PAF—ANTAGONIST INTERACTION IN GUINEA PIG ATRIAL MYOCARDIUM

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Platelet activating factor (PAF), a key mediator of inflammation and shock-related states [1, 8], when acting directly on th guinea pig myocardium (10^{-7} M) reduces the amplitude and also considerably shortens the duration of intracellular action potentials of the myocardial fibers and depresses contractility of isolated myocardial preparations. Other workers also have obtained similar data [9, 11]. In the existing view [2] reduction of cardiac ejection in various shock-related states is largely attributable to a disturbance of the pumping function of the heart, one of the main causes of which is reduction of myocardial contractility. Various shock-related states caused by injection of synthetic analogs of PAF [6, 7, 12, 14] and endotoxins [5] are known to be weakened or completely abolished by some PAF antagonists [5, 8, 12]. However, in an investigation conducted on the isolated guinea pig heart, the PAF antagonist BN 52021 was shown to be unable to restore myocardial contractility when depressed by PAF.

Considering the urgent nature of the search for new drugs preventing the development of the negative inotropic action of humoral factors of inflammation and shock, we decided to compare the effects of three PAF antagonists on electrical and contractile activity of the guinea pig myocardium when disturbed by the action of PAF.

EXPERIMENTAL METHOD

The traditional microelectrode technique was used to measure intracellular transmembrane potentials of the myocardial fibers. A full account of the experimental technique and composition of the standard Tyrode solution was given previously [1, 3]. The left auricle of the atrium was placed in a chamber through which Tyrode solution, warmed to 30-32°C, flowed continuously. For 1 h before the beginning of the experiment and during its course the auricles were stimulated by above-threshold square pulses with a frequency of 0.5 Hz. Slow calcium action potentials (AP) in myocardial fibers were recorded while the K^+ ion concentration in the Tyrode solution was raised to 15.5 mM and the Ca^{++} ion concentration was raised to 6 mM. Cardiodepressive effects in the myocardium were induced by addition of PAF [$(1\text{-}2) \times 10^{-7}$ M], obtained from "Novobiochem" (Switzerland) or the Research Institute of Biomedical Technology, Ministry of Health of the USSR, to the perfusion fluid. No difference was found in the action of the two PAF preparations on the myocardium.

In a special series of experiments the electrical and contractile activity of the myocardium was disturbed by replacing the standard Tyrode solution by the blood serum of a patient with the clinical diagnosis of "sepsis" (five experiments). The patient's serum was diluted beforehand with Tyrode solution in the ratio of 1:1, the free Ca^{++} ion concentration in the serum being measured by means of ion-selective electrodes and adjusted to their level in Tyrode solution. Three PAF antagonists were used in the work: U-66985 ("Novobiochem"), Brotizolam ("Boehringer," West Germany), and BL-8701 (Research Institute of Biomedical Technology, Ministry of Health of the USSR, Moscow). The antagonists U-66985 and BL-8701 are similar in their chemical structure to PAF [4]. All antagonists were used within the concentration range of $(1\text{-}5) \times 10^{-6}$ M.

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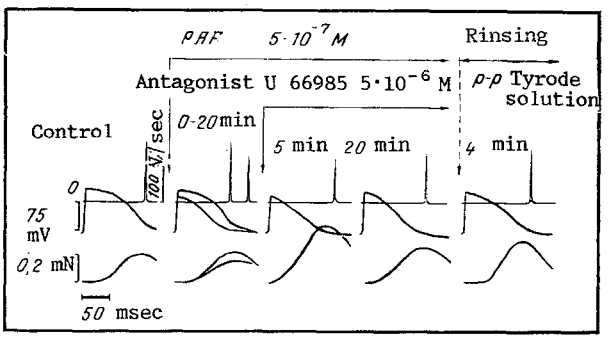

Fig. 1. Restoration of AP and contractions of guinea pig auricle, disturbed by PAF, under the influence of the PAF antagonist U-66985. Curves from top to bottom denote: maximal rate of rise of leading edge of AP, AP itself, contraction. Horizontal line is line of O potential. Control — Tyrode solution. Calibration: vertical axis, from top to bottom: 100 V/sec, 75 mV, 0.2 mN; horizontal axis: 50 msec.

EXPERIMENTAL RESULTS

The effect of PAF antagonists on electrical and contractile activity of the myocardium was studied first. Experiments showed that the antagonist U-66985 in a concentration of (1-5) x 10^{-6} M virtually did not change values of a resting potential (RP) but slightly increased the amplitude and steepness of rise of the leading edge of AP, while the amplitude of contractile responses of the myocardium preparations increased on average by 25% (n = 4). By contrast, Brotizolam (n = 3) and compound BL-8701 (n = 5), in the same concentrations, had no effect on the parameters of RP or AP. Brotizolam likewise did not change the amplitude of the contractions, whereas the antagonist BL-8701 reduced it a little.

Changes in intracellular potentials and isometric contractions of the auricle during the combined action of PAF and antagonist U-66985 are shown in Fig. 1. After 20 min of perfusion of the myocardial preparation, PAF (5 x 10^{-7} M) virtually did not change the amplitude of RP, but reduced the amplitude and steepness of rise of AP and considerably shortened its duration. These changes in the parameters of AP were accompanied by depression of contractility of the myocardium preparation. The antagonist U-66985 (5 x 10^{-6} M), when added to perfusion fluid containing PAF, led to a very small increase in the parameters of AP and completely restored myocardial contractility. After rinsing of the myocardial preparation with Tyrode solution further restoration took place of the original parameters of AP and the contractile responses, preceded at the beginning of rinsing by a sharp increase in amplitude of the contractions. Similar weakening of the cardiodepressor action of PAF also took place in cases when the antagonist was added to the perfusion solution before PAF. Neither Brotizolam nor the antagonist BL-8701 weakened the disturbances of intracellular potentials or the depression of the contractile responses of the myocardial preparations caused by PAF, regardless of the order in which the PAF and its antagonists were added to the perfusion fluid.

Later we studied the effects of separate and combined action of PAF and PAF antagonists during potassium depolarization of the membrane (15.5 mM KCl), which enabled calcium action potentials (Ca-AP) to be recorded. Since the amplitude of Ca-AP and of the corresponding contractile responses in Tyrode solution with an increased K^+ ion concentration depended on the strength of the stimulus, in this series of experiments the electrical and contractile activity of the myocardium was recorded during stimulation at the threshold and twice the threshold strength.

During stimulation at the threshold strength the amplitude of Ca-AP was reduced by the action of PAF (10^{-7} M) after 20 min to 20.2 ± 12.0% of its initial value (100%) in depolarizing solution without the mediator, and the amplitude of contractions was reduced to 25.2 ± 9.0% (n = 4). The amplitude of Ca-AP was increased to 42 ± 12.8% and of the contractions to 69.8 ± 9.8% 2 min after addition of the antagonist U-66985 to the perfusion fluid under the same conditions. At the 20th minute of the combined action of PAF + U-66985 the amplitude of Ca-AP was 81.0 ± 14.3%, and the amplitude of the contractile responses was 82.0 ± 8.7%. By contract, the antagonist BL-8701 caused virtually no change in the amplitude of Ca-AP and of the contractions.

Changes in the amplitude of Ca-AP (a) and of the contractions (b) in response to the separate action of PAF in a concentration of 10^{-7} M (the first 20 min) and the combined action of PAF + antagonist (U-66985 or BL-8701), under conditions when the stimulus was of twice the threshold strength, are demonstated in Fig. 2. Virtually all the

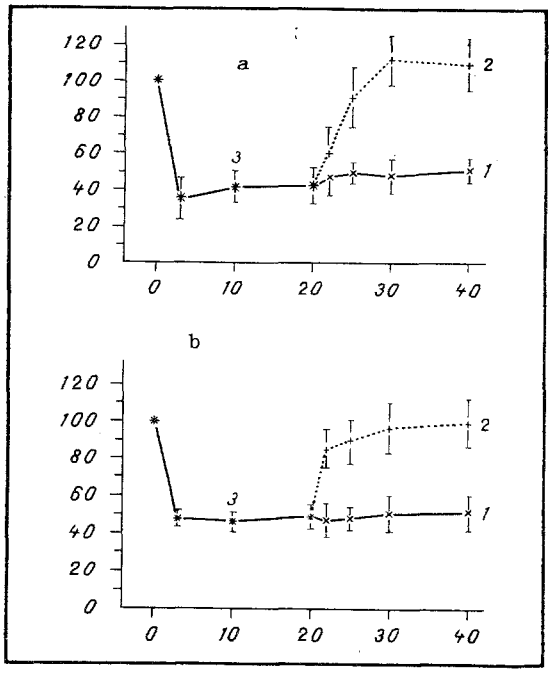

Fig. 2. Effect of two PAF antagonists on Ca-AP (a) and contractions of guinea pig auricle (b) against the background of the action of PAF. 1) PAF (10^{-7} M), 2) antagonist U-66985, 3) antagonist BL-8701. Abscissa, time (in min); ordinate, effect (in %). Antagonists added to solution at 20th minute of action of PAF. AP and contractions recorded during stimulation at twice the threshold strength.

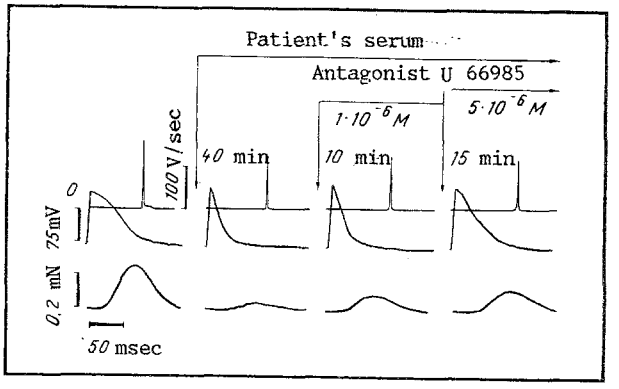

Fig. 3. Restoration of AP and contractions of guinea pig auricle under the influence of antagonist U-66985 and against the background of the cardiodepressive action of serum from a patient with sepsis. Legend as to Fig. 1.

relationships observed during stimulation at threshold strength were preserved, but all the curves were shifted upward along the ordinate.

In the final series of experiments the effect of antagonist U-66985 on the cardiodepressive action of the blood serum from a patient with the clinical diagnosis of sepsis was investigated. The serum was isolated from the patient's blood in different stages of the disease. In all five experiments the serum inhibited the electrical and contractile activity of the myocardium, but the antagonist U-66985 restored the duration of AP and the amplitude of contractions of the myocardial fibers in only one of the five experiments (Fig. 3).

The investigation thus showed that only antagonist U-66985 can abolish the direct cardiodepression action of PAF and of the blood serum of patients with wound infection. The absence of ability to restore electrical and

contractile activity of the myocardium in the other two antagonists tested is not yet understood because all the antagonists mentioned are able to prevent the platelet aggregation reaction induced by PAF [4, 10, 13]. Two suggestions may be put forward: 1) the mechanisms of interaction of PAF and its antagonists on the excitable membrane of the myocardial cells and the inexcitable membrane of blood cells are not identical; 2) unlike U-66985, the PAF antagonists BL-8701 and Brotizolam cannot interact with specific PAF receptors on the myocardial cell membrane and, consequently, they cannot prevent the cardiodepressive action of PAF.

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EFFECT OF THYMOSIN AND B-ACTIVIN ON LATERALIZATION OF SENSOMOTOR CONTROL IN RATS

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Reorganization of brain activity following the development of vascular and traumatic lesions and tumors is a problem many aspects of which remain far from clear, so that there is a consequent lack of methods of targeted correction of the restoration of brain functions. Much evidence has been obtained of significant differences between the cerebral hemispheres, not only from the functional, but also from the biochemical [4-6, 9, 12, 14] and immunologic points of view [7, 10], and also with respect to their electrophysiological characteristics [1-3]. These data demand a differential approach to lesions of the right and left hemispheres, and point out some directions for the search of regulators of compensation and recovery processes in the CNS.

The aim of this investigation was to study the role of endogenous peptide regulators thymosin and B-activin (myelopide) as lateralized (selective) modulators of functions of the right and left hemispheres.

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