CARDIODEPRESSOR ACTION OF PLATELET ACTIVATING FACTOR

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Platelet activating factor (PAF) is a membrane phospholipid derivative. Its high biological activity, beginning with concentrations of 10^{-13}-10^{-12} M, and the broad spectrum of its pathophysiological reactions, manifested as platelet aggregation, spasms of the pulmonary and coronary vessels, and systemic hypotension, have led many investigators to regard PAF as a potential mediator of various states of shock [5, 6, 11].

The direct inhibitory action of PAF has been demonstrated on the isolated guinea pig heart [11] and also on isolated fragments of guinea pig [4, 8, 11] and human [3] myocardium. However, the cellular nature of the disturbances found has not yet been determined.

The aim of this investigation was accordingly to study the cellular mechanisms of the cardiodepressor action of PAF.

EXPERIMENTAL METHOD

Isolated left auricles of the atria of guinea pigs (250-300 g) were placed in a continuous-flow chamber with a capacity of 1 ml and perfused with Tyrode solution at 30°C with continuous oxygenation with carbogen (95% O_2 ± 5% CO_2). The salt composition of the standard Tyrode solution was as follows (in mM): NaCl 136.9, KCl 2.68, $NaHCO_3$ 11.9, $CaCl_2$ 1.8, $NaH_2PO_4 \cdot H_2O$ 0.48, $MgCl_2 \cdot 6H_2O$ 1.0, glucose 5.6, pH 7.3.

Intracellular resting (RP) and action potentials (AP) were recorded by means of "floating" glass microelectrodes filled with 2.5 M KCl solution. Contractions of the myocardial preparations were recorded under near-isometric conditions by means of a 6MKhIS mechanotron.

To study slow calcium AP standard Tyrode solution was used, but with its K^+ ion concentration raised to 15-20 mM. There were two series of experiments. In series 1 the Ca^{++} ion concentration in the solution was incresed to 6 mM. In series 2, isoproterenol (10^{-7} M) was added to the depolarizing solution.

The basic frequency of electrical stimulation of the myocardial preparations in standard Tyrode solution, and in the depolarizing solutions was 0.5 Hz. In some experiments "bursting" stimulation was used: the frequency of electrical stimulation in the "burst" varied from 0.5 to 2 Hz and the interval between "bursts" was 3 min. The frequency was changed after an interval.

The PAF was synthesized at the Institute of Biotechnology (Moscow) [2] or obtained from "Novabiochem" (Switzerland). No difference was found in the action of the Soviet and Swiss preparations on the myocardium. During pharmacological analysis of the effects of PAF, besides the mediator in the solution, histamine, 4-aminopyridine (4-AP), and indomethacin were added.

EXPERIMENTAL RESULTS

In standard Tyrode solution the action of PAF on myocardial function was studied for 20 min. In all concentrations tested (from 10^{-10} to 10^{-6} M) at the 5th-7th minute after the beginning of perfusion PAF induced the development of a negative inotropic effect by

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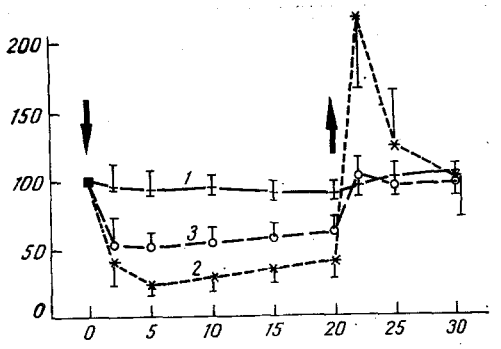

Fig. 1

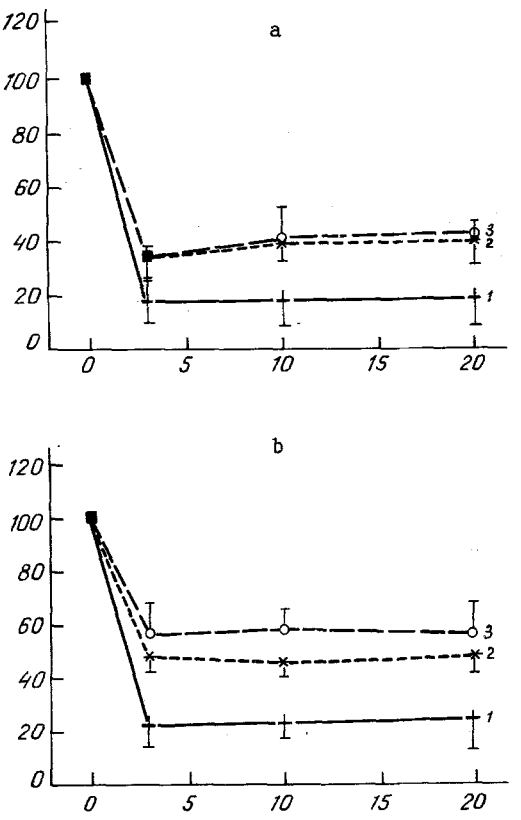

Fig. 2

Fig. 1. Changes in AP and contractile responses of guinea pig auricle under the influence of PAF ($2 \cdot 10^{-7}$ M). Here and in Fig. 3: 1) amplitude of AP; 2) amplitude of contractions; 3) duration of AP at 50% of repolarization phase level. Arrow pointing downward – addition of PAF to perfusion fluid, arrow pointing upward – rinsing with standard Tyrode solution. Here and in Figs. 2 and 3: abscissa, relative changes, in per cent; ordinate, time, in min; n = 8.

Fig. 2. Dependence of amplitude of calcium AP and amplitude of contractions on strength of stimulation under the influence of PAF ($2 \cdot 10^{-7}$ M). a) Amplitude of AP; b) amplitude of contractions. 1, 2, 3) Strength of stimulation in thresholds, respectively.

the myocardium. The degree of inhibition of contractivity of the myocardial preparations increased steadily during subsequent transition through an order of magnitude from low to high mediator concentrations. However, the character of the frequency–force dependence, just as in perfusion fluid without mediator, remained positive, i.e., with an increase in the frequency of stimulation from 0.5 to 2.0 Hz the amplitude of the contractions also increased. During "bursting" stimulation, Bowditch's "staircase" also was positive. In the course of development of the effect of the mediator, irrespective of its concentration in the perfusion fluid, failure of the imposed rhythm took place, manifested by episodes of spontaneous activity of the myocardial preparations. After rinsing with standard Tyrode solution to abolish the cardiodepressor effect of PAF in a concentration of 10^{-10} M, the amplitude of the contractile responses in Bowditch's "staircase" increased temporarily at all frequencies of stimulation compared with the original values before application of PAF.

The action of PAF ($2 \cdot 10^{-7}$ M) on the amplitude and duration of AP and on the amplitude of contractions of the auricle in standard Tyrode solution (frequency of stimulation 0.5 Hz) is shown in Fig.1. Clearly PAF reduced the amplitude of AP a little and significantly shortened its duration, measured at the 50% level of the repolarization phase. On average (of eight experiments) the duration of AP was reduced to 50 ± 8.1% from 100% in Tyrode solution without the mediator ($p < 0.01$). The amplitude of contractions also decreased virtually

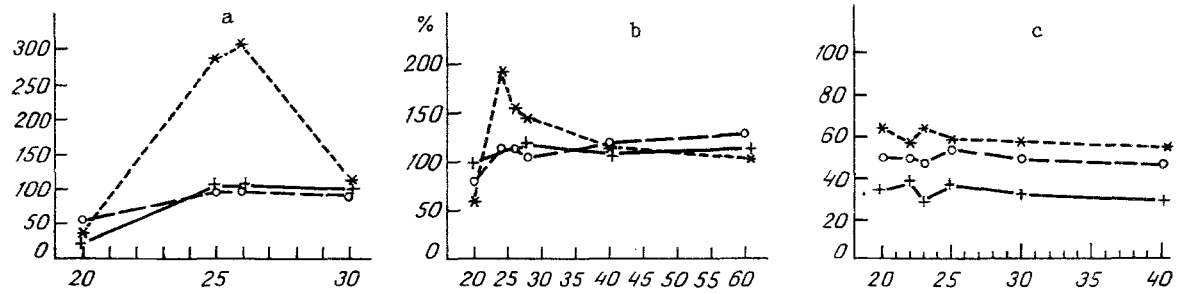

Fig. 3. Effect of histamine, 4-AP, and indomethacin on parameters of calcium AP and contractions against the background of the action of PAF. a) Histamine (10^{-4} M), n = 3; b) 4-AP (3 mM), n = 3; c) indomethacin (10^{-4} M), n = 3. In the course of the experiment stimulus strength remained constant.

parallel to the decrease in the duration of AP. At the 5th minute of action of PAF the amplitude of contractions was reduced to 23.5 ± 2.9% from normal. The negative inotropic action of the mediator then gradually weakened, and by the 20th minute the amplitude of contractions increased to 39.6 ± 8.8% of normal. This phenomenon was conventionally called the "slipping effect."

In all experiments, after only 1 min of rinsing to remove the cardiodepressor action of PAF, the amplitude of the contractile responses rose sharply to reach 200-250% of the normal level. The original duration of AP was restored, and in some experiments it exceeded the normal value by 8-10%. The positive inotropic effect was reduced by rinsing out of the PAF to the original values in the course of 5-10 min.

The effect of PAF on slow calcium AP arising in solution with an increased K^+ ion concentration and with addition of Ca^{++} or isoproterenol, was investigated in the experiments of series 2. In depolarizing solution RP fell from 81.2 ± 2.8 mV to 54.2 ± 2.9 mV. In response to stimulation slow AP appeared, and their amplitude remained virtually unchanged after a two-threefold increase in the threshold strength of stimulation. The amplitude of the contractile responses also did not change significantly.

Addition of PAF to the depolarizing solution led to well marked changes of activity depending on the threshold of stimulation. Changes in amplitude of the slow AP and contractile responses under the influence of PAF ($2 \cdot 10^{-7}$ M) are shown in Fig. 2. Maximal depression of electrical and contractile responses developed at the 2nd-3rd minute, and thereafter remained virtually unchanged. In the presence of mediator, at the 20th minute of its action, and with a stimulus of threshold strength, the amplitude of AP was 20.2 ± 12.0% and the amplitude of contractions 25.2 ± 9.0% of normal (n = 5). With a twofold increase in the strength of stimulation the amplitude of AP rose to 41.2 ± 9.8% and the amplitude of contractions rose to 48.7 ± 6.4% of normal.

It was interesting to discover how the affect of PAF on the myocardium was modified by the presence of histamine, for we know that during the development of an anaphylactic reaction of the isolated guinea pig heart, both mediators are secreted virtually simultaneously into the perfusion fluid [11]. Addition of histamine ($f \cdot 10^{-4}$ M) to the depolarizing solution (15.5 mM K^+ and 6 mM Ca^{++}) at the 20th minute of action of PAF ($2 \cdot 10^{-7}$ M) is shown in Fig. 3a. It can be seen that after 5-6 min histamine considerably increased both the amplitude and the duration of the slow AP, the amplitude of contractile responses being increased by 5-6 times (mean results of five experiments). Similar results were obtained in depolarizing solution with the addition of isoproterenol.

A similar increase in the amplitude and duration of slow AP and the amplitude of contractions of the myocardial preparations took place when 4-AP (3 mM) was added to both types of depolarizing solutions. One such experiment is illustrated in Fig. 3b. When 4-AP was added to the perfusion fluid before the addition of PAF, the cardiodepressor effect of the latter did not develop.

The investigation thus showed that PAF (10^{-10}-10^{-6} M) inhibits electrical and contractile activity of the myocardium. The main cause of development of the cardiodepressor effect under the influence of PAF is evidently linked with inhibition of the inward calcium current during the plateau phase of AP. This is shown by reduction of the calcium AP, induced by PAF, and restoration of the parameters of AP and contractile responses during the

combined use of PAF and histamine, which increases the inflow of Ca^{++} ions into the myocardial cells during excitation [1, 10]. Evidence of this is given by abolition of the cardiodepressor action of PAF when the outward flow of K^+ ions is blocked by 4-AP, causing an increase in the inflow of Ca^{++} ions into the cell due to an increase in the duration of the AP plateau. However, the marked shortening of AP under the influence of PAF may also be the result of an increase in potassium conductance of the myocardial cell membrane.

Prostaglandin PGD_2, which is an end product of arachidonic acid metabolism and has a negative inotropic action on the myocardium [7], does not make any contribution itself to the development of the cardiodepressor effect. This is also confirmed by the fact that in the presence of indomethacin (10^{-4} M), which lowers activity of the enzyme cyclo-oxygenase, catalyzing the conversion of arachidonic acid to prostaglandins [13], the amplitude of the calcium AP and of the contractile responses of myocardial preparations, when depressed beforehand by PAF, was not restored (Fig. 3c).

The absence of "slipping" effects during exposure to the combined action of PAF and indomethacin in the present experiments can evidently be explained by a progressive rise of the positive inotropic action of arachidonic acid [12] or of prostacycline [9] on the myocardium.

The marked increase in myocardial contractility after rinsing to remove the effects of PAF may be connected either with the positive inotropic action of low concentrations of PAF [8, 14] or with the effect of arachidonic acid metabolites [9].

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