that spermine and Ca^{2+} effects are not additive. This suggests that spermine may act as a substitute for Ca^{2+} at high concentrations.

Two amino acids, arginine and ornithine, known to be the precursors of PA in plant cells[2], were also assayed. In the absence of Ca^{2+} they were without effect on peroxidase secretion. In the presence of Ca^{2+} (fig. 2), the general shape of the curve obtained with increasing concentrations of these 2 amino acids resembles that obtained with PA. The inhibition by arginine is about 50%. Ornithine, which is the most effective substance assayed in these experiments, reduces the effect of Ca^{2+} by more than 80%. It is not yet known whether the amino acids directly reduce the Ca^{2+}-mediated secretion of peroxidase or are previously transformed by cells into an active form. A kinetic study, which shows that both PA and amino acids are already active a few minutes after their addition (data not shown) suggests that the amino acids act directly on secretion without being metabolized.

It has already been shown with the same material that auxins have a short-term activating effect on the peroxidase secretion[9]. As a relationship between auxins and the level of PA in plant tissues has been reported[11], it may be envisaged that auxins control this secretory process by modifying the endogenous level of PA. The recent demonstration of arginine decarboxylase activity induction and PA accumulation by low pH[12] is in favor or this assessment. The mechanism of the effect of the interaction of PA with Ca^{2+} on enzyme secretion is not known, but it could be related to the functions of these compounds in the stabilization of membranes[2]. An electrostatic binding of the amine groups with the membrane phospholipids is likely, and this would be analogous to the electrostatic interaction found with the phosphate residues of nucleic acids[2].

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Effect of platelet activating factor on guinea-pig papillary muscle

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Summary. Platelet activating factor (PAF) induces a biphasic effect on guinea-pig papillary muscle: 1. a transient positive inotropic effect preceded by an increase in action potential duration (APD); 2. a marked negative effect on inotropism and on APD. Since Ca^{++} slow action potentials were initially enhanced by PAF and then markedly depressed, it is suggested that PAF specifically interferes with the Ca^{++} slow channel.

The platelet activating factor (PAF), a polar lipid mediator of inflammation was originally described as being released from basophils sensitized with Immune globulin E (IgE) after challenge with the specific antigen[2]. PAF has been recently defined as an acetyl glyceryl ether phosphorylcholine[3,4]. Subsequent in vitro studies have shown that besides basophils, polymorphonucleonuclear neutrophils (PMNs)[5], monocytes[5], macrophages[6], platelets[7] and endothelial cells[8] are capable of releasing PAF under immunologic and non-immunologic stimuli. An intravascular release of PAF has been documented not only during anaphylactic shock[9] but also in experimentally-induced immunocomplex pathology[10].

PAF, initially described as a potent platelet activator[2], is now known to possess a broad spectrum of biological activities, such as PMN aggregation, PMN and monocyte chemotaxis, stimulation of oxygen radical generation and lysosomal enzyme release (for review see Pinckard[11]). Recently an in vitro direct receptor-mediated spasmogenic effect on smooth muscle has been demonstrated[12].

The i.v. infusion of synthetic PAF not only induces a marked thrombocytopenia and neutropenia, but also reproduces the cardiovascular and respiratory alterations associated with the anaphylactoid reaction[13]. The mechanisms of PAF-induced cardiovascular alterations are as yet unknown. However, Burke et al.[14] recently demonstrated that PAF exerts a direct negative inotropic effect on isolated, perfused guinea-pig heart. The aim of the present report was to evaluate the effect of PAF on the electrical and mechanical activities of guinea-pig papillary muscle.

Materials and methods. Small papillary muscles isolated from the left ventricles of guinea-pigs (300–500 g, male) were placed in a small perspex chamber and perfused at 30 °C with gassed (95% O_2 and 5% CO_2) standard Tyrode solution (Na^+ 144.42 mM; K^+ 4 mM; Ca^{++} 2 mM; Mg^{++} 1.15 mM; Cl^- 141.25 mM; $H_2PO_4^-$ 0.42 mM; HCO_3^- 11.9 mM; D-glucose 5.6 mM; pH = 7.40). In order to study the Ca^{++} slow action potential, a K^+-enriched (K^+ = 22 mM) solution of identical composition was used.

Stimulation rate was 60 pulses/min in standard Tyrode solution and 10 pulses/min in K^+-enriched solution. Mechanical and electrical activities were evaluated respectively by an RCA transducer tube 5734 and a floating glass microelectrode as

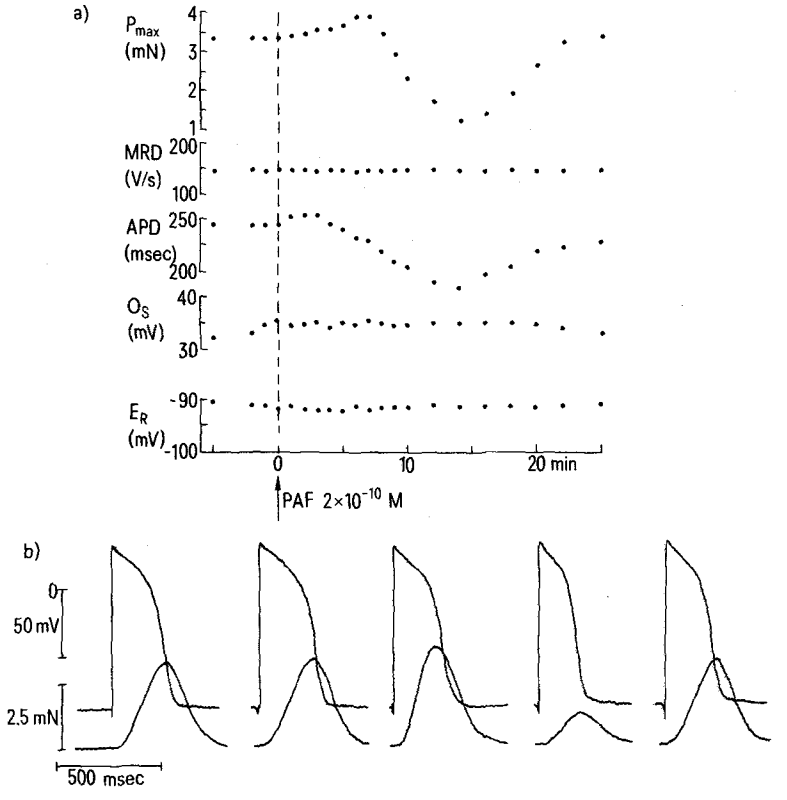

Figure 1. Typical mechanical and electrical alterations induced by PAF (protocol 2, 1 ml of Tyrode solution containing 2×10^{-10} M of PAF instilled in flowing solution) in guinea-pig papillary muscle (standard Tyrode solution). *a* P_{max}, mechanical tension; MRD, maximum rate of depolarization; O_s, overshoot; APD, action potential duration; E_R, resting membrane potential. Action potential from a persistent impalement. *b* Action potentials and mechanograms respectively 2 min before and 2,6,14 and 25 min after PAF challenge.

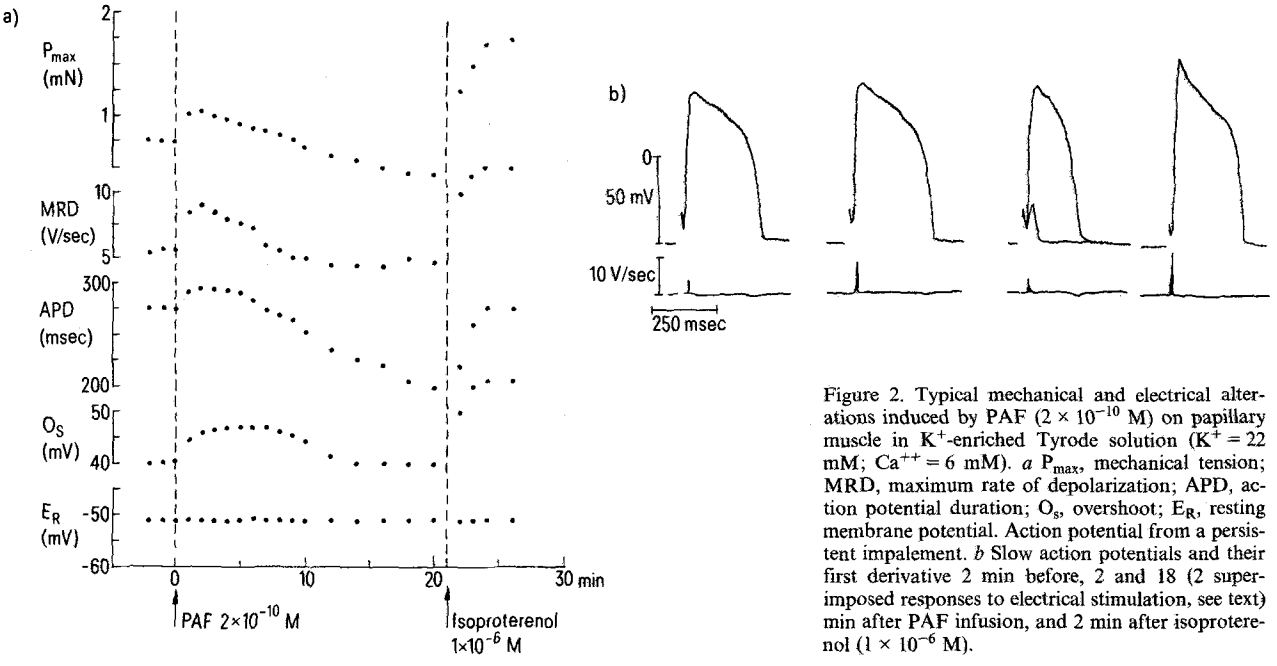

Figure 2. Typical mechanical and electrical alterations induced by PAF (2×10^{-10} M) on papillary muscle in K^+-enriched Tyrode solution ($K^+ = 22$ mM; $Ca^{++} = 6$ mM). *a* P_{max}, mechanical tension; MRD, maximum rate of depolarization; APD, action potential duration; O_s, overshoot; E_R, resting membrane potential. Action potential from a persistent impalement. *b* Slow action potentials and their first derivative 2 min before, 2 and 18 (2 superimposed responses to electrical stimulation, see text) min after PAF infusion, and 2 min after isoproterenol (1×10^{-6} M).

detailed in a previous publication[15]. In K^+-enriched Tyrode solution, impalements were performed at a distance from stimulating electrodes sufficient to maintain the separation of the rising phase of the action potential from the stimulus.

The 1-O-octadecyl-2 acetyl-sn-glyceryl-3 phosphoryl-choline (synthetic PAF) (Bachem, Bubendorf, Switzerland), further pufiried by TLC on silica gel plates (60 F_{254}, Merck, Darmstadt, FRG) exhibited: 1. an R_f of 0.22 using chloroform-methanol-water (65:35:6, v/v) (IO); 2. a minimal effective dose of 5×10^{-11} M on washed rabbit platelet aggregation in the presence of indomethacin (1×10^{-5} M) (Sigma, St. Louis, USA) and creatine phosphate (31.25 µg/ml, Sigma) and creatine phosphokinase (15.25 µg/ml, Sigma).

The 2-lyso derivative of PAF (1-O-octadecyl-sn-glyceryl 3 phosphorylcholine), inactive on washed rabbit platelets, was obtained from synthetic PAF after alkaline methanolysis or

phospholipase A_2 treatment[16]. In selected experiments, the following drugs were used: propranolol (2×10^{-7} M) (Sigma) and isoproterenol (1×10^{-6} M) (Sigma).

The effects of PAF on papillary muscle were studied by 3 different experimental protocols. Papillary muscles were challenged 1. in standard Tyrode solution with continous infusion of PAF (from 1×10^{-10} M to 5×10^{-10} M) for 15 minutes; 2×10^{-10} M of PAF was selected as the optimal concentration, i.e. the minimal concentration giving maximal biological effect (protocol 1); 2. in standard Tyrode solution with a single dose of PAF (1 ml of Tyrode solution containing from 4×10^{-11} M to 4×10^{-10} M of PAF) instilled in flowing solution immediately before the perfusion chamber (2×10^{-10} M was the optimal dose) (protocol 2); 3. in K^+-enriched solution with continuous infusion of PAF (2×10^{-10} M) (protocol 3).

Results. In protocols 1 and 2 PAF (2×10^{-10} M), 1 min after the beginning of the challenge, induced a positive inotropic effect, with an increase of $20.0 \pm 4.51\%$ (mean ± SE, 6 experiments) of the prechallenged values, which reached its peak within 2–3 min and was reversed after 5 min. This first phase was followed by a progressive decrease of the tension developed, which was reduced by $56{,}0 \pm 6.91\%$ in respect to prechallenged values after 10–15 min. As shown in figure 1, the positive inotropic effect (maximum at 6 min) was preceded by a slight augmentation (maximum at 2 min) of the action potential duration (APD) (calculated at 50% of depolarization, $+ 2.3 \pm 0.37\%$, 6 experiments). Subsequently, APD decreased concomitantly with the negative inotropic effect (fig. 1).

In contrast, the resting membrane potential (E_R), the overshoot (O_s) and the maximum rate of depolarization (MRD) were unaffected during the whole duration of the experiments. The mechanical and electrical alterations induced by PAF were nearly completely reversed after challenge with a single PAF dose (protocol 2), while recovery was incomplete after continuous PAF infusion (protocol 1). Pretreatment with 2×10^{-7} M propranolol (2 experiments) completely prevented the positive inotropic effect and alteration of APD.

The 2-lyso-PAF tested under the same experimental conditions failed to induce significant mechanical and electrical changes of papillary muscle. Protocol 3 was performed to study the effect of PAF on the Ca^{2+}- slow action potentials. PAF induced within 1 min a positive inotropic effect, that reached its peak ($-24.0 \pm 4.40\%$, 3 experiments) after 2–3 min, followed after 5 min by a marked negative inotropic effect ($-50.6 \pm 6.18\%$) and, after 15 min, by inability of the preparation to follow the imposed stimulation frequency (fig. 2). While E_R was unchanged, APD and MRD values were first increased in concomitance with the positive inotropic effect, and subsequently reduced during the negative inotropic effect.

Pretreatment of papillary muscle with isoproterenol (1×10^{-6} M), which has per se a positive inotropic effect ($233 \pm 18.5\%$ of the prechallenged values; mean ±SE of 5 experiments) allowed us to appreciate the positive but not the negative effects on inotropism and on slow action potential induced by PAF. The positive effect of isoproterenol was therefore transiently enhanced but not subsequently depressed by PAF. Furthermore, the negative effect of PAF on the electrical and mechanical activities was completely reversed within 2 min, when 1×10^{-6} M isoproterenol was added after the stimulation with PAF.

Discussion. It has recently been shown that the i.v. infusion of synthetic PAF mimicked the respiratory and cardiovascular alterations associated with the anaphylactoid reaction[13]. Circulatory alterations included a transient bradycardia and an initial increase in ventricular pressure, followed by a marked decrease associated with systemic hypotension.

Preliminary results obtained by Burke et al.[14] in guinea-pig isolated heart (Langendorff preparation) indicated that PAF induced a marked reduction in ventricular contractile force and coronary flow. Using the same Langendorff preparation, we confirmed a negative inotropic effect induced by PAF (from 1×10^{-11} M to 4×10^{-10} M), in addition to a significant reduction of APD and conduction arrhythmias (unpublished results), that could be ascribed either to the coronary constriction or to the direct effect of PAF on cardiac muscle.

The results of the present study indicated that PAF had a biphasic direct effect on papillary muscle. The first increase in APD, as well as the positive inotropic effect could be related either to direct agonism on β-receptors or to release of endogenous catecholamines, as inferred from the inhibitory effect of propranolol. The second phase, characterized by a marked negative inotropic effect associated with a consistent reduction in APD and inhibited by isoproterenol, could be ascribed to a specific inhibitory effect on the Ca^{++} slow channel, since in this phase the E_R,O_s and MRD values were unchanged.

From these observations and concomitant measurement of slow action potentials for the study of the transmembrane Ca^{++} inflow[17] it is postulated that PAF induced a first transient influx of Ca^{++}, as shown for platelets[18], followed by a marked reduction as result of an inhibitory effect on the Ca^{++} slow channel. These effects were not observed after stimulation with 2-lyso-PAF, inactive on platelets[9], indicating that the effects observed after PAF stimulation were related to its biological activity. These results indicated that PAF has salient effects on cardiac muscle that could have a relevance in immunopathological states involving an in vivo PAF release.

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