

Effects of Hydrocortisone on Peritoneal Phagocytizing Cells in Rats under Conditions of β -Adrenoceptors Blockade

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Hydrocortisone decreased the count of peritoneal mononuclear phagocytes and mast cells and total phagocytic activity of peritoneal phagocytes in rats, but had no effect on the nitroblue tetrazolium test. β -Adrenoceptor blockade abolished the suppressive effect of hydrocortisone on phagocytosis and prevented the decrease in the count of mast cells, but markedly reduced the number of neutrophils and parameters of stimulated nitroblue tetrazolium test.

Key Words: *hydrocortisone; propranolol; β -adrenoceptors; phagocytosis*

Our previous studies showed that β -adrenoceptor blockade modifies the effect of acute stress on phagocytizing cells and causes their activation [7]. These changes can be realized through modulation of β -adrenoceptor expression by glucocorticoids, which produce permissive effects on target cells in various organs (in relation to the influence of catecholamines) [3]. Here we studied the effect of hydrocortisone on phagocytizing cells under conditions of β -adrenoceptor blockade.

MATERIALS AND METHODS

Experiments were performed on male Wistar rats weighing 198.4 ± 16.5 g. Group 1 animals were intraperitoneally injected with hydrocortisone acetate in a single dose of 50 mg/kg (ImBio). Group 2 rats received 50 mg/kg hydrocortisone during β -adrenoceptor blockade with propranolol hydrochloride (obsidan, ISIS Pharma GmbH). Propranolol hydrochloride was injected subcutaneously in a dose of 5 mg/kg 30 min before treatment with hydrocortisone and then at 3-h intervals (4 times). The treatment schedule and doses of prepara-

tions were selected in previous experiments [2,4]. Intact animals served as the control. The animals were euthanized 48 h after treatment with hydrocortisone.

The suspension of peritoneal cells was obtained by washing of the peritoneum with medium 199 containing 20 U/ml heparin, 10 mM HEPES, and 2 mM L-glutamine. The cell composition was estimated microscopically. We used buffered medium 199 containing 2 mM L-glutamine and 10 mM HEPES (pH 7.36). The oxygen-dependent mechanisms underlying bactericidal properties of phagocytizing cells were studied spectrophotometrically by the nitroblue tetrazolium (NBT) test with modifications [1]. The stimulated sample contained 25 μ l peritoneal cells (10^7 /ml medium), 25 μ l autologous plasma, 25 μ l opsonized zymosan (3 mg/ml 0.15 M NaCl, Sigma), 25 μ l medium, and 50 μ l 0.15% NBT in 0.15 M NaCl.

In the spontaneous test the sample contained similar components and 25 μ l 0.15 M NaCl (instead of zymosan). The measurements were performed as described previously [1], except for a 1.5-fold higher volume of reagents.

To estimate phagocytic activity of peritoneal cells, we mixed 10 μ l cell suspension (10^7 /ml medium), 10 μ l autologous plasma, and 10 μ l washed formalinized sheep erythrocytes (objects of phagocytosis, 2×10^8 cells/ml medium) in microtubes coated with antiadhe-

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sive materials. The samples were incubated at 37°C for 20 min and studied microscopically.

The absolute count of phagocytized objects (product of the phagocytic number by the absolute count of cells), phagocytic index (average number of objects phagocytized by each cell), percent of activated phagocytes (ingested 2 and more objects), and specific contribution (percent of objects phagocytized by cells of the certain type in relation to the total number of objects ingested by all phagocytizing cells) were estimated individually for mononuclear phagocytes, neutrophils, and mast cells [6]. The ability of mast cells and blood basophils to phagocytize and kill microorganisms was demonstrated previously [8,10]. The results were analyzed by Student's *t* test and Wilcoxon *W* test.

RESULTS

Hydrocortisone decreased the absolute count of rat peritoneal monocytes, macrophages, and mast cells, but did not change the number of neutrophils (Table 1). This is consistent with published data that glucocorticoids decrease the count of skin mast cells [9] and suppress clearance functions of mononuclear phagocytes [5]. Hydrocortisone decreased the total count of mononuclear phagocytes and granulocytes. β -Adrenoceptor blockade abolished the hydrocortisone-induced decrease in the count of mast cells. However, the absolute number of peritoneal neutrophils in animals treated with hydrocortisone during β -adrenoceptor blockade was much lower than in animals receiving hormone alone. Administration of hydrocortisone to animals during blockade of β -adrenoceptors decreased the total count of nucleated cells.

Hydrocortisone decreased the relative (phagocytic index, percent of phagocytosis, and percent of active

phagocytes) and absolute parameters reflecting the total phagocytic activity of peritoneal cells (Table 2). Hydrocortisone-induced changes in the relative phagocytic activity were partially abolished during β -adrenoceptor blockade. In hydrocortisone-receiving rats mononuclear phagocytes played a less important role, while the contribution of neutrophils into total phagocytosis increased. This was related to the inhibition of phagocytic activity in mononuclear phagocytes. Hydrocortisone had no effect on the absolute phagocytic activity of neutrophils, but decreased their relative activity. β -Adrenoceptor blockade partially abolished the suppressive effects of hydrocortisone on monocyte/macrophage-mediated phagocytosis and relative phagocytic activity of neutrophils. However, the absolute phagocytic activity of neutrophils was suppressed due to the decrease in cell count (Table 2). Hydrocortisone did not change the relative phagocytic activity of mast cells, but decreased their absolute activity (Table 2). This was associated with the decrease in mast cell count (Table 1). β -Adrenoceptor blockade abolished this effect of hydrocortisone. In animals treated with hydrocortisone parameters of the NBT test did not differ from the control. However, hydrocortisone decreased absolute parameters of the HCT test during blockade of β -adrenoceptors (109.9 ± 20.4 vs. 212.7 ± 35.4 rel. opt. units in the control, $p < 0.05$, Student's *t* test). This effect was probably related to a decrease in the number of neutrophils and total count of peritoneal granulocytes and mononuclear phagocytes (Table 1), since the relative parameters of the NBT test remained practically unchanged ($p > 0.05$).

Our results indicate that the effects of glucocorticoids on phagocytizing cells are mediated by endogenous catecholamines that modulate β -adrenoceptor

TABLE 1. Peritoneal Cells (10^6 Cells per Peritoneum) in Rats 48 h After Treatment with Hydrocortisone during β -Adrenoceptor Blockade ($M \pm m$)

Parameter	Control (<i>n</i> =10)	Hydrocortisone (<i>n</i> =10)	Hydrocortisone and β -adrenoceptor blockade (<i>n</i> =10)
Total count of nuclear cells	126.7 \pm 10.9	113.4 \pm 15.6	89.1 \pm 11.4 ^{*,**}
Monocytes	22.1 \pm 3.9	11.0 \pm 1.7 ^{*,**}	11.3 \pm 1.5 ^{*,**}
Macrophages	11.3 \pm 2.0	5.1 \pm 0.7 ^{*,**}	4.3 \pm 0.5 ^{*,**}
Total count of mononuclear phagocytes	33.4 \pm 5.0	16.1 \pm 2.2 ^{*,**}	15.6 \pm 2.0 ^{*,**}
Neutrophils	13.2 \pm 2.3	14.5 \pm 2.1	9.1 \pm 1.3 ⁺
Mast cells	8.7 \pm 1.9	4.5 \pm 0.8 ^{**}	4.9 \pm 0.9
Total count of granulocytes and mononuclear phagocytes	55.4 \pm 8.3	35.1 \pm 4.1 ^{*,**}	29.7 \pm 3.9 ^{*,**}
Lymphocytes	71.3 \pm 5.6	78.3 \pm 12.7	59.4 \pm 8.0

Note. Here and in Table 2: $p < 0.05$ compared to the control: ⁺Student's *t* test, ^{**}Wilcoxon *W* test; $p < 0.05$ compared to group 1 rats receiving hydrocortisone: ⁺Student's *t* test, ^{**}Wilcoxon *W* test.

TABLE 2. Phagocytic Activity of Various Peritoneal Cells in Rats 48 h After Treatment with Hydrocortisone during β -Adrenoceptor Blockade ($M \pm m$)

Parameter	Control	Hydrocortisone	Hydrocortisone and β -adrenoceptor blockade
Total phagocytosis			
Phagocytic index	1.99 \pm 0.20	1.29 \pm 0.24*,**	1.52 \pm 0.15
Percent of phagocytosis	69.3 \pm 2.6	55.9 \pm 5.3*,**	64.1 \pm 1.8
Percent of activated phagocytes	49.1 \pm 4.0	31.9 \pm 5.9*,**	38.7 \pm 2.7*,**
Absolute count of objects, $\times 10^6$	107.7 \pm 16.8	45.8 \pm 9.7*,**	47.8 \pm 10.9*,**
Absolute count of phagocytizing cells, $\times 10^6$	38.4 \pm 5.7	19.9 \pm 3.2*,**	19.1 \pm 2.7*,**
Mononuclear phagocytes			
Phagocytic index	2.18 \pm 0.22	1.36 \pm 0.26*,**	1.62 \pm 0.16*,**
Percent of phagocytosis	72.4 \pm 2.5	58.1 \pm 5.2*,**	68.1 \pm 2.2
Percent of activated phagocytes	52.7 \pm 4.3	34.4 \pm 5.9*,**	41.0 \pm 3.0*,**
Absolute count of objects, $\times 10^6$	69.1 \pm 8.8	22.7 \pm 5.1*,**	26.6 \pm 5.7*,**
Absolute count of phagocytizing cells, $\times 10^6$	23.8 \pm 3.1	9.6 \pm 1.7*,**	10.6 \pm 1.4*,**
Specific contribution, %	67.7 \pm 2.6	48.8 \pm 4.5*,**	57.9 \pm 1.8*,**
Neutrophils			
Phagocytic index	1.93 \pm 0.26	1.31 \pm 0.25**	1.52 \pm 0.22
Percent of phagocytosis	67.2 \pm 2.8	55.3 \pm 5.5	59.8 \pm 2.5**
Percent of activated phagocytes	48.7 \pm 4.2	31.3 \pm 6.3*	38.1 \pm 4.4
Absolute count of objects, $\times 10^6$	26.4 \pm 6.4	19.0 \pm 4.4	14.8 \pm 4.2**
Absolute count of phagocytizing cells, $\times 10^6$	9.2 \pm 1.9	8.1 \pm 1.5	5.4 \pm 0.9**
Specific contribution, %	23.8 \pm 2.2	42.2 \pm 3.9*,**	30.5 \pm 2.4*,**,+**
Mast cells			
Phagocytic index	1.14 \pm 0.18	0.88 \pm 0.20	1.05 \pm 0.12
Percent of phagocytosis	57.2 \pm 5.7	46.5 \pm 6.4	55.6 \pm 4.1
Percent of activated phagocytes	30.0 \pm 6.2	25.0 \pm 6.7	29.5 \pm 4.4
Absolute count of objects, $\times 10^6$	10.8 \pm 3.3	3.9 \pm 1.1**	4.80 \pm 0.97
Absolute count of phagocytizing cells, $\times 10^6$	5.3 \pm 1.4	2.1 \pm 0.4*,**	2.7 \pm 0.6
Specific contribution, %	8.5 \pm 1.4	9.1 \pm 2.3	11.5 \pm 1.5

Note. Absolute parameters are calculated per total peritoneal cavity.

expression. These data open a new area in the study of relationships between immunomodulatory activity of catecholamines and glucocorticoids.

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