The State of Mast Cells and Hemocoagulation during Chronic Norepinephrine Treatment in Mountain Dogs

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The active product of mast cells heparin, has an effect on homeostasis and cell metabolism and is in general an antistressive and antihypoxic agent [3,10]. Our investigations confirmed that the state of the coagulation hemostatic system correlates with the functional activity of mast cells both under normal conditions and for norepinephrine (NE) injections [11,12]. It is well known that high-altitude "aborigines" express a somewhat different level of physiological indexes, including the state of hemocoagulation [4,7,11], and a different progression of pathological processes [1,2].

The aim of this investigation was to study special features of the state of mast cells and hemocoagulation during chronic NE injection in mountain dogs.

MATERIALS AND METHODS

Experiments were carried out on four groups of mongrel dogs: the first group comprised low-altitude untreated dogs (Bishkek, 760 m), the second group low-altitude animals treated with NE, the third group untreated medium-altitude dogs (1650 m), and the fourth group medium-altitude dogs treated with NE. Norepinephrine was injected through a catheter pre-implanted in the jugular vein in a dose of 0.56 μg/kg/min during two hours per day over a period of six days. One day after withdrawal of NE, blood was taken from the vein; the animals were sacrificed with thiopental, and skin specimens were dissected for morphological study. The methods of processing and analyzing the mast cells and hemocoagulation parameters were described previously [12].

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RESULTS

A comparison between the groups of untreated low-altitude and medium-altitude animals showed that the latter had an increase of 22% in the number of mast cells. A high percentage of young forms with a diameter of up to 13 μ (71±3.1% versus 18±2.2% $p<0.001$) and a low percentage of intermediate and mature forms were noted in the medium-altitude animals. Degranulation of cells, mainly third-degree, was better expressed in the low-altitude group. The mast cells of both groups were evenly distributed in the connective tissue, with no signs of grouping, with granules of uniform size; they stained well with toluidine blue. The mast cells of the medium-altitude animals had a medium diameter (more then 18 μ) and were mainly observed in the immediate vicinity of blood vessels (Fig. 1). This testifies with a high probability that their secretion enters the blood.

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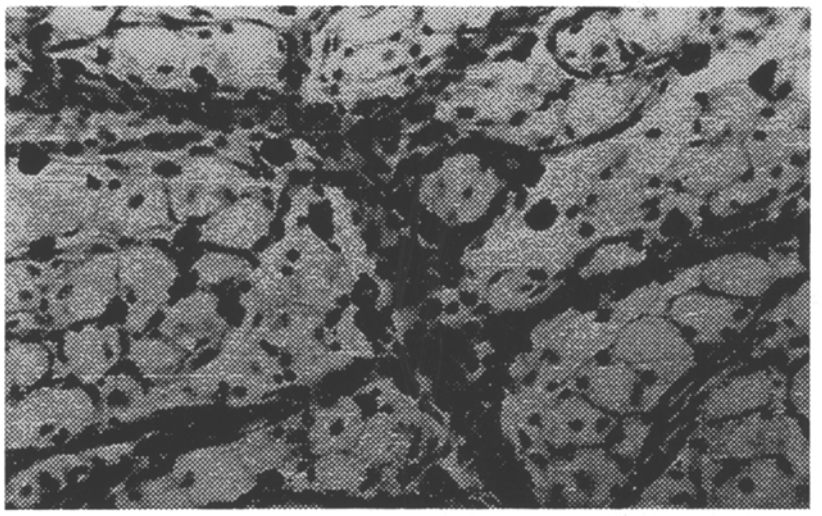

Fig. 1

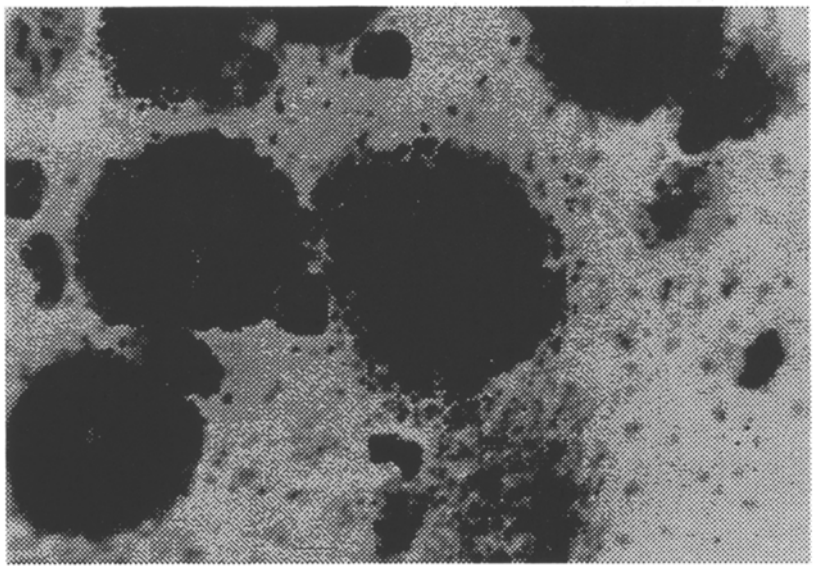

Fig. 2

Fig. 1. Mast cells along the blood vessels in mountain dogs (control). Toluidine blue staining, ×160.

Fig. 2. Group of mast cells in mountain dogs (experiment). Toluidine blue staining, ×400.

Norepinephrine injection resulted in qualitative changes of mast cell state in the medium-altitude group, and in comparison with the results obtained for the treated low-altitude animals [12] some special features were observed. The number of mast cells with respect to the untreated group decreased less dramatically among the medium-altitude treated animals (by 15% versus 37% in the low-altitude group). The average diameter practically did not change. The number of young and mature cells decreased, whereas the number of intermediate cells increased. It may be assumed that the decrease of young forms is related to a decrease of their differentiation from precursor cells. In the group of treated low-altitude animals the distribution of mast cells according to their degree of maturity was as follows: the number of young forms increased, while that of mature forms decreased. The frequency of pronounced degranulation significantly increased. It should be noted that the number of cells with third-degree degranulation was lower than among the treated low-altitude animals. In our view, this was because the mast cells of the treated medium-altitude group mainly had first-degree degranulation. Preserved cells looked friable, lost their distinctness, and became less intensively colored. Less frequent were clumps of cells and large areas full of metachromatic granules. There was a tendency for the mast cells to form groups and chains during chronic NE treatment (Fig. 2). The appearance of degranulated cells with vacuoles and fine granulation and the decreasing of color intensity testify to mast cell hypofunction.

A comparison of coagulograms from the untreated groups of low and medium altitude showed (Table 1) that the medium-altitude dogs had more active plasma coagulation factors, involved in the phase of contact activation (the recalcification activated time was shorter), a higher heparin blood level, a lower content of b-fibrinogen and a lower thromboplastin inactivation index, and a weaker aggregation capacity in spite of an increased number of platelets. This suggests that the anticoagulation properties of the blood were enhanced. After chronic NE treatment the medium-altitude dogs demonstrated a decrease of maximal coagulation activity assayed by the anticoagulation test, attesting to the development of hypocoagulation. The level of fibrinogen and b-fibrinogen increased and free plasma heparin decreased,in contrast to the case with the low-altitude animals. An increase of fibrinogen degradation products characterized the reinforcement of the anticoagulation system. Enhanced platelet aggregation was noted with no change in the adhesive properties of the platelets.

Thus, intact medium-altitude dogs have an increased total number of mast cells. The fourfold percentage augmentation of young forms strongly points to stepped-up production of mast cells. It may be assumed that the increased production under mountain condi-

TABLE 1. Main Indexes of Mast Cell Morphofunctional State and Hemocoagulation under Chronic Injection of NE

Index	Low-altitude groups		Medium-altitude groups	
	control (n=10)	experiment (n=8)	control (n=10)	experiment (n=10)
Mast cells:				
number	65±4.8	41±3.2*	79±2.9+	67±5.4
diameter, μ	15.8±0.4	13.9±0.5*	12.8±0.1+	12.8±0.8
degree, of maturity, %				
Young	18±2.1	42±5.1*	71±3.1+	44±4.2*
Intermediate	51±3.6	47±3.9	13±2.9+	48±3.7*
Mature	31±4.1	11±2.6*	16±4.6+	8±2.3
Degree of degranulation, %				
I	63±4.5	28±2.8*	77±4.1+	55±3.9*
II	26±2.8	37±1.2*	17±2.4+	29±2.6*
III	11±1.1	35±2.1*	6±2.1	16±4.1
Blood plasma:				
free heparin, sec	12±1.1	18±2.3*	22±2+	15±1.1*
Recalcification activated time, sec	44±3.6	32±1.9*	27±1.4+	26±5
maximal coagulative activity, %	66±5.1	44±7.2*	72±2.1	61±3.5*
inactivated thromboplastin index, c.u.	1.5±0.08	1.2±0.05*	1.2±0.04+	1.2±0.07
fibrinogen, mg%	396±72	446±96	217±24	690±120*
b–fibrinogen, mg%	60±5.8	86±6.3*	47±4.0+	79±8.4*
fibrinolitic activity, %	35±6.2	48±11.7	30±3.9	40±3.8
fibrinogen degradation products, mg%	70±9.7	159±25.9*	60±8.3	130±32.3*
Blood platelets:				
maximal aggregation percentage	78±2.8	76±6.4	55±5.1+	74±5.9*
aggregation time, sec	288±54	340±64	408±73	640±129
deaggregation time, sec	506±72	350±59	672±76	310±137*
adhesiveness index, c.u.	1.2±0.1	1.2±0.2	1.2±0.05	1.3±0.1

Note: A plus sign – reliably in relation to control–control; an asterisk –reliably in relation to treatment–control; c.u. – conventional unit.

tions arises from the activation of a neurohumoral factor such as heparin cytotropic hormone [5].

Under NE treatment an external similarity in the reactions of low-and medium-altitude dogs was observed, but the mast cells and hemocoagulation system of the latter exhibited more tolerance to the damaging effect of catecholamine. The medium-altitude dogs underwent a significantly smaller decrease of mast cells , and demonstrated an increase of mature forms and a decrease of the number of III-degree degranulation cells. The reduction of young forms under chronic NE treatment correlated with a drop of the free heparin level in the medium-altitude dogs. This drop in the level of heparin can be partly related to the fact that its release occurs at the stage of incomplete synthesis [9]. This also may explain the appearance of incompletely mature mast cells in the connective tissue. These alterations took place along with the strengthening of the anticoagulation system and thrombinogenesis. In this connection it may be suggested that the drop of the heparin level is due to the enhancement of nonfermentative fibrinolysis [6]. Perhaps as a result of hypocoagulation, heparin cannot be functionally used and is deposited by endothelial and mast cells or captured by macrophages [8].

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