol.*, **102**, 282–291.
- PALMER, R.M.J., FERRIGE, A.J. & MONCADA, A. (1987). Nitric oxide release accounts for the biological activity of endothelium-derived relaxant factor. *Nature*, **327**, 524–526.
- PATMORE, L., DUNCAN, G.P. & SPEDDING, M. (1989). The interaction of palmitoyl carnitine with calcium-antagonists in embryonic chick myocytes. *Br. J. Pharmacol.*, **97**, 443–450.
- RUBANYI, G.M., SCHWARTZ, A. & VANHOUTTE, P.M. (1985). The calcium agonists Bay K 8644 and (+)202,791 stimulate the release

- of endothelial relaxing factor from canine femoral arteries. *Eur. J. Pharmacol.*, **117**, 143–144.
- RUBANYI, G.M. & VANHOUTTE, P.M. (1985). Hypoxia releases a vasoconstrictor substance from the canine vascular endothelium. *J. Physiol.*, **364**, 45–56.
- SAITO, T., WOLF, A., MENON, N.K., SAEED, M. & BING, R.J. (1988). Lysolecithins as endothelium-dependent vascular smooth muscle relaxants that differ from endothelium-derived relaxing factor (nitric oxide). *Proc. Natl. Acad. Sci. U.S.A.*, **85**, 8246–8250.
- SPEDDING, M., ANDERSON, A.J. & PATMORE, L. (1989). Definition of the interaction of acyl carnitines, Bay K 8644 and diphenylalkylamine calcium-antagonists. *Proceedings of the 4th Int. Symposium on Calcium-antagonists, Florence: Giovanni Lorenzini*, p. 207.
- SPEDDING, M. & MIR, A.K. (1987). Direct activation of Ca^{2+} channels by palmitoyl carnitine, a putative and endogenous ligand. *Br. J. Pharmacol.*, **92**, 457–468.
- SPEDDING, M., SCHINI, V., SCHOEFFTER, P. & MILLER, R.C. (1986). Calcium channel activation does not increase release of endothelial-derived relaxant factors (EDRF) in rat aorta though tonic release of EDRF may modulate calcium channel activity in smooth muscle. *J. Cardiovasc. Pharmacol.*, **8**, 1130–1137.
- TAKEDA, K., SCHINI, V. & STOECKEL, H. (1987). Voltage-activated potassium, but not calcium currents in cultured bovine aortic endothelial cells. *Pflügers Arch.*, **410**, 385–393.

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