Role of Prostaglandins and Cyclic Nucleotides in the Mechanism of Development of Drug-Induced Thrombocytopenia

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Hormonal regulation plays an important role in the complex chain of regulatory mechanisms of thrombocytopoesis [1-5]. It has recently been established that hormonal effects are mediated by cyclic nucleotides (cAMP and cGMP) which are hormonal messengers and intercellular regulators [7] participating in the regulation of cell proliferation and differentiation [9,10,12-15]. Antagonistic interrelations have been shown to exist between cAMP and cGMP [8].

In previous research we showed that the adrenals and the insulin-producing tissues of the pancreas have a marked influence on thrombocytopoesis both in health and during drug-induced thrombocytopenia [1-3]. However, the intimate mechanisms of these effects are still to be studied.

In the present work we aimed to study the blood content of some prostaglandins (PG) and cyclic nucleotides (CN) as hormonal messengers during the development of drug-induced thrombocytopenia and to investigate the role of these bioactive compounds in the mechanism of thrombocytopoesis regulation.

MATERIALS AND METHODS

Experiments were carried out on mongrel dogs (male and female) weighing 14-16 kg. Thrombocytopenia was induced by intravenous injections of 0.7 mg/kg rubimycin daily, five times a day. The blood platelet count and concentration of CN as well as of PGE and $PGF_{2\alpha}$ (by radioimmunoassay) were determined both before and after rubimycin administration. Amersham standard kits (England) were used for cAMP and cGMP measurements; PG were measured using the DRG kit (USA) for PGE determination and a Hungarian kit for $PGF_{2\alpha}$. Radioactivity was assessed using an LKB-Wallac β-counter (Finland). Bone marrow counts of megakaryocytes and myelokaryocytes were determined. The day of the first rubimycin injection was considered the first day of the experiment. All the results were compared to the initial values and processed statistically using the Student *t* test.

RESULTS

The results of the investigation showed a significant decrease of the platelet count on day 5, the maximum drop being attained on day 7.

More noticeable changes were revealed in the megakaryocyte count, which was reduced fivefold on day 5 as against the initial level. The maximal decrease of the megakaryocyte count was noted on the 7th day after intravenous rubimycin injection. Later, the megakaryocyte count somewhat increased, remaining, however, appreciably lowered during the whole period of observation.

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TABLE 1. Changes of Some Parameters of Megakaryocyte–Platelet System and of Blood PG and CN Content during Development of Drug–Induced Thrombocytopenia ($M \pm m$, $n = 6$)

Parameter	Basic level	Time of investigation, days						
		5	7	10	15	20	25	30
Platelets, 10^9 per liter	120±8.2	39.0±1.4*	25.7±0.9*	66.0±1.8*	63.4±2.3*	60.2±2.1*	63.6±2.5*	87.0±2.2*
Megakaryocytes, 10^6 per liter	68.7±4.5	12.5±1.04*	9.4±0.7*	16.4±1.1*	22.7±1.1*	17.6±0.9*	18.7±0.7*	25.0±1.5*
Myelokaryocytes, 10^9 per liter	124.4±3.4	54.8±1.8*	49.2±1.2*	80.2±1.8*	82.4±1.3*	70.3±1.1*	66.1±2.8*	90.6±1.1*
cAMP, pmole/ml	11.5±0.7*	29.4±1.7*	48.1±1.3*	37.4±1.4*	31.6±1.2*	32.1±1.2*	30.3±1.2*	46.1±1.3*
cGMP, pmole/ml	8.7±0.6	8.7±0.4	7.4±0.3	16.1±1.4*	15.6±1.2*	6.1±0.4	18.3±1.1*	15.3±0.8*
PGE, ng/ml	5.9±0.7	5.2±0.5	3.9±0.3*	4.7±0.4	4.7±0.4	2.9±0.28*	6.7±0.03	3.8±0.33*
$PGF_{2\alpha}$, ng/ml	0.8±0.1	0.3±0.01*	0.46±0.01*	0.39±0.01*	0.31±0.02*	0.38±0.08*	0.6±0.02	0.59±0.01

Note: asterisk: reliable differences of values compared to the basic level.

The curve of the changes in the myelokaryocyte count was similar to that of the megakaryocyte number, but with a less marked decrease. Thus, on day 5 the myelokaryocyte count was only half of the initial level. Its gradual recovery was observed beginning from day 10, the number of megakaryocytes comprising 24% of the initial level and the myelokaryocyte number attaining 64% by this time.

The results also showed that rubimycin administration was associated with marked disturbances of the blood levels of CN and their ratio. A significant increase of the cAMP content was noted starting from day 5, whereas the cGMP content had undergone practically no change by this time.

The maximal cAMP rise (more than four times surpassing the basal level) was observed on the 7th day. A noteworthy fact is that the concentration of this nucleotide remained significantly elevated during the whole period of observation. mA less marked increase in the cGMP content was noted beginning from day 10.

A sharp drop of the $PGF_{2\alpha}$ level in comparison with the initial level was revealed starting from the 5th day (Table 1). The changes in PGE were less significant, and this resulted in an increase of the $PGE/PGF_{2\alpha}$ ratio.

Thus, the bone marrow megakaryocyte count is reduced sevenfold, the blood cAMP level is increased significantly, while cGMP is unchanged in drug-induced thrombocytopenia, resulting in an increase of the cAMP/cGMP ratio; the $PGF_{2\alpha}$ and PGE levels drop simultaneously. A remarkable fact is that the maximal decrease of the $PGF_{2\alpha}$ content was observed as early as on the 5th day.

One may suppose that rubimycin has an effect on the receptors of the megakaryocyte membranes and of hemopoetic precursors in general. According to published data [5], the adenylate cyclase-cAMP system is responsible for the realization of certain functions and effects. It seems that rubimycin causes a rise of the cAMP level, suppressing cell proliferation in general, including that of hemopoetic cells. It can be stated that megakaryocytes are the most sensitive to cAMP in comparison with other hemopoetic cells, as evidenced by our data on the bone marrow megakaryocyte count being only 13% vs. the initial level, while the fraction of other bone marrow nuclear elements constitutes almost 40% of their initial level. This is also confirmed by the time course of thrombocytopoesis recovery, namely, by a pronounced decrease of the megakaryocyte count on day 30 (37% of the initial level), while the myelokaryocytes count is 73% of the initial value. It is also noteworthy that beginning from day 10 there is a rise of the blood cGMP level, this nucleotide reportedly stimulating proliferation and inhibiting cAMP. As far as the levels of the PG studied are concerned, the maximal decrease of the $PGF_{2\alpha}$ content and an increase of the $PGE/PGF_{2\alpha}$ ratio are observed on day 5, i.e., before maximal thrombocytopenia.

Consequently, the above shifts can be considered as one of the mechanisms of the organism's adaptive reaction directed at the restoration of the quantitative and qualitative composition of the megakaryocyte-platelet system.

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BIOPHYSICS AND BIOCHEMISTRY

β-Adrenoreceptors of the Cerebral Cortex in Experimental Neurosis

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It is common knowledge that stress factors exert their effects on the brain by alterating the lipid component of cell membranes [1,2,5]. However, the interpretation of the adaptive, compensatory, and disadaptive changes of the lipid bilayer on the level of membranes, cells, parts of the brain, and the whole brain is not quite clear, especially in chronic stress, when contrasting processes are taking place. For example, the model of experimental neurosis reveals that after 1-3 weeks of combined stress, on the one hand, the capacity of the antiradical system is enhanced, leading to an inhibition of free-radical processes and to the depletion of cholesterol in the cell membrane, while, on the other hand, pathological changes in the cerebral membrane phospholipid pattern are observed, primarily as a result of a diminished proportion of readily oxidized lipids [2]. Thus, experimental neurosis, possessing incontestable features of free-radical pathology, can also be considered as an example of pathological adaptation (what has been termed adaptation with a high structural price [5]) of brain membranes to chronic stress. The revealed changes in membrane structure and composition point to probable functional alterations which result from the development of experimental neurosis. Nevertheless, the findings offer no direct information on the functional changes in membrane-associated processes. It should be very useful to analyze the β-adrenoreceptors (β-AR), which are the most widespread receptors in the brain. The indexes of β-AR activity are determined by the state of the lipid component of membranes.

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