

Effects of beta-adrenergic receptor blockade on stress-induced changes in hematological parameters of rats

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ABSTRACT

This study was carried out to examine the effects of acute and chronic stress on hematological parameters in combination with β -adrenergic receptor blockade in rats. In the present study, acute stress is a form of short-term stress induced by a single footshock session, and chronic stress is a form of long-term stress induced by multiple and repetitive footshock sessions.

Male Wistar rats were treated with propranolol (5 mg/kg s.c.) and were subjected to an acute stress [intermittent session of footshock: 0.5 mA, during 60s (5s shock, 5s pause) at one and three days after the moment of propranolol administration] and to a chronic stress [intermittent session of footshock: 0.5 mA, during 60s (5s shock, 5s pause) over three consecutive days from the moment of propranolol administration]. Control group rats were subjected to the same conditions without the propranolol administration and were treated with saline solution.

Four days after the drug administration, we assessed the number of total erythrocytes, the erythrocyte indexes (mean cell volume, MCV; mean cell hemoglobin, MCH; mean cell hemoglobin concentration, MCHC) and the number of thrombocytes.

The results indicate that hematological parameters differed under acute and chronic stress conditions in combination with adrenergic receptor blockade. β -adrenergic receptor blockade with propranolol enhances the total number of erythrocytes under acute stress influences and the total number of thrombocytes under chronic stress influences. Acute and chronic stress in combination with β -adrenergic blockade has no significant effect on erythrocyte indexes.

Propranolol administration in low doses during different forms of stress could prevent erythrocyte disorders after stress exposure and symptoms in post-traumatic stress disorders. Propranolol markedly suppressed the tachycardia induced by footshock stress after chronic administration. On the whole, the obtained data indicate the important role of β -adrenoreceptor mechanisms in the regulation of erythrocyte dynamics.

Key words: Propranolol, erythrocyte, thrombocyte, erythrocyte indexes

INTRODUCTION

Adrenergic compounds are extremely important factors in neuroendocrine regulation of immune system function in the norm and in the case of different pathological conditions. Concurrently, β - and α -adrenoreceptors are expressed on the cells of the immune system and on the erythrocyte system. The presence of adrenergic receptors on white blood cells demonstrates a modulatory role of noradrenergic system in hematopoiesis [1].

Stress exerts different effects on neuroendocrine reactions. Acute and chronic stress exposure activates both the hypothalamo-pituitary-adrenocortical (HPA) and sympatho-adrenomedullary systems [2]. In turn, through their secretion, glucocorticoids and catecholamines suppress a variety of immune functions [3]. Some studies have shown that actions of stress may result in inhibition of cell-mediated and humoral activity, and also of the uptake activity of the system of mononuclear phagocytes. Role of adrenergic mechanisms in these changes has not been clarified, although they are important in the redistribution of T lymphocytes and the regulation of medullar hemopoiesis under stress. These mechanisms act through the α - and β -adrenoreceptors that are expressed on cells of the immune system and on the erythrocyte system.

The aim of this study was to investigate the influence of the blockade β -adrenoreceptors on the number of total erythrocytes, the erythrocyte indexes (mean cell volume, MCV; mean cell hemoglobin, MCH; mean cell hemoglobin concentration, MCHC) and the number of thrombocytes in rats exposed to acute and chronic footshock stress.

MATERIALS and METHODS

Male Wistar rats weighing $200\text{g} \pm 25\text{g}$ at the beginning of experiments were used. The animals were housed in groups of six in a temperature- and light-controlled room (22°C , 12-h cycle starting at 08:00h).

They were fed and allowed to drink water ad libitum. Rats were treated in accordance with institutional guidelines of animal bioethics from the Act of Animal Experimentation and Animal Health and Welfare Act from Romania.

Propranolol hydrochloride was purchased from Sigma. All test compounds were dissolved in sterilized pyrogen-free physiological saline. Rats were divided into three groups: 1. saline with acute and chronic footshock treatment; 2. propranolol with acute footshock treatment; and 3. propranolol with chronic footshock treatment.

Prior to the acute footshock stress, rats in group 2 were treated with propranolol (5 mg/kg, s.c.) and then were subjected to an intermittent session of footshock: 0.5 mA, during 60s (5s shock, 5s pause) one and three days from the moment of the propranolol administration, and then returned to the home cage. Control group rats were subjected to the same conditions and were treated with physiological saline.

The rats in group 3 were treated with propranolol (5 mg/kg, s.c.) and then were subjected to chronic footshock stress [intermittent session of footshock: 0,5 mA, during 60s (5s shock, 5s pause)] over three consecutive days from the moment of propranolol administration, and then returned to the home cage. Control group rats were subjected to the same conditions without the propranolol administration and were treated with physiological saline.

Four days after the drug administration, whole heparinized blood was collected.

To determine erythrocyte and thrombocyte (mm^3) counts, blood sample was taken with an erythrocytes pipette and diluted (1/200) with Hayem solution. One drop of hemolyzed blood was transferred onto Neuberg's hemocytometer, onto the counting area, and then cover-slipped. The blood sample was therefore monolayered in a space of 0.1 mm height. The total number of erythrocytes and thrombocytes in a 5 mm^2 area was counted and expressed as the number of erythrocytes and thrombocytes from 1 mm^3 whole blood.

The blood samples were examined in a light microscope KRÜSS model with a magnification of 400x.

To determine erythrocyte indexes (MCV, MCH, MCHC), we used a COULTER® A^C-T™ 5 diff CP blood analyzer, due to the high sensitivity of this method.

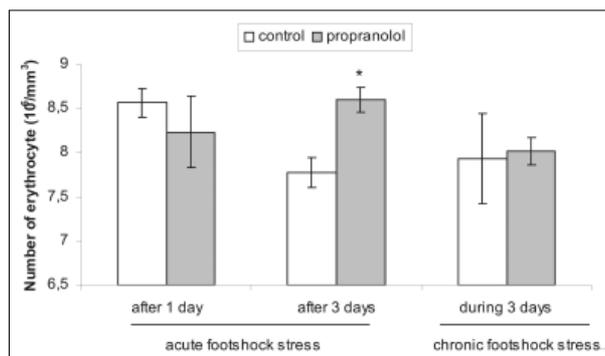


Figure 1. Changes in the total number of erythrocytes after one and three days of propranolol administration under acute and chronic footshock stress influences. Values are expressed as mean \pm SEM (n = 6). *P<0.05 vs. control.

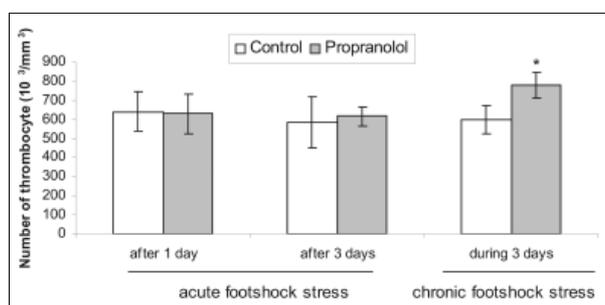


Figure 2. Changes in the total number of thrombocytes after one and three days of propranolol administration under acute and chronic footshock stress influences. Values are expressed as mean \pm SEM (n = 6). *P<0.05 vs. control.

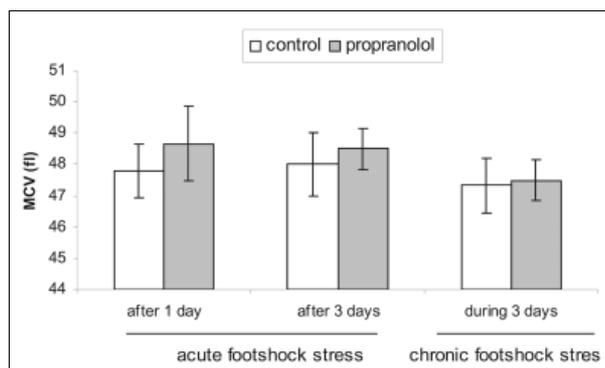


Figure 3. Variation in MCV (mean cell volume) after one and three days of propranolol administration under acute and chronic footshock stress influences. Values are expressed as mean \pm SEM (n = 6).

Statistical methods

Data were expressed as mean \pm SEM. The data were analyzed statistically by means of the Student's-t test. P values less than 0.05 were considered significant. Number of observation was 30.

RESULTS

The total number of erythrocytes decreased significantly in the control group after three days

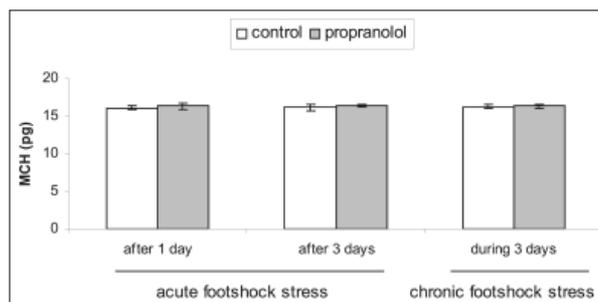


Figure 4. Variation in MCH (mean cell hemoglobin) after one and three days of propranolol administration under acute and chronic footshock stress influences. Values are expressed as mean \pm SEM (n = 6).

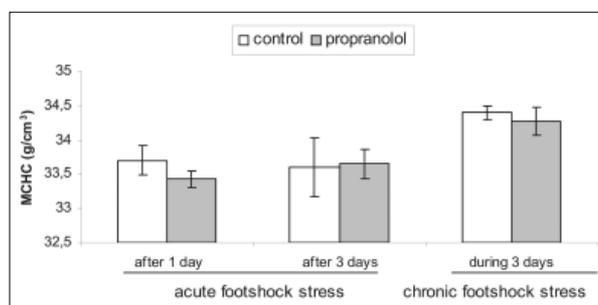


Figure 5. Variation in MCHC (mean cell hemoglobin concentration) after one and three days of propranolol administration under acute and chronic footshock stress influences. Values are expressed as mean \pm SEM (n = 6).

under both the acute and chronic stress influence. In the groups treated with propranolol, the total number of erythrocytes increased significantly ($p < 0.05$) only after three days under the acute stress influence; the number decreased in the chronic stress condition (Figure 1). The total number of thrombocytes increased significantly ($p < 0.05$) in the group treated with propranolol under chronic stress influences (Figure 2).

There were no statistically significant differences in the erythrocyte indexes (MCV, MCH, and MCHC) in animals treated with propranolol under acute and chronic stress conditions (Figures 3, 4, 5).

DISCUSSION

The data obtained in this study indicates that the suppressive actions of stress may be antagonized under conditions of pharmacological blockade of β -adrenoreceptors by means of propranolol administration (5 mg/kg, s.c.) during a consecutive three days at the beginning of the experiment.

While acute stress exerts stimulative effects three days after the propranolol administration, chronic

stress induced suppressive effects on erythrocyte distribution but not on thrombocyte distribution.

Catecholamines during stress apparently may serve as synergists in respect to other hormones, like glucocorticoids, which have been studied to the greatest extent in the development of stress factor immunodepression. It is known that glucocorticoids enhance the expression of β -adrenoreceptors^[4] and the number of total erythrocytes^[5]. Since the effect of the blockade of β -adrenoreceptors is directly opposite to the actions of endogenous catecholamines^[6], it may be presumed that a part of the effects of glucocorticoids during stress may be mediated through the actions of catecholamines on β -adrenoreceptors, as we observed after three days of propranolol administration under acute stress influences, but not under chronic stress influences.

β -adrenoreceptor blockade does not have significant influences on erythrocyte indexes during the action of acute and chronic stress.

With such processes like stress, the adrenergic mechanisms may play a key role in changes in the immune system, the redistribution of T lymphocytes and the regulation of medullar hemopoiesis.

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Stress may have a bi-directional effect on immune function such that under certain (acute, physiologically adaptive, eustress) conditions, stress may result in immuno-enhancement, whereas under other (chronic, physiologically maladaptive, distress) conditions it may be immunosuppressive^[7, 8, 9, 10, 11]. Our data demonstrated that acute footshock stress results in a rapid, significant, and rapidly reversible decrease in absolute numbers of erythrocytes after one day of propranolol administration, which returns to normal number after three days of propranolol administration.

On the basis of our results obtained by acute and chronic stress influences in combination with β -adrenoreceptors blockade, we can conclude that in rats, acute stress has a significant influence, less than that of chronic stress, and the adrenergic mechanism plays a key role in regulation of hemopoiesis.

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