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THE ROLE OF PLATELET-ACTIVATING FACTOR (PAF) ANTAGONISTS AND NITRIC OXIDE IN CARDIAC ACTIONS OF PAF. ELECTROPHYSIOLOGICAL AND MORPHOLOGICAL STUDY

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Electrophysiological and ultrastructural effects of platelet-activating factor (PAF) antgonists, WEB 2086 and BN 52021 were compared in isolated guinea-pig hearts preparations. We studied the possible role of nitric oxide (NO) in electromechanical actions of PAF. Isometric twitches and intracellular action potentials (APs) were recorded from guinea-pig right ventricular papillary muscles and left atria. For electron microscopic study the hearts were perfused according to Langendorff technique. WEB 2086 ($5 \times 10^{-9} - 5 \times 10^{-7}$ M) significantly shortened the duration of atrial AP without changing the ventricular one, however, BN 52021 decreased both of them. The shortening of atrial and ventricular AP duration (APD) by both PAF antagonits were abolished by 4-aminopyridine (10^{-3} M), a blocker of one type of K^+ channels ($I_{K_{to}}$). Glibenclamide (10^{-6} M) the blocker of ATP-dependent K^+ channels prevented the shortening effect of BN 52021 (10^{-6} M) on ventricular APD. Electron microscopic study of myocardial samples from hearts subjected to 30 min hypoxia/reoxygenation showed intracellular oedema, intramitochondrial swelling and fragmentation of mitochondrial christae, separation of intercalated disc. Pretreatment with WEB 2086 (5×10^{-7} M) warded off nearly all damage caused by hypoxia/reoxygenation. Both WEB 2086 and NO synthase inhibitor N^G-nitro-L-arginine methyl esther (L-NAME) (10^{-3} M) abolished the negative inotropic effect of PAF (10^{-7}, 10^{-6} M). L-NAME prevented the shortening of APD induced by 10^{-7} M PAF. These results suggest that PAF may be responsible for myocardial ischemia and the beneficial effects of PAF antagonists in this pathological process could be due to their possible K^+ channel stimulator property. These data support the possibility that NO contributes to the cardiac electromechanical alterations induced by PAF.

Key words: *cardiac action potential; cardiac ultrastructure; platelet activating factor; platelet activating factor antagonists; nitric oxide; glibenclamide.*

INTRODUCTION

Platelet activating factor (PAF) has been shown to implicate as a mediator/modulator in different pathophysiological events (inflammation, thrombosis, cardiovascular diseases) (1—4). As far as cardiovascular diseases

are concerned PAF seems to be involved in myocardial ischemia, arrhythmia, anaphylactic and cardiovascular shock (2—3, 5—7). PAF administration induced coronary vasoconstriction, negative inotropic action, arrhythmia and ischemic-like severe morphological effect in myocardium (8—12). These actions of PAF are receptor mediated as shown by the use of different PAF receptor antagonists (2—5, 13—14). Several mechanisms (platelets/neutrophils, electrophysiological, ionic currents alterations, free radicals) have been suggested to be the underlying mechanisms of these PAF-induced actions (1, 15—25). Recent studies indicate that nitric oxide (NO) participates, as a final mediator in the PAF-dependent cardiac alterations of tumor necrosis factor alpha (TNFα) (26). The aim of this work is to study the electrophysiological and ultrastructural effects of the PAF antagonist, WEB 2086 and the role of NO on PAF-induced cardiac effects in isolated guinea-pig hearts.

MATERIALS AND METHODS

Electrical and mechanical measurements on myocardial preparations

Guinea-pigs (n = 35), weighing 320—380 g, were used in the experiments. Following cervical dislocation, right ventricular papillary muscles and left atria were rapidly excised and superfused (8—10 ml/min) with modified Krebs solution containing (in mM): NaCl, 137; KCl, 4.0; $CaCl_2$, 1.8; $MgCl_2$, 1.05; $NaHCO_3$, 11.9; NaH_2PO_4, 0.42 and glucose, 5.5. The temperature of this superfusate was 36.5 ± 0.2°C, and pH was set to 7.4 ± 0.05 when gassed with a mixture of 95% O_2 and 5% CO_2. Contractile parameters were determined under isometric conditions using mechano-electrical transducer (Experimetria) The preparations were stretched so as to produce maximum force of contraction when stimulated with rectangular current pulses (having a duration of 1 ms and an amplitude of twice the diastolic threshold) at a constant pacing frequency of 1 Hz. These stimuli were delivered to the preparations through a pair of platinum electrodes.The resting tension at which the contractile responses reached the maximum value was determined. The contractile force (CF), the maximum rate of rise ($+dT/dt$) and fall ($-dT/dt$) of developed tension were determined. Transmembrane potentials were recorded with conventional glass microelectrodes (having tip resistance of 7—18 MOhms), filled with 3 M KCl. The electrodes were electrically coupled to the input of a high impedance amplifier (INTR-01, Experimetria, Hungary) with capacitance compensation. Records were digitized and analyzed using a computer-based data acquisition and analyzing system (Intrasys, Experimetria, Hungary) allowing on-line determination of the resting membrane potential, action potential amplitude, overshoot potential, maximum rate of depolarization, and duration of the action potential measured at 20%, 50% and 90% level of repolarization (APD_{20}, APD_{50} and APD_{90}, respectively). Each preparation was equilibrated for 120 min, and impalements were maintained for at least 30 min before recording. The drugs were added to the bathing solution in a cumulative manner (for 30 min at each concentration). NO synthase (NOS) inhibitor N^G-nitro-L-arginine methyl esther (L-NAME, 10^{-3} M) was applied for 2 h before PAF application. Results were expressed as means ± SEM. Statistical significance was determined using Student's t-test for paired data. P values of less than 0.05 were considered significant.

Ultrastructural method

Following cervical dislocation of heparinized (1000 I.U.,i.p.) guinea-pigs the hearts were rapidly removed and perfused with oxygenated (95% O_2+5% CO_2) Krebs solution for 15 min at 37°C through the aorta (Langendorff technique). After 15 min normoxic Krebs perfusion the hearts were subjected to 30 min of ischemia by using anaerobic solution (90% N_2+10% CO_2) and this was followed by 30 min of reoxygenation. The hears were pretreated by WEB 2086 ($5 \times 10^{-9} - 5 \times 10^{-7}$ M) for 15 min and after 30 min of ischemia and 30 min of reoxygenation were used. At the end of the experiments biopsy specimens of the left and right ventricle free wall were excised. For the electron microscopic study the tissue samples were treated as Post et al. and we have previously described (27, 12).

The drugs used were PAF (1-0-alkyl-sn-glycero-3-phosphorylcholine), 4-aminopyridine (4-AP), N^G-nitro-L-arginine methyl ester (L-NAME), glibenclamide obtained from Sigma-Aldrich Co., BN 52021 (IHB Research Lab., France) WEB 2086 (Boehringer Ing.). The solubility of PAF, BN 52021 and glibenclamide was previously detailed (21, 28).

Principles of Laboratory Animal Care met the standards set by the 'European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes' (Council of Europe No 123, Strasbourg 1985) and Ethical Committee of Semmelweis University

RESULTS

WEB 2086 significantly shortened the duration of atrial AP at 20 and 50% of repolarization (APD_{20} and APD_{50}), without causing any significant alterations in the other parameters (*Fig. 1*). This shortening effect of WEB 2086 on the repolarization phase was prevented by 4-aminopyridine (4-AP) (10^{-3} M), the blocker of one type of K^+ channels (I_{Kto}).The shortening of ventricular APD was, however, slight and not significant (*Fig. 2*). BN 52021 (10^{-6} M) markedly shortened the APD in both guinea-pig atrial and ventricular papillary muscles and this shortening was partially prevented by 4-AP, as we previously reported (21). In order to study the role of other type of K^+ channels in the electrophysiological actions of PAF antagonists, the effect of BN 52021 on APD was studied in the presence of glibenclamide, the well known inhibitor of ATP-dependent K^+ channels (*Fig. 3*). *Fig. 4* demonstrates that glibenclamide (10^{-6} M) caused a modest prolongation of both APD_{50} and APD_{90} and nearly completely prevented the strong shortening of APD induced by BN 52021 (10^{-6} M). The characteristic morphological changes (intracellular oedema with intramitochondrial swelling and flocculation and fragmentation of mitochondrial christae, separation of intercalated disc (z) of ischemic/reperfused myocardium) are seen in *Fig. 5*. WEB 2086 (10^{-7} M) pretreatment warded off the reperfusion induced damage (*Fig. 6*). The intracellular oedema decreases and there are no mitochondrial alterations. *Fig. 7* shows that PAF-induced a concentration-dependent effect on the mechanical activities of isolated papillary muscles. PAF (10^{-7} and 10^{-6} M) caused a concentration-depedent reduction of contractile force (CF), the

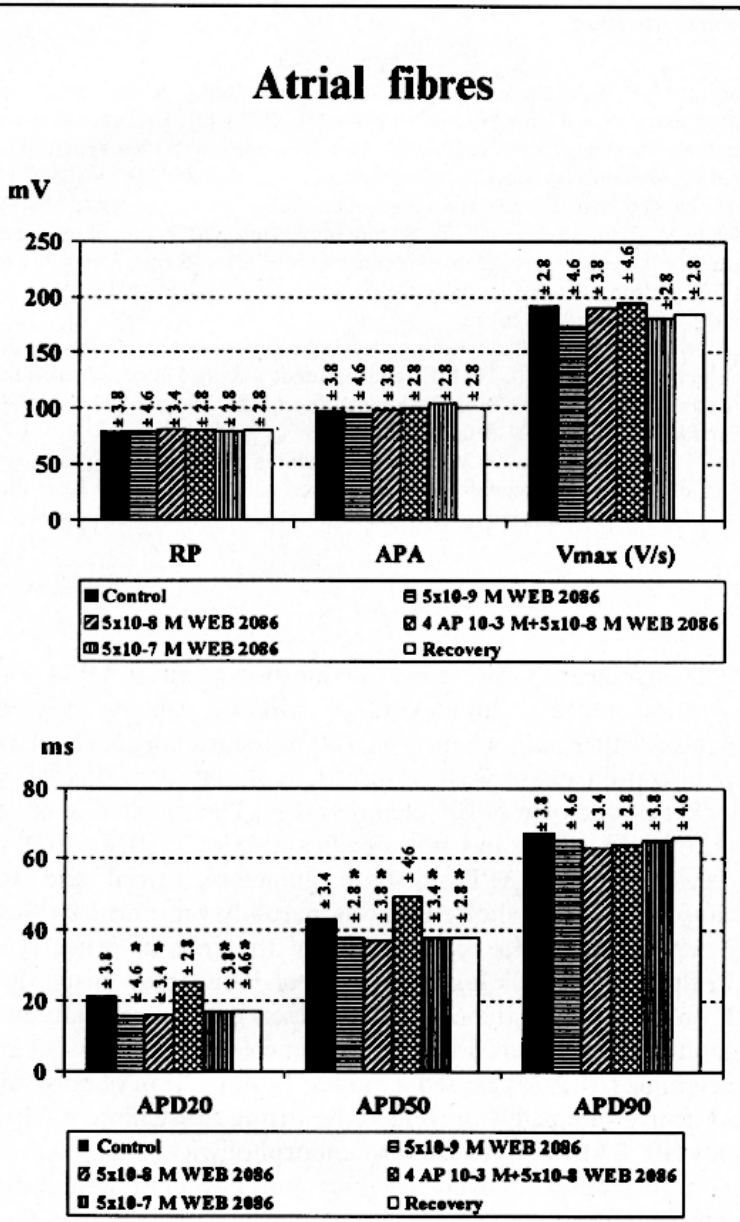

Fig. 1. Effects of WEB 2086 ($5 \times 10^{-9} - 5 \times 10^{-7}$ M) on guinea-pig atrial action potential parameters. RP = resting membrane potential; APA = amplitude of action potential; V_{max} = maximum rate of rise depolarization phase; APD_{20}, APD_{50}, APD_{90} = duration of action potential measured at 20, 50 and 90% of repolarization; 4-AP = 4-aminopyridine (10^{-3} M).

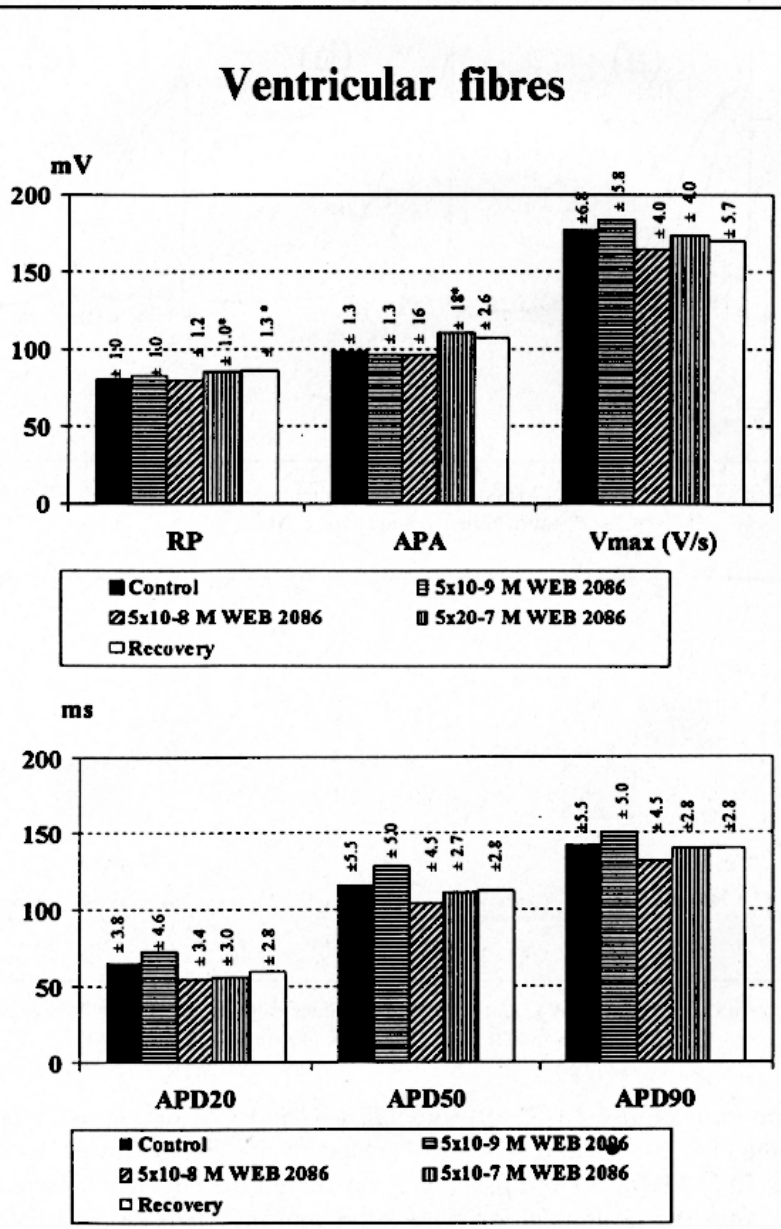

Fig. 2. Effects of WEB 2086 on guinea-pig ventricular action potential parameters.

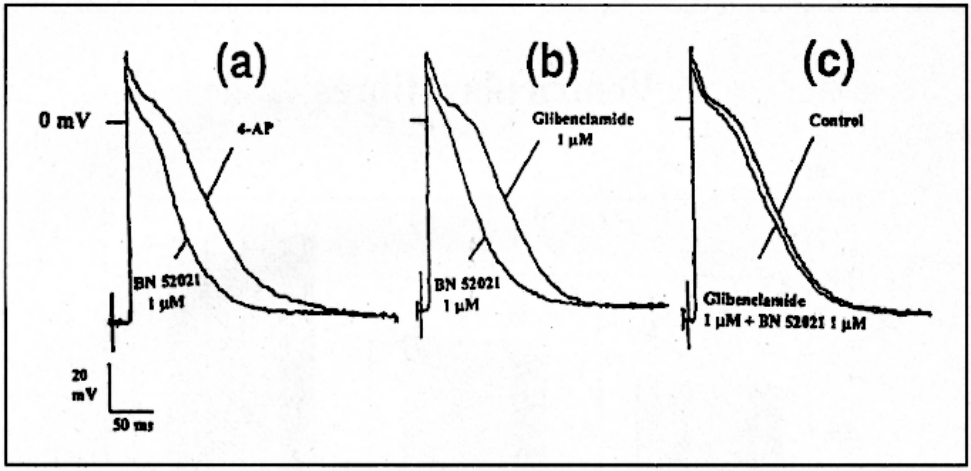

Fig. 3. Effects of BN 52021 (10^{-3} M) on atrial action potential pretreated with 4-AP (10^{-3} M) (b) and glibenclamide (10^{-6} M)(c).

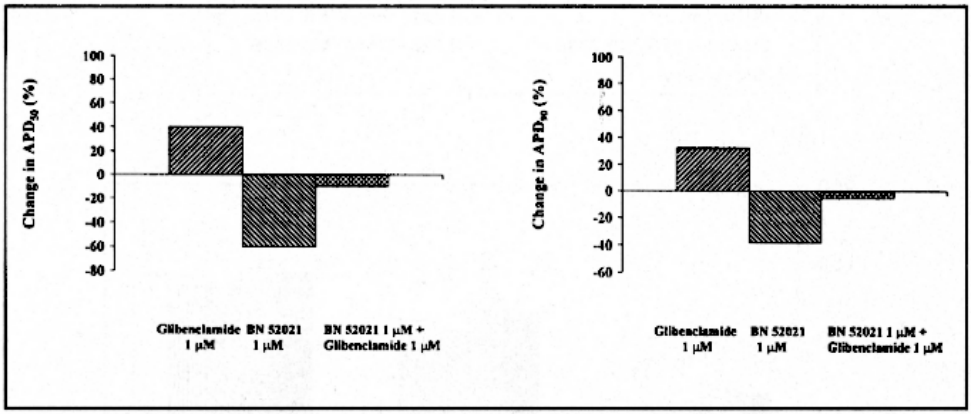

Fig. 4. Per cent change in $APDW_{50}$ and $APDW_{90}$ in guinea-pig ventricular papillary muscle in the presence of BN 52021 (10^{-6} M) and glibenclamide (10^{-6} M).

maximum rate of rise (+dT/dt) and fall (−dT/dt) of developed tension by approximately 23—25 and 47—50%, respectively. Pretreatment with WEB 2086 (5×10^{-7} M) for 30 min practically prevented the negative inotropic effect of PAF. After the 2h incubation with NOS inhibitor L-NAME (10^{-3} M) the negative effects of PAF on contractile parameters were completely blocked. The effects of NOS inhibitor L-NAME (10^{-3} M) on PAF ($10^{-9}-10^{-7}$ M) — induced changes in the parameters of ventricular AP are summarized in *Fig. 8* Neither PAF nor L-NAME modified significantly the values of resting membrane potential (RP) and the maximum rate of rise of depolarization phase (V_{max}). L-NAME pretreatment prevented the significant increase in the

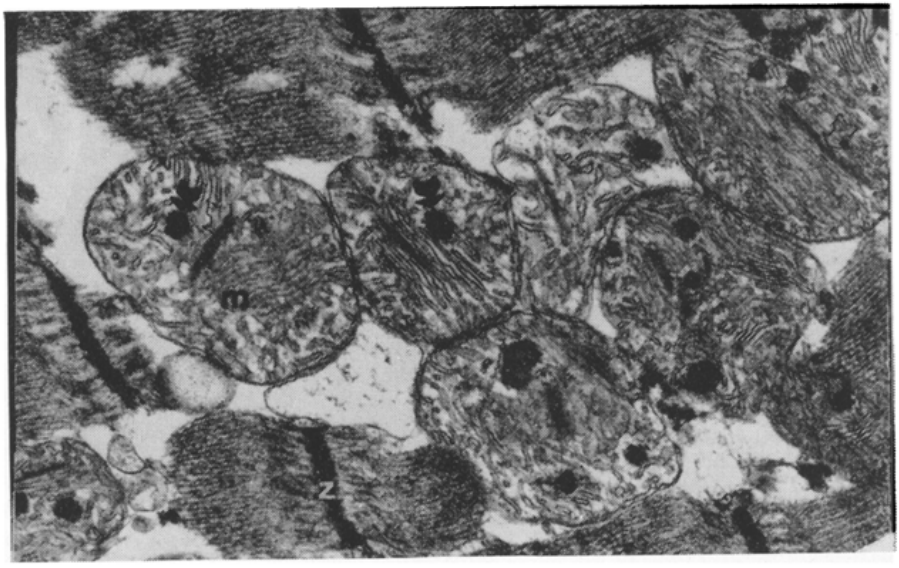

Fig. 5. Cardiac muscle from hypoxic/reoxygenated guinea-pig heart. Intracellular oedema, intramitochondrial swelling (⇐) and fragmentation of mitochondrial cristae (⇐) separated intercalated disc (z), m = mitochondria. Magnification: 42.000 ×.

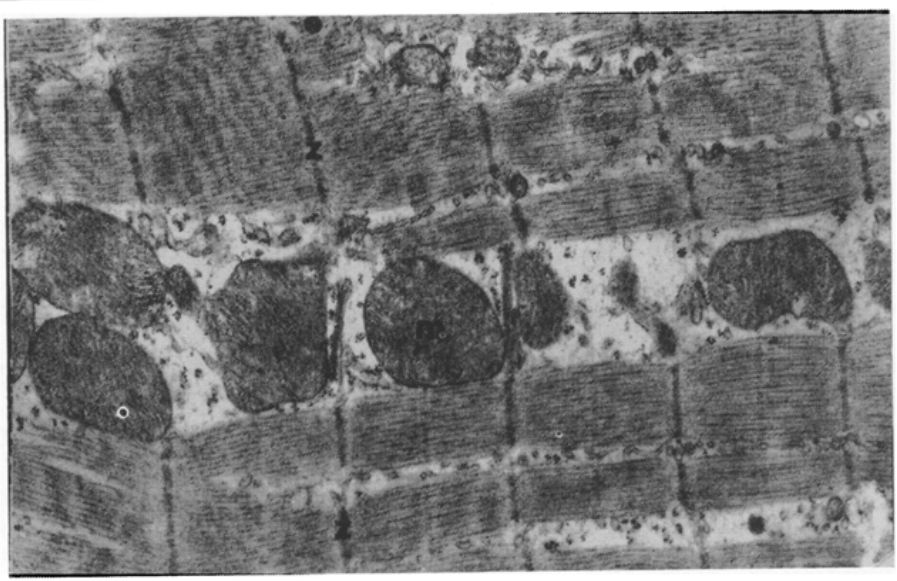

Fig. 6. Preatment of WEB 2086 (5×10^{-7} M) for 30 min before hypoxia/reoxygenation. Magnification: 21.600 ×.

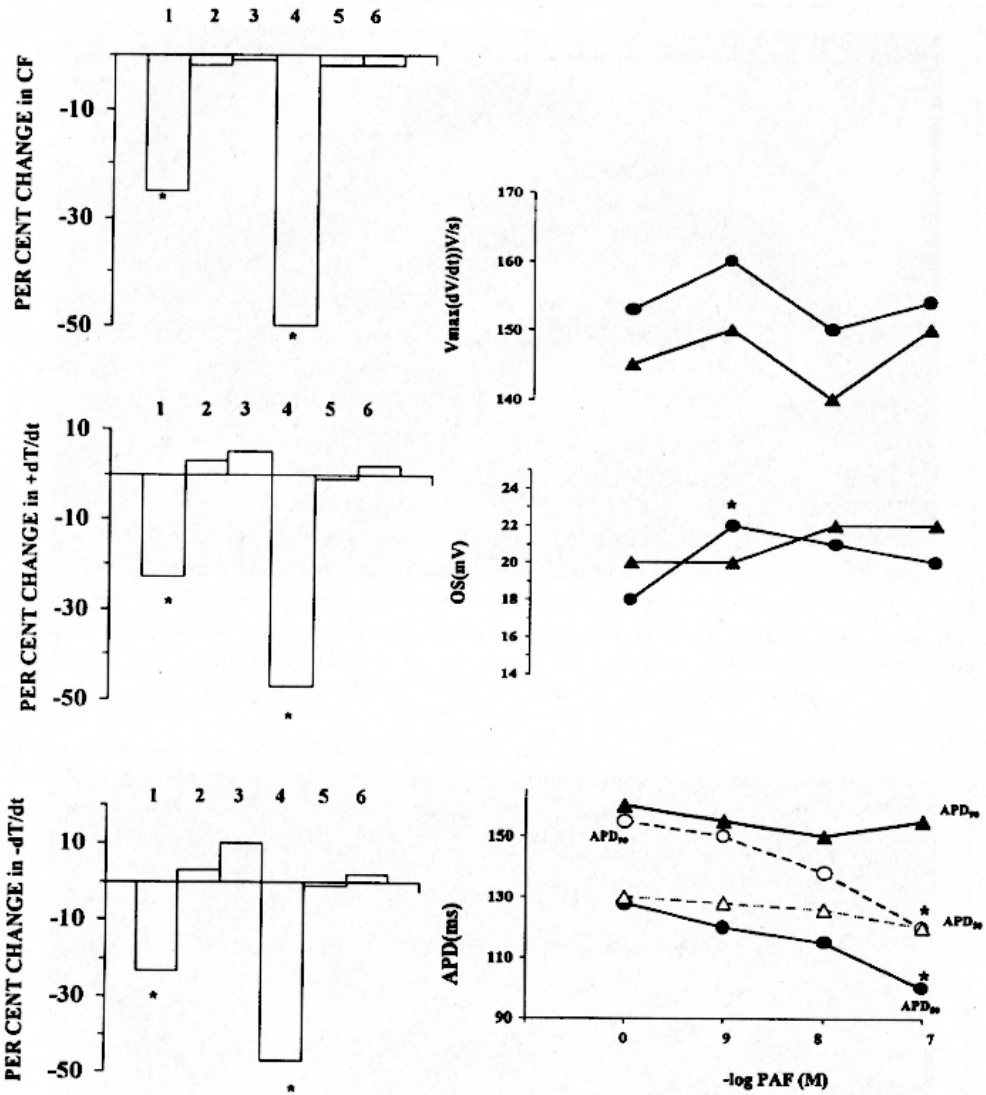

Fig. 7. Effect of PAF (10^{-7} and 10^{-6} M, columns 1, 4; n = 5), pretreatment of WEB 2086 (5×10^{-7} M) (columns 2, 5; n = 5) and L-NAME (10^{-3} M) (columns 3, 6; n = 4) on contractile parameters (CF, +dT/dt, dT/dt) on guina-pig right ventricular papillary muscle. Data show the percent change of mechanical parameters induced by drugs. Baseline 0 represents the control values.

Fig. 8. L-NAME (10^{-3} M) pretreatment induced modification of electrophysiological effects of PAF (10^{-9} to 10^{-7} M) in guinea-pig ventricular papillary muscle. Each points reperesent the mean values from 5 experiments. RP = resting potential; OS = overshoot; VW_{max} = maximum rate of rise of depolarization phase; $APDW_{50}$ and $APDW_{90}$ = AP duration measured 50 and 90% level of repolarization, respectively.

overshoot (OS) caused by low concentration of PAF (10^{-9} M) as well as the shortening of action potential duration (measured at 50% and 90% repolarization phase, APD_{50}, APD_{90}) caused by higher concentration (10^{-7} M) of PAF.

DISCUSSION

It is generally accepted that PAF is released under ischemia, anaphylaxia (2—6, 15). The cardiovascular shock caused by PAF can be explained by its vascular and cardiodepressant action (1—3, 15). PAF-induced decrease in myocardial contractility is due to its negative inotropic as well as its coronary constrictor action (8—11, 17). The direct negative inotropic effect of PAF in isolated cardiomyocytes can be found to be the result of a decrease in systolic $[Ca^{2+}]_i$ (24). Such decrease of $[Ca^{2+}]_i$ has been found to be the responsible mechanism for the shortening effect of PAF on APD (29). In this study we have shown that NO could be involved in the decrease of contractility and APD induced by PAF in isolated guinea-pig ventricular papillary muscle (*Fig. 7*). The cardiac electrophysiological effects of PAF are concentration dependently different. Our and other previous data demostrated that low concentrations ($<10^{-9}$ M) of PAF caused a slight increase of OS and APD in ventricular muscle, however, higher concentrations had no effect on OS and shortened APD (17—18, 21, 29). The shortening of APD can be explained by both an enhancing effect on K^+ currents and a reduction on Ca^{2+} current induced by PAF, as shown by direct ionic currents measurements (22—23). Our present results confirm previous data of Alloatti and coworkers (26) who found that both PAF and NO contribute to the electrical and mechanical alterations induced by TNF_α and NO is the final mediator in the PAF-induced cardiodepressant actions. This observation was confirmed by others (30—31) who had found that hypotensive and vasoactive effects of PAF were dependent on NO generation. It has been shown that NO reduces myocardial contractility and shortens AP and it decreases Ca^{2+} current (32—34).

Several data proved that PAF antagonists prevented ischemic and TNF_α-induced changes in cardiac functions (2—5, 14, 26). PAF-induced negative inotropic effect (14, 24) and ischemic-like morphological actions (12) can be antagonized by different PAF antagonists (WEB 2086, 2170, BN 52021). In present study WEB 2086 antagonized the negative inotropic action of PAF and prevented the hypoxia/reoxygenation induced morphological alterations, but its effect on APD was similar to that of PAF (*Figs. 5—7*). WEB 2086 similarly to PAF caused a significant shothening of atrial APD and a weaker decrease of ventricular one (*Figs 1—2*). In a previous study we and others demonstrated that BN 52021 significantly shortened both atrial and ventricular APD (21, 35). The experiments with 4-aminopyridine showed that this blocker

of one type of K^+ channels (transient outward current, I_{Kto}) prevented the acceleration of atrial repolarization phase induced by both BN 52021 and WEB 2086 (*Figs. 1, 3*). These data suggest that this shortening of APD may be related to an increase in the transient outward K^+ current (I_{Kto}), dominant in atrial fibers. The duration of cardiac AP is a result of a fine balance of inward (slow inward Ca^{2+}, "window" Na^+ current) and outward (inward rectifying K^+, I_{K1}; transient outward, I_{Kto}; two types of delayed rectifying K^+, $I_{Kr/s}$ currents) currents (36—39). The contribution of these currents to the repolarization phase of AP is different, depending on the cardiac tissue and species (37—39). Among the various K^+ channels the ATP-sensitive K^+ channels are of great interest because they are essential to myocardial function during hypoxia/ischemia (40—41). During hypoxia or/ischemia the cytostolic concentration of ATP decreases, which directly results in opening of K_{ATP} channels. The subsequent efflux of K^+ induces hyperpolarization of the cell membrane which results in a shortening of myocardial APD, reduction of contractility and relaxation of vascular smooth muscles. Thus ATP-dependent KW^+ channels play a role in the mechanism of cardioprotection (40). Glibenclamide the well known inhibitor of the ATP-dependent K^+ channels preventing the increase in extracellular K^+ concentration , preserves the APD and other electrophysiological parameters during myocardial ischemia (42—45). In this experiments (*Fig. 4*) glibenclamide increased moderately the ventricular APD, as the ATP-sensitive K^+ channels are likely to be partly activated during normoxia (46), but it was able to prevent the strong APD shortening effect of BN 52021. These data suggest that beyond its PAF receptor antagonist action, BN 52021 should have a stimulatory effect on ATP-depedent K^+ channels.

In summary, our data further support the role of PAF in myocardial ischemia. The protective, beneficial effects of PAF antagonists in this pathological condition could be explained by their receptor antagonist activity as well as by their possible stimulatory action on K^+ currents. These results support the possibility that in cardiac muscles NO contributes to the electromechanical alterations induced by PAF.

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