Electrophysiological effects of acetyl glyceryl ether phosphorylcholine on cardiac tissues: comparison with lysophosphatidylcholine and long chain acyl carnitine

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1   Electrophysiological effects of synthetic platelet activating factor, acetyl glyceryl ether phosphorylcholine (AGEPC), were examined and compared with those of lysophosphatidylcholine (LPC) and long chain acyl carnitine (AC) in canine Purkinje fibres and guinea-pig papillary muscles, by use of standard microelectrode techniques.

2   In canine Purkinje fibres, AGEPC at concentrations higher than 3×10^{-5} M, decreased maximum diastolic potential, action potential amplitude and the maximum upstroke velocity of phase 0. AGEPC also induced abnormal automaticity arising from depolarized membrane potentials.

3   LPC and AC in concentrations higher than 3×10^{-5} M also produced virtually identical electrophysiological alterations in Purkinje fibres.

4   Although twitch tension was slightly decreased by low concentrations (10^{-6}–10^{-5} M) of these amphiphilic lipids, a transient positive inotropic response appeared at the beginning of a progressive depolarization after exposure to higher concentrations of the amphiphiles.

5   In guinea-pig papillary muscles, AGEPC in concentrations higher than 3×10^{-5} M produced slight decreases in resting membrane potential, action potential amplitude and action potential durations, concomitantly with a positive inotropic response. These electrophysiological and mechanical changes were also induced by LPC and AC at comparable concentrations.

6   In guinea-pig papillary muscles depolarized with 25 mM $[K^+]_o$, AGEPC, LPC and AC all evoked slow action potentials at a concentration of 10^{-4} M.

7   It is concluded that in isolated cardiac tissues AGEPC exerts electrophysiological effects similar to those of LPC and AC only at high concentrations, and that the non-specific interaction of amphiphiles with sarcolemmal membrane may be responsible for the electrophysiological and mechanical effects.
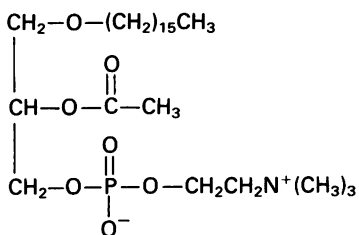
Introduction

In recent years, considerable effort has been directed toward understanding the pathophysiological role of several amphiphiles in various diseases (Pinckard *et al.*, 1982; Corr *et al.*, 1984). Extensive studies by Corr *et al.* (1981, 1984) have shown that lysophosphoglycerides and long chain acyl carnitine, which are reported to accumulate in the ischaemic myocardium (Idell-Wenger *et al.*, 1978; Liedtke *et al.*, 1978; Shaikh & Downar, 1981; Corr *et al.*, 1982), induce electrophysiological disturbances in isolated cardiac tissues. These findings led them to postulate that these amphiphiles may play a role in the genesis of malignant dysrhythmias during myocardial ischaemia. Acetyl glyceryl ether phosphorylcholine (AGEPC, platelet-
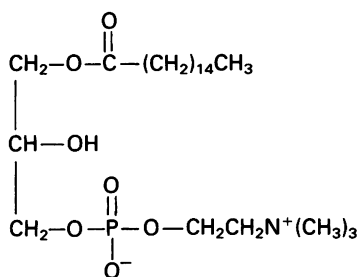
activating factor) is an amphiphilic phospholipid, which has been suggested as an important biochemical mediator of anaphylactic reactions (Halonen *et al.*, 1980; McManus *et al.*, 1980; Pinckard *et al.*, 1982). AGEPC is structurally somewhat similar to lysophosphatidylcholine and long chain acyl carnitine, as shown in Figure 1. In addition to causing platelet aggregation, this phospholipid produces a variety of biological actions in the living system such as neutrophil activation, contraction of bronchial and intestinal smooth muscle and hypotension (Blank *et al.*, 1979; Vargaftig *et al.*, 1980; Findlay *et al.*, 1981; Shaw *et al.*, 1981; Pinckard *et al.*, 1982). Moreover, Levi *et al.* (1984) have recently reported that exogenously administered AGEPC can induce arrhythmias in the guinea-pig isolated heart. Therefore, the purpose of the

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AGEPC (Hexadecyl Paf)

$$CH_2-O-(CH_2)_{15}CH_3$$
$$CH-O-\overset{\overset{O}{\parallel}}{C}-CH_3$$
$$CH_2-O-\overset{\overset{O}{\parallel}}{\underset{\underset{O^-}{\parallel}}{P}}-O-CH_2CH_2N^+(CH_3)_3$$

Palmitoyl lysophosphatidylcholine

$$CH_2-O-\overset{\overset{O}{\parallel}}{C}-(CH_2)_{14}CH_3$$
$$CH-OH$$
$$CH_2-O-\overset{\overset{O}{\parallel}}{\underset{\underset{O^-}{\parallel}}{P}}-O-CH_2CH_2N^+(CH_3)_3$$

Palmitoyl carnitine

$$CH_2-\overset{\overset{O}{\parallel}}{C}-OH$$
$$CH-O-\overset{\overset{O}{\parallel}}{C}-(CH_2)_{14}CH_3$$
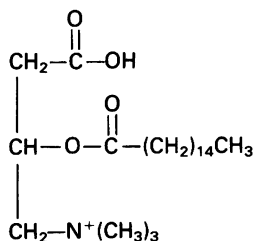$$CH_2-N^+(CH_3)_3$$

Figure 1 Chemical structures of acetyl glyceryl ether phosphorylcholine (hexadecyl Paf), palmitoyl lysophosphatidylcholine and palmitoyl carnitine.

present study was to examine and compare the electrophysiological effects of AGEPC with those of lysophosphoglycerides and long chain acyl carnitine, putative arrhythmogenic amphiphiles, in isolated cardiac tissues.

A preliminary account of this work has appeared in abstract form (Nakaya *et al.*, 1985).

Methods

Tissue preparations and solutions

Hearts were removed from mongrel dogs of either sex, 6–10 kg body weight, anaesthetized with pentobar-

bitone sodium (30 mg kg^{-1}, i.v.), and immersed in a modified Tyrode solution. Free running false tendons (less than 1 mm in diameter and 5–15 mm in length) were carefully dissected from either ventricle in a dish containing oxygenated Tyrode solution. Hearts were also removed from 250–400 g guinea-pigs killed by a blow on the head. The hearts were placed in the dissection bath and papillary muscles less than 1 mm in diameter were obtained from the right ventricle. The cardiac tissues were transferred to a tissue bath of 5 ml volume and superfused at a rate of 10 ml min^{-1} with a modified Tyrode solution, gassed with 95% O$_2$ plus 5% CO$_2$. The modified Tyrode solution had the following composition (in mM): NaCl 125, KCl 4, NaHCO$_3$ 25, NaH$_2$PO$_4$ 1.8, MgCl$_2$ 0.5, CaCl$_2$ 2.7 and glucose 5.5. The bath temperature was kept constant at $36.0 \pm 1.0°C$ for canine Purkinje fibres and at $33.0 \pm 1.0°C$ for guinea-pig papillary muscles. In certain experiments, an elevated K$^+$ solution (25 mM) was prepared by isotonic substitution of NaCl with KCl.

Tension measurements and electrical arrangements

One end of the Purkinje fibre or the papillary muscle was hooked to an extension of the lever arm of a force transducer (Nihon Kohden, TB 651T, Tokyo, Japan) and the other end was pinned to the bottom of the tissue chamber. The transducer was mounted on a micromanipulator and resting tension was progressively increased to 200–400 mg.

The preparations were electrically driven by rectangular pulses of 1 ms duration, delivered through the platinum field electrodes. The stimulus intensity was twice the diastolic threshold and the stimulus frequency was 1 Hz for Purkinje fibres and 0.5 Hz for papillary muscles. The stimuli were delivered from an electronic stimulator (Nihon Kohden SEN 3101) through an isolation unit (Nihon Kohden SS 101J). Transmembrane potentials were recorded with glass microelectrodes filled with 3 M KCl (resistance 10–30 MΩ). The microelectrode was coupled via an Ag/AgCl junction to a high input impedance capacitance-neutralizing amplifier (Nihon Kohden MEZ 8201). An electronic differentiator whose output was linear from 50 to 1000 Vs^{-1} was used for measuring the maximum rate of rise ($\dot{V}_{max}$) of fast action potentials and another differentiator with a linearity from 0 to 50 Vs^{-1} was used for $\dot{V}_{max}$ of slow action potentials. The amplified signals were displayed on an oscilloscope (Nihon Kohden VC-10), photographed on 35 mm film and recorded on a chart recorder (Watanabe Sokki WR-3101, Tokyo, Japan).

Drugs and experimental protocol

Synthetic pure platelet-activating factor (1-α-phos-

phatidylcholine-β-acetyl-γ-0-hexadecyl, AGEPC or Paf), lysophosphatidylcholine (1-α-lysophosphatidyl-choline palmitoyl, LPC) and long chain acyl carnitine (palmitoyl-l-carnitine chloride, AC) were obtained from Sigma Chemical Co. (St. Louis, MO, U.S.A.). These amphiphiles were dissolved in the modified Tyrode solution and diluted with oxygenated solution to achieve the desired concentrations just before use. In certain experiments with guinea-pig papillary muscles, AGEPC was dissolved in modified Tyrode solution containing 0.25% bovine serum albumin (Wako, Osaka, Japan).

After an equilibration period of 2 h, control recordings were made and the preparations were exposed to a solution containing the lowest concentration of an amphiphile. The amphiphile concentration of the superfusion medium was increased stepwise at intervals of 20 min until it reached 10^{-4} M or the maximum diastolic potential became more positive than -60 mV. Only the results of experiments in which a stable impalement was maintained are described here, the others were discarded.

Statistics

All data are presented as mean ± standard errors (± s.e.). Statistical analyses of amphiphile-induced changes in action potential parameters were performed using paired Student's t test. Significance was established when the probability value was less than 0.05.

Results

Electrophysiological effects of amphiphiles on canine Purkinje fibres

Marked alterations in transmembrane action potentials of canine Purkinje fibres were produced by acetyl glyceryl ether phosphorylcholine (AGEPC), lysophosphatidylcholine (LPC) and long-chain acyl carnitine (AC) in concentrations higher than 3×10^{-5} M. Figure 2 shows representative examples of amphiphile-induced action potential changes. Exposure to AGEPC resulted in decreases in the maximum diastolic potential (MDP), action potential amplitude (APA) and the maximum upstroke velocity of phase 0 depolarization ($\dot{V}_{max}$). These action potential changes were very similar to those induced by LPC and AC.

Spontaneous activity arising from reduced membrane potentials (around -50 mV) consistently developed in all fibres exposed to 3×10^{-5}–10^{-4} M AGEPC. An example of spontaneous activity observed after AGEPC 3×10^{-5} M is shown in Figure 2. Such rhythmic automatic depolarizations from depolarized membrane potentials were also induced
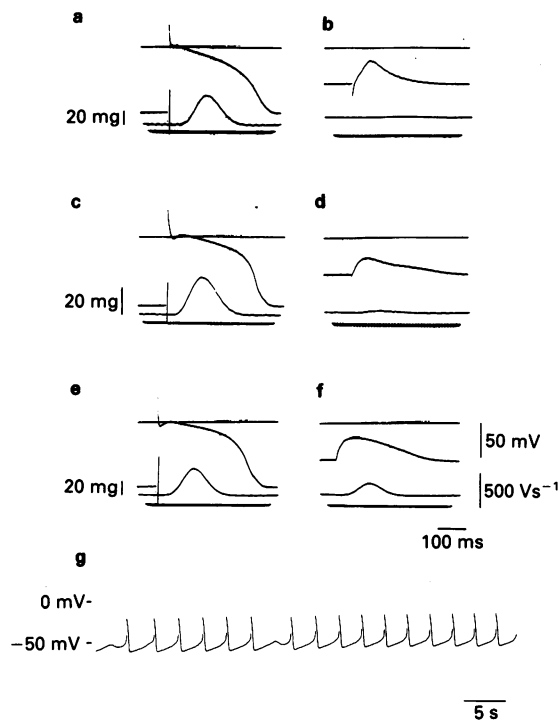
Figure 2 Effects of amphiphiles on transmembrane potentials of canine Purkinje fibres. Effects of acetyl-glyceryl ether phosphorylcholine (AGEPC), lysophosphatidylcholine (LPC) and long chain acyl carnitine (AC) on the action potential and twitch tension are shown in the first, second and third row, respectively. Panels (a), (c) and (e) depict control records in different preparations. Records taken 20 min after 10^{-4} M AGEPC (b), 10^{-4} M LPC (d) and 10^{-4} M AC (f) are shown in the right column. The same cell was impaled in each experiment. In panels (a)–(f) traces from top to bottom represent zero potential, action potential, twitch wave form and dV/dt. Time, voltage and dV/dt calibrations at the bottom of panel (f) apply to panels (a)–(f). Tension calibration is given in left side of each row. Panel (g) demonstrates rhythmic automatic depolarizations from depolarized membrane potentials in a fibre exposed to 3×10^{-5} M AGEPC. Note time and voltage scales are different from other panels.

by LPC and AC in concentrations higher than 3×10^{-5} M.

The changes of action potential parameters induced by these amphiphiles are summarized in Figure 3. During the control period there were no significant differences in any of these parameters between the subgroups (6 fibres in each group) which were exposed to the different amphiphiles. The three amphiphilic compounds produced essentially identical alterations in the action potentials. MDP, $\dot{V}_{max}$ and APA were

concentration-dependently decreased by the amphiphiles and the decreases in these parameters were statistically significant in concentrations of 3×10^{-5} M and 10^{-4} M (Figure 3). In 4 out of 6 fibres exposed to AGEPC, MDP became more positive than -60 mV after 3×10^{-5} M and in the other 2 fibres after 10^{-4} M. In one of 6 fibres exposed to LPC, MDP became more positive than -60 mV after 3×10^{-5} M and in the other 5 fibres after 10^{-4} M. In all the 6 fibres exposed to AC, MDP became more positive than -60 mV after

10^{-4} M. Action potential duration at 50% (APD_{50}) and 90% repolarization (APD_{90}) were evaluated only when MDP remained more negative than -80 mV (Figure 3). These amphiphiles at a concentration of 10^{-5} M or 3×10^{-5} M produced a slight prolongation of APD_{90}, which preceded a progressive depolarization.

Complex inotropic responses were produced by all three amphiphilic compounds. A representative example observed after the superfusion of AGEPC is
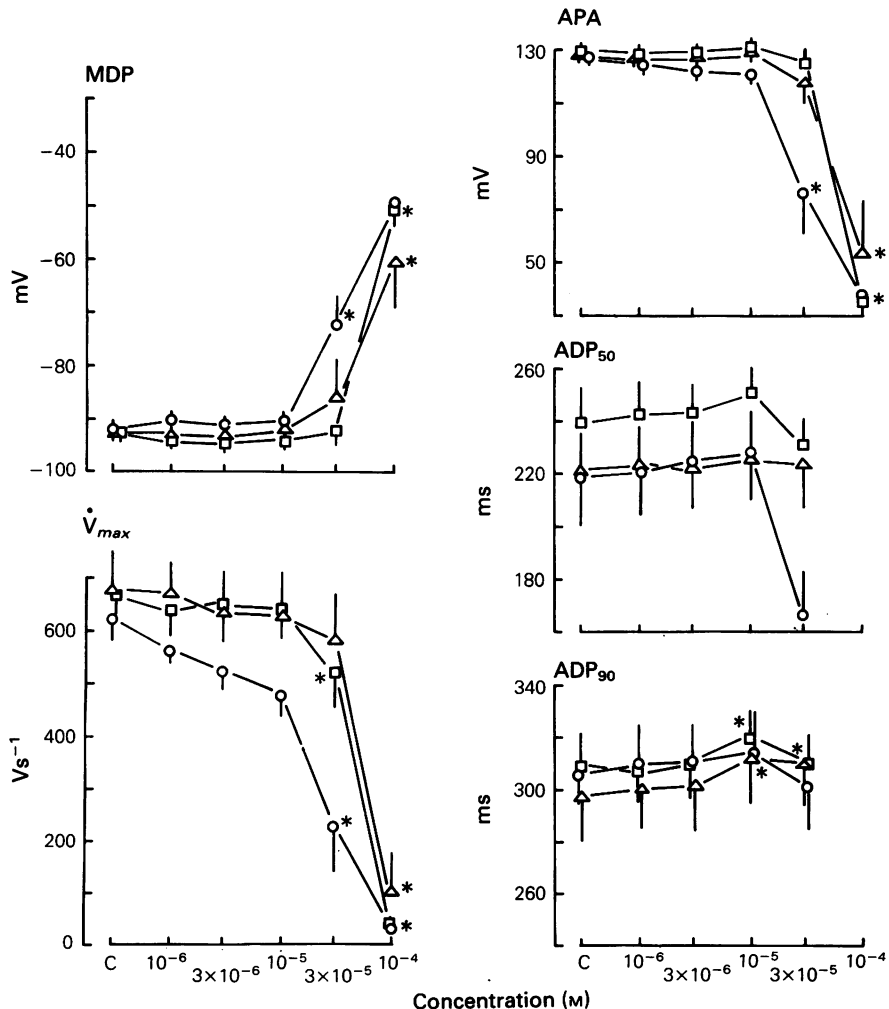

Figure 3 Effects of acetyl glyceryl ether phosphorylcholine (AGEPC, O), lysophosphatidylcholine (LPC, △) and long chain acyl carnitine (AC, □) on action potential parameters of canine Purkinje fibres. Maximum diastolic potential (MDP), maximum upstroke velocity of phase 0 ($\dot{V}_{max}$), action potential amplitude (APA), action potential duration at 50% (APD_{50}) and 90% repolarization (APD_{90}) are indicated on the ordinates. Concentrations of amphiphiles are on the abscissae. C indicates the control value of each parameter. Each point represents the mean of 5-6 fibres except for the data point of 10^{-4} M AGEPC ($n = 2$), and vertical lines show 1 s.e.mean. *Significantly different from the control ($P < 0.05$).

demonstrated in Figure 4. AGEPC at low concentrations $(10^{-6}–10^{-5}\,M)$ produced a slight decrease in twitch tension without any effects on MDP. After the introduction of $3 \times 10^{-5}\,M$ AGEPC a transient positive inotropic response appeared concurrently with a progressive decrease in MDP. Further increase in the concentration to $10^{-4}\,M$ resulted in a marked depolarization, associated with a significant negative inotropic response. At times, an increase in resting tension was also observed. Such an initial slight decrease followed by a transient increase, and then a marked decrease in twitch tension was observed not only in the fibres exposed AGEPC, but also in those exposed to LPC and AC.

Electrophysiological effects of amphiphiles on guinea-pig papillary muscles

Recently Tamargo et al. (1985) reported that low concentrations $(10^{-11}–10^{-7}\,M)$ of AGEPC (Paf) produced increases in resting membrane potential (RMP), APA and $\dot{V}_{max}$, and decreases in APD_{50} and APD_{90} in guinea-pig papillary muscles. They also reported that AGEPC at a concentration of $10^{-10}\,M$ induced slow action potentials in papillary muscles partially depolarized by a high K^+ solution. Accordin-

gly, we examined the electrophysiological effects of these amphiphiles on guinea-pig papillary muscles. In contrast to their report, AGEPC in concentrations of $10^{-11}–10^{-6}\,M$ did not exert any appreciable change of action potential in 4 preparations. In two other experiments AGEPC was dissolved in the modified Tyrode solution containing bovine serum albumin (BSA), as described by Tamargo et al. (1985). However, AGEPC again failed to cause changes in action potential at these concentrations. AGEPC in concentrations higher than $3 \times 10^{-5}\,M$ produced slight decreases in RMP, APA and APDs, and a marked increase in twitch tension, as shown in Figure 5. The positive inotropic response was usually associated with a slight increase in resting tension. LPC and AC also produced similar electrophysiological and inotropic responses only in their high concentrations (Figure 5).

Exposure to the high K^+ (25 mM) solution resulted in decreased RMP around -45 mV, which is sufficient to inactivate the fast Na^+ channels. The stimulus frequency was decreased to 0.1 Hz, and the intensity and the duration of stimuli was increased to 100 V and 3 ms, respectively. These conditions failed to induce slow action potentials despite intense field stimulation. Contrary to the report of Tamargo et al. (1985),
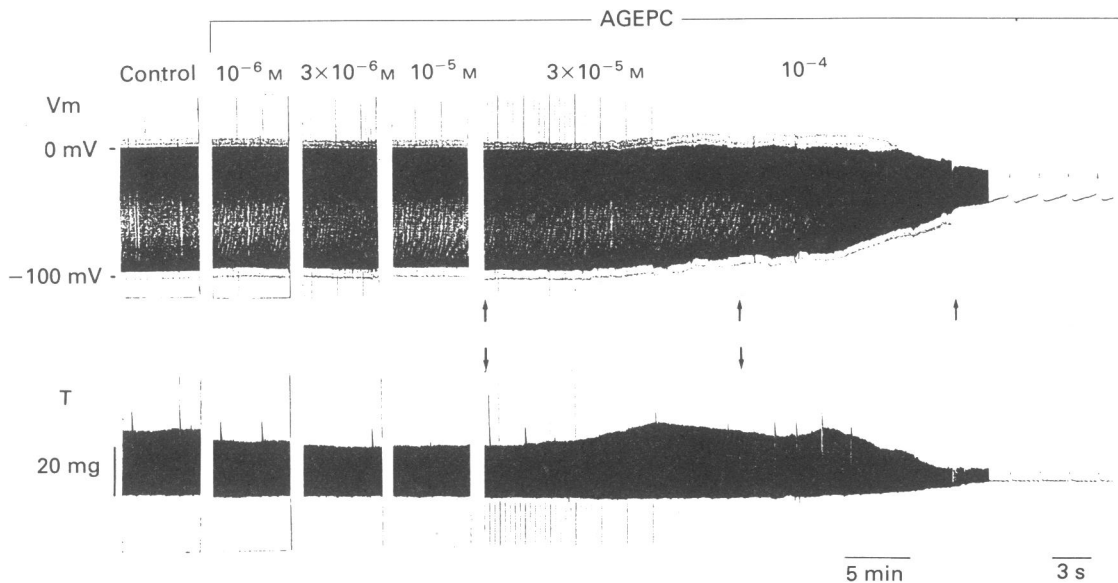

Figure 4 Effects of acetyl glyceryl ether phosphorylcholine (AGEPC) on the electrical and mechanical activities of a canine Purkinje fibre. The upper and lower trace represents membrane potential (Vm) and twitch tension (T), respectively. The first and second arrow indicates the beginning of the superfusion with $3 \times 10^{-5}\,M$ and $10^{-4}\,M$ of AGEPC, respectively. The third arrow indicates the interruption of electrical stimulation to observe the spontaneous activity from depolarized membrane potentials. Note that a slight decrease in twitch tension was observed with low concentrations $(10^{-6}\,M–10^{-5}\,M)$ of AGEPC without any changes of membrane potentials, and that upon the introduction of $3 \times 10^{-5}\,M$ AGEPC a transient positive inotropic response appeared with a gradual depolarization.

AGEPC in concentrations of $10^{-10}-10^{-5}$ M with or without BSA did not evoke slow action potentials in three K^+ -depolarized papillary muscles. AGEPC at the highest concentration (10^{-4} M) induced slow action potentials (accompanied by contractions) within
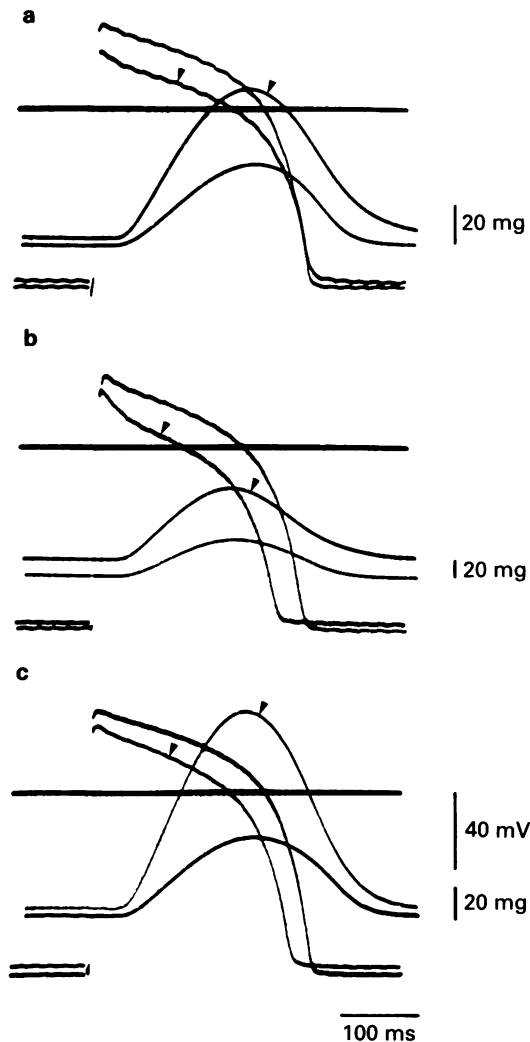
15 min, as shown in Figure 6. After washout of AGEPC the slow action potentials ceased and the addition of the same concentration of LPC and AC also produced slow action potentials.

Figure 5 Effects of acetyl glyceryl ether phosphoryl-choline (AGEPC), lysophosphatidylcholine (LPC) and long chain acyl carnitine (AC) on action potentials and twitch tension of guinea-pig papillary muscles. Records of action potentials and twitch wave form before and after exposure to amphiphiles are superimposed. Arrow heads indicate records taken 20 min after 3×10^{-5} M AGEPC (a), 10^{-4} M LPC (b) and 10^{-4} M AC (c). The same cell was impaled in each experiment. Tracings in each panel from top to bottom indicate zero potential, twitch wave form and action potential. Tension, voltage and time calibrations are given on the right side and below.

Discussion

Consistent with previous reports (Corr et al., 1979; 1981), lysophosphatidylcholine and long chain acyl carnitine caused various electrophysiological derangements, such as decreases in MDP, APA and $\dot{V}_{max}$, and abnormal automaticity arising from depolarized membranes, in canine Purkinje fibres. The present study has revealed that acetyl glyceryl ether phosphorylcholine (AGEPC), which has been implicated as an important mediator of systemic anaphylaxis (Pinckard et al., 1982), induces essentially identical electrophysiological alterations in comparable concentrations. These findings may not be too surprising if we take into account the fact that AGEPC is structurally similar to lysophosphatidylcholine (LPC) or long chain acyl carnitine (AC). It has been reported that AGEPC can exert other biological actions, such as platelet aggregation and neutrophil activation, at low concentrations ($10^{-10}-10^{-5}$ M) (Demopoulos et al., 1979; Shaw et al., 1981; McManus et al., 1981; Pinckard et al., 1982). However, the present study showed that higher concentrations of AGEPC were required to induce the electrophysiological alterations. Therefore, the electrophysiological effects of AGEPC might stem from non-specific amphiphile-membrane interaction rather than from activation of specific Paf receptors. It has been also reported that high concentrations ($10^{-5}-3 \times 10^{-4}$ M) of AGEPC were needed to induce mechanical responses in isolated vascular preparations (Cervoni et al., 1983; Kamitani et al., 1984; Vanhoutte & Houston, 1985). Vanhoutte & Houston (1985) have suggested that the Paf-induced relaxation of the isolated coronary artery of the dog is not receptor-mediated, since the mechanical response was not blocked by a Paf-antagonist.

Recently Levi et al. (1984) have reported that Paf is released into the coronary effluent by antigen challenge to isolated hearts of sensitized guinea-pigs, and that synthetic Paf, i.e., AGEPC, when administered to non-sensitized isolated hearts, produces cardiac dysfunction, mimicking cardiac anaphylaxis, with decreased coronary flow, depression of contractile force and arrhythmias. In their study, exogenously administered AGEPC elicited conduction arrhythmias in the isolated hearts which were perfused not only at constant pressure but also at constant flow, suggesting that arrhythmogenic action of AGEPC might be due to the direct electrophysiological action of AGEPC rather than to myocardial ischaemia secondary to the reduced coronary flow. Since they
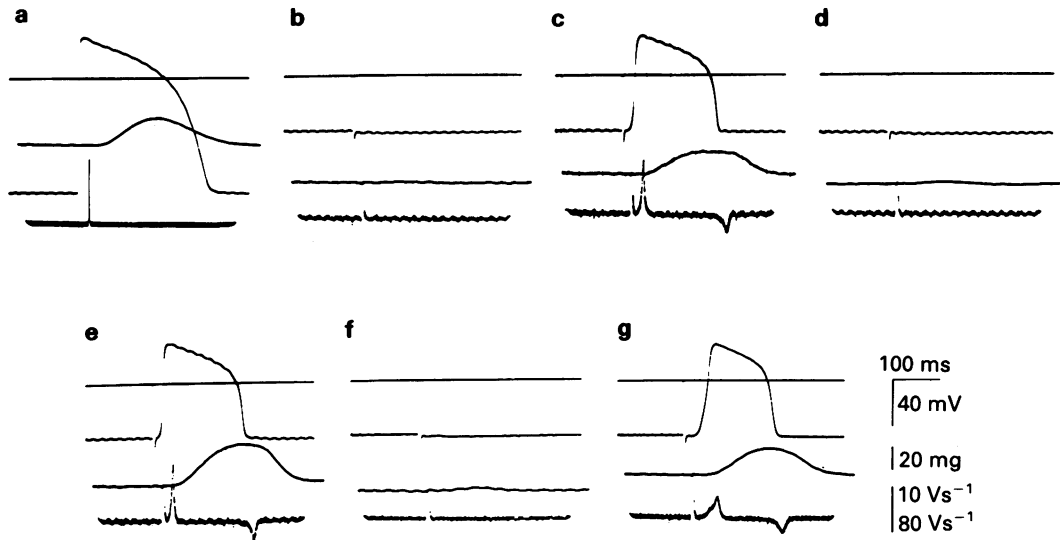
Figure 6 Induction of slow action potentials by acetyl glyceryl ether phosphorylcholine (AGEPC), lysophosphatidyl-choline (LPC) and long chain acyl carnitine (AC) in a K^+-depolarized papillary muscle. (a) Control electrical and mechanical activity in normal Tyrode solution; (b) 30 min after high K^+ (25 mM) solution; (c) 15 min after 10^{-4} M AGEPC; (d) 30 min after washout of AGEPC; (e) 9 min after 10^{-4} M LPC; (f) 50 min after washout of LPC and (g) 12 min after 10^{-4} M AC are shown. All electrical records are taken from the same cell. In panel (a), from top to bottom, zero potential, twitch wave form, transmembrane potential and dV/dt are presented. In other panels traces from top to bottom represent zero potential, transmembrane potential, twitch wave form and dV/dt. The dV/dt calibration for the fast action potential (80 V s^{-1}) is different from that for the slow acton potentials (10 V s^{-1}).

gave a bolus intra-aortic injection of AGEPC in doses of 10^{-9}–10^{-8} mol, the concentrations of AGEPC in the perfusate might reach approximately 10^{-5} M. Therefore, the direct electrophysiological effects of AGEPC, observed in the present study, might play a role in the genesis of arrhythmias in isolated hearts. However, it is presumptuous to conclude that AGEPC can also be arrhythmogenic *in vivo*, because the concentrations of AGEPC required to exert electrophysiological effects are much higher than those to cause platelet aggregation, neutrophil activation and hypotension (Blank *et al.*, 1979; Shaw *et al.*, 1981; McManus *et al.*, 1981; Pinckard, 1982).

As far as we are aware, there has been only one study which examined the electrophysiological effects of AGEPC in cardiac tissues (Tamargo *et al.*, 1985). They reported that AGEPC in concentrations of 10^{-10}–10^{-7} M increased APA and $\dot{V}_{max}$, shortened APD and hyperpolarized RMP in guinea-pig papillary muscles. They also reported that 10^{-10} M AGEPC induced slow action potentials in papillary muscles depolarized by a high K^+ solution. However, in the present study, AGEPC in the concentrations mentioned above failed to produce changes in the fast action potentials and to induce slow action potentials in guinea-pig papillary muscles. The source of dis-

crepancies between our and their studies is not known. As observed in Purkinje fibres, AGEPC as well as LPC and AC in concentrations higher than 3×10^{-5} M caused decreases in RMP and APA and a positive inotropic response. The mechanical and electrophysiological alterations with LPC and AC are in line with the previous studies on feline and avian ventricular muscles (Snyder *et al.*, 1981; Clarkson & Ten Eick, 1983; Inoue & Pappano, 1983). LPC at a concentration of 2×10^{-4} M reportedly induces slow responses in canine Purkinje fibres (Corr *et al.*, 1982). In this study, not only LPC but also AGEPC and AC evoked slow action potentials in the K^+-depolarized papillary muscles only at high concentrations (10^{-4} M). Whether the induction of the slow action potentials is due to enhancement of the slow inward current or to inhibition of the K^+ outward current cannot be determined from the present study.

As concerns the inotropic effects of AGEPC, apparently conflicting results have been reported. Levi *et al.* (1984) demonstrated that AGEPC in concentrations of 10^{-10}–10^{-5} M produced a slight negative inotropic effect in guinea-pig isolated left atria and papillary muscles. On the contrary, a positive inotropic effect of AGEPC at a concentration of 10^{-4} M in rat atria was reported from two laboratories

(Cervoni *et al.*, 1983; Kamitani *et al.*, 1984). In the light of the present observations, however, these reports may not be contradictory. In Purkinje fibres AGEPC produced a small negative inotropic response at low concentrations and a positive one at high concentrations. Such a transient positive inotropic response following a negative one was also produced by LPC and AC. Therefore, the inotropic effect of AGEPC, especially the positive one, might stem from a non-specific action of the amphiphilic compound. In this context, it is noteworthy that amphiphiles can change sarcolemmal Ca^{2+} binding and contractility (Philipson *et al.*, 1985). It has been reported that AC affects action potentials and contractility of avian ventricular muscles in a similar way to elevated extracellular Ca^{2+} (Inoue & Pappano, 1983), and that LPC causes intracellular Ca^{2+} accumulation in cardiac myocytes (Sedlis *et al.*, 1983).

Recently it has been demonstrated that three amphiphilic moieties, viz. LPC, AC and AGEPC, increase myocardial sarcolemmal membrane fluidity when similar amounts of the amphiphiles were incorporated into the sarcolemma (Fink & Gross, 1984). Accordingly, the changes of membrane fluidity as well as changes of surface Ca^{2+} binding might contribute to the electrophysiological and mechanical alterations observed in the present study. Proof or disproof of this hypothesis awaits further experimentation.

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