BLOCKING ACTION OF PLATELET ACTIVATING FACTOR (PAF) ON ATRIAL FIBER CALCIUM CURRENTS IN FROGS AND GUINEA PIGS

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Experiments on an atrial guinea pig preparation have shown that the shock mediator [5, 11], otherwise known as platelet activating factor (PAF), causes a decrease in the duration of the transmembrane action potential (AP) but does not affect the resting potential (RP) and reduces the amplitude of contractile responses [1]. It was then shown that blocking K channels with 4-aminopyridine leads to weakening or complete abolition of the above-mentioned effects of PAF, evidence of the important role of K channels in the mechanism of action of the mediator. Effects of PAF have a great resemblance to changes in the duration of AP and amplitude of contractions observed during the use of calcium current blockers, especially when partial depolarization of the myocardial preparations is present [3]. Calcium current activators can abolish PAF-induced depression of the electrical and mechanical parameters of the myocardium [12]. Most workers therefore suggest that PAF-induced disturbances of cardiac tissue function are connected with blockage of the inward Ca-current during the development of the plateau phase of AP. However, no reference could be found in the literature to the direct recording of changes in Ca-transmembrane currents under the influence of the action of PAF and (or) its antagonists on the myocardium.

The aim of this investigation was to study the effect of PAF and its antagonists directly on transmembrane Ca-currents in frog atrial fibers, and also on Ca-AP coupled with contractile responses in the atrial myocardium of the guinea pig.

EXPERIMENTAL METHOD

Some of the experiments were done on atrial trabeculae of the frog Rana ridibunda. Membrane currents or potentials were clamped by means of a four-electrode circuit, using a double sucrose gap [6, 7]. The resting potential was conventionally taken to be 0. When combined calicum and potassium currents were recorded the inward sodium currents were inactivated by a preliminary square pulse 100 msec in duration, depolarizing the membrane by +40 mV relative to the resting potential. The frog myocardial preparations were perfused externally with standard Ringer's solution of the following composition (in mM): NaCl 110, KCl 2.5, $CaCl_2$ 1.8, pH 7.6-7.8, at 18-20°C. The myocardial preparations were stimulated in accordance with an assigned program, AP and ionic currents were recorded, and the data were displayed and processed automatically by means of a CM-3 computer, connected to the experimental apparatus through the "Camac" module [2]. PAF was used in this series in a concentration of 2×10^{-7} M and the PAF antagonist BN 52021 in a concentration of 4×10^{-6} M. In a separate series of experiments changes in Ca-AP (indirect method of measuring Ca-currents) and in the corresponding contractile responses were studied in isolated auricles of guinea pig atria under the influence of PAF and after addition of the PAF antagonist U-66985 and of histamine. Ca-AP were obtained by the use of standard Tyrode solution with K^+ ion concentration in the perfusion solution increased to 15-20 mM. To increase the inflow of Ca^{++} into

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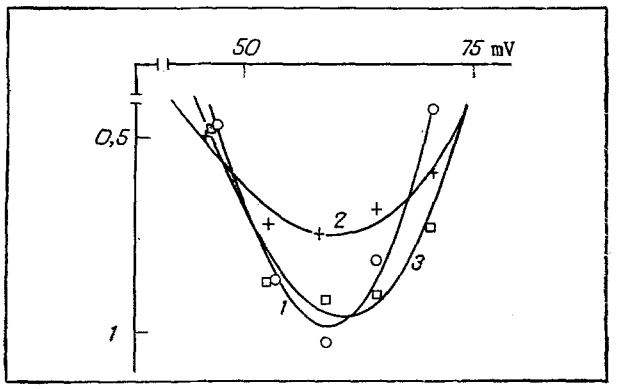

Fig. 1. Changes in current-voltage characteristic curve of slow calcium current of frog atrial fibers under the influence of PAF. 1) Control; 2) PAF 2 × 10^{-7} M, 10th minute of action; 3) after rinsing for 1 min with original solution. Abscissa, clamped voltage (in mV); ordinate, slow calcium current (×10^{-7} A).

the partially depolarized preparation during its stimulation (0.5 Hz), the Ca^{++} ion concentration in the solution was raised in one group of experiments of this series to 6 mM, and in another group, isoproterenol (10^{-7} M) was added to a solution containing the standard Ca^{++} concentration. The technique of simultaneous recording of electrical and mechanical activity of the guinea pig myocardium was described by the writers previously [1]. The PAF was obtained from "Novabiochem" (Switzerland), the BN 52021 from IHB Research Laboratories (France), and the compound U-66985 from "Novabiochem" (Switzerland), the BN 52021 from IHB Research Laboratories (France), and the compound U-66985 from "Novabiochem."

EXPERIMENTAL RESULTS

PAF lowered the amplitude and shortened the duration of AP (measured at the level of 50% repolarization of AP) in frog atrial trabeculae on average by 9.5 ± 0.5% and 27.6 ± 8.1% respectively (n = 3). No changes in the maximal values of the inward Na-current were found by measuring ionic curents. By contrast to this, the peak value of the calcium current (I_{Ca}) was reduced almost by half, on average by 46.9 ± 10.8% (n = 8) and the outward holding K^+-ion current (I_K) was increased by approximately the same degree. With voltage clamping at +73 mV above resting potential, I_K rose by 49.2 ± 22.0% in the presence of PAF (n = 4).

Changes in peak values of I_{Ca} depending on the membrane potential under the influence of PAF are shown in Fig. 1. Compared with the control (curve 1) PAF lowered I_{Ca} by 40-50% (curve 2). Curve 2, it will be noted, was recorded at the 10th minute of action of PAF. Almost complete recovery of the peak values of I_{Ca} took place 1 min after the beginning of rinsing the preparation with the original Ringer's solution (curve 3).

The PAF antagonist BN 52021 itself caused an increase in the inward I_{Ca} on average by 30.0 ± 9.8% (n = 3), as may be seen in the first half of Fig. 2a, trace 2 (BN 52021) and trace 1 (control), and also in curves 1 and 2 of Fig. 2b. Under the combined influence of PAF and BN 52021 no inhibitory action of PAF on the amplitude of I_{Ca} could be observed (Fig. 2b, curves: 1 – control, 2 – BN 52021, 3 – BN 52021 + PAF). Replacing the perfusion fluid containing PAF and BN 52021 by solution containing PAF only caused a marked decrease in I_{Ca} (Fig. 2a and b, curves 4).

Recordings from the frog myocardial preparation thus revealed the ability of PAF both to depress I_{Ca} and to potentiate the outward I_K. Changes in both I_{Ca} and I_K opposite to those observed under the influence of PAF also were recorded under the influence of the PAF antagonist BN 52021.

The study of the action of PAF and another antagonist U-66985 on Ca-AP and contractile response of the auricles of the guinea pig atrium yielded similar data on changes in the values of I_{Ca}. It will be clear from Fig. 3 that PAF considerably reduced the steepness and amplitude of the Ca-AP both in the presence of isoproterenol (frame a) and when the Ca^{++} concentration in the depolarizing solution was increased (frame b). The PAF antagonist

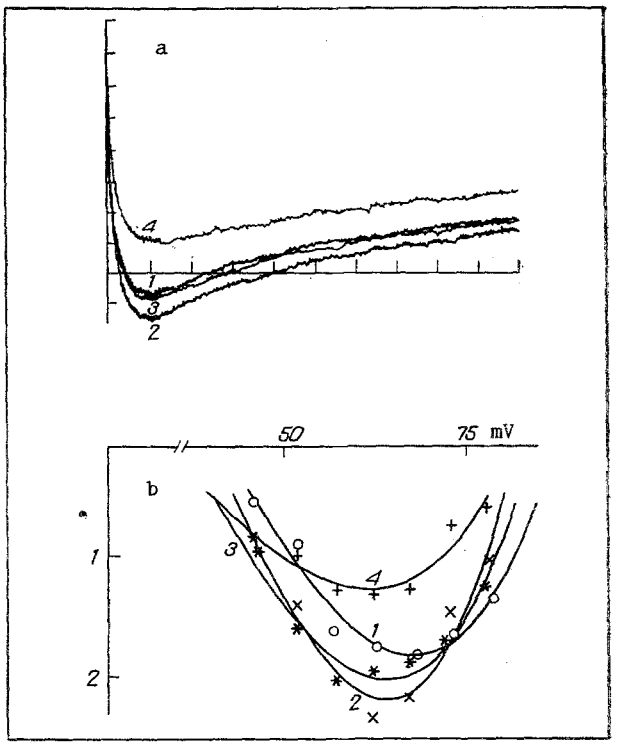

Fig. 2. Effect of PAF antagonist BN 52021 and of a combination of it with PAF on combined calcium and potassium currents (a) and current-voltage characteristic curve of calcium current (b) of frog atrial fibers. a) Original traces of combined currents. Abscissa, time (in msec, one division = 8 msec); ordinate, amplitude of combined currents (I_{Ca} + I_K, one division = 7 × 10^{-8} A). Testing potential 70 mV. Clamping voltage +40 mV (curves 1, 2, 3) and +73 mV (curve 4). Peak value of I_{Ca} for all conditions taken as 8 msec, maximal value of I_K as 80 msec. 1) Control; 2) BN 52021 (4 × 10^{-6} M, 10th minute of action); 3) BN 52021 + PAF (4 × 10^{-6} M and 2 × 10^{-7} M respectively, 10th minute of action); 4) PAF (2 × 10^{-7} M), 10th minute of action. b) Current-voltage characteristic curves. Curves 1, 2, 3, and 4 plotted under the same conditions as for Fig. 2a. Key to axes the same as in Fig. 1.

U-66985 was able to restore (partly, in the first case) the original parameters of the electrical and contractile responses. Complete restoration of contraction in the experiment illustrated in Fig. 3a was achieved after the addition of histamine to the solution containing PAF and U-66985. Histamine is known to be one of the most powerful activators of myocardial Ca-channels [8].

This investigation conclusively confirmed the hypothesis of the important role of I_{Ca} during the action of PAF on the myocardium, based on results obtained by recording AP in preparations of the atrium [1, 11] and ventricle [11] of warm-blooded animals. The interconnected but opposite changes in I_{Ca} and I_K which we recorded during the action of PAF and its antagonist BN 52021 on the frog myocardium indicate that the mechanism of changes in the myocardium of vertebrates (both warm- and cold-blooded) are probably similar in principle. These results also confirmed our previous hypothesis that the PAF-induced shortening of the duration of AP can be explained by strengthening of I_K [1].

The existence of at least two types of calcium channels (T and L) has recently been established in the surface membrane of the myocardial cells of warm-blooded animals [12]. There are two corresponding types of I_{Ca}: fast and slow. The fast I_{Ca} is the signal for

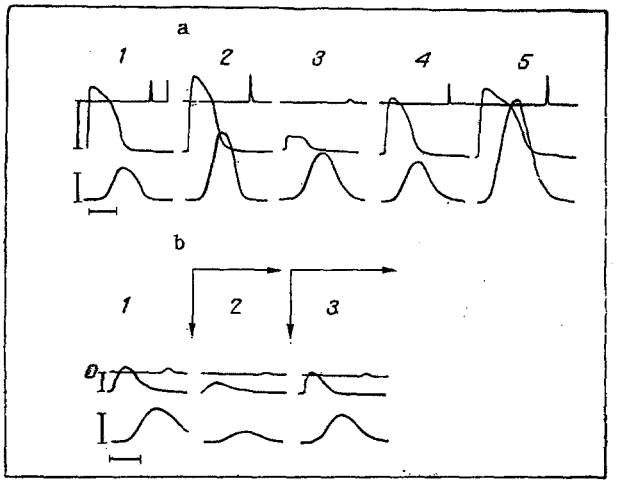

Fig. 3. Restoration of calcium action potentials and amplitude of contractions of auricles of guinea pig atrium, when inhibited by PAF, under the influence of the PAF antagonist U-66985. a) Original traces obtained in experiment in which isoproterenol acted on partially depolarized cells. Each trace reflects changes in electrical and mechanical activity after consecutive addition of the following components to the same solution: 1) control depolarizing solution with $[K^+]$ = 20 mM, 2) isoproterenol 2 × 10^{-8} M, 15th minute of action; 3) PAF 5 × 10^{-7} M, 10th minute of action; 4) U-66985 5 × 10^{-6} M, 15th minute of action; 5) histamine 1 × 10^{-3} M, 5th minute of action. b) Traces obtained in experiment in which Ca^{++} concentration in depolarizing solution was increased. 1) Control depolarizing solution with $[K^+]$ = 15.5 mM and $[Ca^{++}]$ = 6 mM; 2) action of PAF 1 × 10^{-7} M, 20th minute of action; 3) effect of addition to solution of U-66985 (5 × 10^{-6} M), 15th minute of action. a and b: Electrical parameters recorded above: RP, AP, dv/dt (calibration 50 mV and 5 V/sec); below – corresponding contractile responses (calibration: a = 0.2 mN, b = 1.0 mN, time 50 msec).

release of Ca^{++} ions from the sarcoplasmic reticulum (SR) of the myocardial cells, whereas the role of the slow I_{Ca} is the direct activation of contraction induced by calcification of troponin C and replenishing of Ca^{++} in SR [9]. An electrical stimulus induces the fast inward I_{Ca} in partially depolarized guinea pig atrial fibers, whereas under these same conditions it induces a slow I_{Ca} in frog atrial fibers [9].

The main pathway for transmission of the signal from PAF inside cells is considered to be the phosphoinositol system [4, 6]. This system exists in the myocardium but has received little study [10]. Our own results cannot be explained by activation of protein kinase C, as the end product of the inositol cycle, for it causes stimulation of I_K in the myocardium without any change in I_{Ca} [14]. The effects of two other end products of the cycle, namely diacylglycerol and inositol triphosphate, on ionic currents have not been investigated in the myocardium. Simultaneous blockage of Ca channels and stimulation of K channels under the influence of PAF has been obtained in experiments on yeast cells [13]; that investigation showed that activated G protein (this activation is the first stage of the biochemical mechanism of the action of PAF in any cell) possesses multifunctional properties, i.e., it participates in processes of simultaneous blockage of Ca channels and stimulation of K channels. It may be that the effects of PAF in the myocardium also take place on account of the multifunctional properties of G protein.

LITERATURE CITED

1. E. G. Vornovitskii, V. B. Ignat'eva, M. Gollasch, et al., Byull. Éksp. Biol. Med., No. 1, 27 (1989).
2. E. M. Kobrinskii, "Mechanism of disturbance of membrane and contractile properties of heart cells under conditions of calcium overloading and the possibility of restoring them," Dissertation for the degree of Candidate of Biological Sciences, Minsk (1988).
3. G. Alloatti, G. Montrucchio, F. Mariano, et al., J. Cardiovasc. Pharmacol., 9, 181 (1987).
4. C. L. Ashendel, Biochim. Biophys. Acta, 822, 219 (1985).
5. P. Braquet, T. Y. Shen, L. Tonquil, and B. B. Vargaftig, Pharmacol. Rev., 39, 97 (1987).
6. H. F. Brown, A. Clark, and S. J. Noble, J. Physiol. (London), 258, 521 (1976).
7. A. K. Filippov and V. I. Porotikov, Gen. Physiol. Biophys., 2, 93 (1983).
8. J. Hescheler, M. Tang, B. Jastoff, and W. Trautwein, Pflügers Arch., 410, 23 (1987).
9. E. Honore, E. Challice, P. Guilbault, et al., Can. J. Physiol. Pharmacol., 64, 1153 (1986).
10. L. G. Yoness, D. Goldstein, and Y. H. Brown, Circulat. Res., 62, 299 (1989).
11. R. Levi, J. Burke, Z. Guo, et al., Circulat. Res., 54, 117 (1984).
12. R. Mitra and M. Morad, Proc. Natl. Acad. Sci. USA, 93, 5340 (1986).
13. O. H. Petersen, Nature, 336, 528 (1988).
14. K. B. Walsh and R. S. Kass, Science, 242, 67 (1988).