AMPHE TAMINE ENHANCES NATURAL KILLER CYTOTOXIC ACTIVITY VIA β-ADRENERGIC MECHANISM

Although addiction to amphetamine (AMPH) is a serious social and medical problem, the data concerning AMPH – immune interactions are still not numerous. To analyze the mechanism of AMPH-induced changes in the function of the immune system, rats were pretreated with β-adrenergic receptor antagonist propranolol (PROP; 5 mg/kg, i.p.) prior to AMPH (1 mg/kg, i.p.) administration. Natural Killer cells cytotoxicity (NKCC) ($\text{^{51}}\text{Cr}$-release assay), the number of LGLs (NK cells) (Timonen method), leukocytes, lymphocytes and monocytes, and plasma corticosterone level (CORT) (RIA) were evaluated in the peripheral blood and spleen. In the peripheral blood increases in NKCC (+331%), as well as in LGL (+33%) and monocyte (+65%) number observed after AMPH were partially inhibited by PROP (respectively by 30%, 19%, and 30%) in contrast to lymphopenia (-19%) and granulocytosis (+65%) which were not affected by β-blockade. In the spleen AMPH-induced decreases in NKCC (-25%) and in all the leukocyte populations number (approximately -30%) were completely blocked by PROP. Plasma CORT level, highly elevated by AMPH (+337%), was attenuated nearly by 50% under β-adrenergic blockade. These data indicate that AMPH-induced enhancement of cytotoxic activity of NK cell is related to β-adrenergic mechanism.

Key words: amphetamine, Natural Killer cells cytotoxicity, large granular lymphocytes, propranolol, adrenergic system, immune system, peripheral blood, spleen, rats

INTRODUCTION

Amphetamine (AMPH), besides its well known addictive properties, was found to influence the immune functions as a potent immunosuppressor. AMPH and its derivatives (eg. 3,4-metylenedioxymethamphetamine and fenfluramine) cause a decrease in leukocyte and lymphocyte (particularly T helper
lymphocytes) numbers in the peripheral blood (1 - 3). In addition, amphetamines were found to suppress cytokine and antibody production, lymphoproliferative responses, as well as to decrease in natural killer cells cytotoxicity (NKCC) and induction of cytotoxic T lymphocytes (1, 2, 4). However, opinions concerning the influence of AMPH on the immune system are not uniform. Some authors (3, 5, 6) pointed to a possibility of the enhancement of the immune system responses. In our recent study (7) we demonstrated that AMPH can lead to an increase in NKCC and the number of large granular lymphocytes (LGL) identified with NK cells. The precise mechanism of AMPH-immune interactions is still not fully understood.

AMPH can act either directly on peripheral cells (6) or indirect by affecting the neuroendocrine pathway. Acute and chronic AMPH administrations cause a marked stimulation of the hypothalamic-pituitary-adrenal axis and sympathetic nervous system resulting in the elevation of glucocorticoids (e.g. corticosterone, CORT) and catecholamines (epinephrine and norepinephrine) levels (7, 8). Glucocorticoids and catecholamines are known to have strong immunomodulating properties (9, 10).

In search for a possible mechanism behind the immunomodulating effect of AMPH, in the present study we tested an involvement of the adrenergic system – its β-adrenergic component. After pretreatment with propranolol (PROP), a β-adrenergic antagonist, we evaluated the effect of AMPH on NKCC, the numbers of LGL and leukocyte populations (lymphocyte, granulocyte, and monocyte) in the peripheral blood and spleen.

MATERIAL AND METHODS

Animals

The experiments were performed on 40 male Wistar rats, weighing approximately 250 g at the beginning of the experiment. The animals were housed in individual cages with free access to food and water under a 12 h light/12 h dark illumination cycle. They were handled daily for about two weeks before the experiment to minimize stress evoked by the experimental procedures. The animals were divided randomly to 4 experimental groups: (I) PROP-AMPH rats received β-adrenergic antagonist PROP 30 min prior to AMPH injection (n=10); (II) SAL-AMPH rats received saline (SAL) 30 min prior to AMPH injection (n=10); (III) PROP-SAL rats received PROP 30 min prior to SAL injection (n=10); (IV) SAL-SAL rats received two SAL injections (n=10).

All the protocols were reviewed and approved by the Local Ethical Committee for the care and use of laboratory animals in Gdańsk.

Drug administration

d-Amphetamine sulfate (Sigma, USA) was dissolved in 0.9% SAL and administered by i.p. injection in a dose of 1 mg/kg and volume of 1 ml. Propranolol hydrochloride (Sigma, USA) was dissolved in 0.9% SAL and administered by i.p. injection in a dose of 5 mg/kg and volume of 1 ml. The control injections of SAL were performed by the same route and in the same volume.
**Blood and spleen sampling**

The rats were killed by decapitation 30 min after the second injection and 4 ml of trunk blood and spleen were collected from each animal. NKCC and number of leukocytes, lymphocytes, granulocytes, monocytes and LGLs were determined in each blood and spleen sample.

**$^{51}$Cr-release assay**

Cytotoxicity of the peripheral blood and spleen NK cells was quantified using a $^{51}$Cr-release assay according to the procedure described previously (7). Briefly, the YAC-1 tissue culture cell line labeled with 100 µCi of Na$_2^{51}$CrO$_4$ (Radio Chemical Centre, Otwock-Swierk, Poland) was used as target cells, and peripheral blood mononuclear cells and spleen mononuclear cells were used as the effector cells for the determination of peripheral blood and spleen NKCC respectively. Target cells were cultured with effector cells under standard culture conditions for 4 h. Isotope count and the percent of specific lysis (specific $^{51}$Cr release) were performed with gamma counter. All results are presented in lytic units (LU$_{20}$).

**LGL number**

The percentage and total number of LGL was determined in the peripheral blood mononuclear cells and spleen mononuclear cells of each individual. This procedure was performed according to the method of Timonen et al. (11) with minor modifications which have been described previously (12).

**WBC and leukocyte subsets**

Total WBC (white blood cells) counts were determined with the use of the hematology analyzer (Baker System 9120 CP, Biochem Immunosystems). The percentage of lymphocytes, granulocytes, and monocytes was determined by counting 200 WBC with a microscope (May-Grünwald/Giemsa staining). The number of each leukocyte subset was calculated as WBC number × percentage of individual leukocyte subset.

**Corticosterone measurement**

Plasma CORT concentration was measured by radioimmunoassay using commercially available kit (ICN Biomedicals Inc., USA) and Wizard 1470 gamma counter (Pharmacia-LKB, Turku, Finland).

**Statistics**

The results are presented as mean ± SE. One-way analysis of variance (ANOVA) followed by the post-hoc Tukey test was used for the statistical analysis.

**RESULTS**

**Peripheral blood**

*Fig. 1* presents AMPH-induced changes in the peripheral blood NKCC and leukocyte populations in rats pretreated with PROP. AMPH caused an increase in NKCC (p<.001 vs. SAL-SAL group), which was partially blocked by PROP.
AMPH-induced increases in the numbers of LGL (p<.001 vs. SAL-SAL) and monocyte (p<.001 vs. SAL-SAL) were also significantly attenuated by PROP pretreatment (p<.001 vs. SAL-AMPH in both cases). In contrast, β-adrenergic antagonist did not change AMPH-induced decrease in lymphocyte number (p<.001 vs. SAL-SAL) neither it changed AMPH-induced increase in granulocytes (p<.001 vs. SAL-SAL). The total number of WBC remained unchanged after AMPH injection.

As shown in Fig. 2, plasma CORT level, highly elevated by AMPH (p<.001 vs. SAL-SAL), were attenuated nearly by 50% under β-adrenergic blockade (p<.001 vs. SAL-AMPH).

**Spleen**

Fig. 3 shows the effects of β-adrenergic blockade on AMPH-induced changes in splenic NKCC and leukocyte populations. PROP abrogated a decrease in NKCC (p>.05, comparison between PROP-AMPH and SAL-AMPH) and LGL number (p<.001, comparison between PROP-AMPH and SAL-AMPH) evoked
by AMPH. AMPH-induced decreases in the numbers of WBC (p<.001 vs. SAL-SAL) and of all the leukocytes populations (p<.05 vs. SAL-SAL in case of lymphocytes; p<.001 vs. SAL-SAL in case of granulocytes and monocytes) were completely blocked by β-adrenergic antagonist PROP (p<.01 vs. SAL-AMPH in
In contrast to the peripheral blood, AMPH injection resulted in the suppression of splenic NKCC and NK cell number. Both effects were completely blocked in rats pretreated with PROP. It was suggested that stress-induced sympathetic activation can cause a reduction of NK cells and NKCC in the spleen (18).

It is generally believed that the rise in circulating NK (and thus NKCC) cells resulted from its recruitment from the spleen via stimulation of β-adrenergic receptors (e.g. 19). However, other studies reported that catecholamine-induced NK lymphocytosis also occurred in splenectomized subjects (e.g. 15), indicating that there must be other than the spleen sources of mobilized immune cells. The data from in vitro studies indicate that catecholamines may be involved in recruiting NK cells from the blood vessels marginal pool into the circulation (16).

Above mentioned data suggest that AMPH-induced increase in NK activity may be due to the rise of NK cell number. However, an alteration in functional capacity of NK cells can not be excluded. The studies of Schedlowski et al. (13, 14) demonstrated that NKCC stimulation in stress to be a large extent resulted from increased lytic activity of individual cells. In this context, about 330% rise of NKCC (from 4.6 LU$_{20}$ to 25.1 LU$_{20}$, vide Fig. 1) observed in this study, can not be solely explained by 30% increase in effector cells (LGL – NK) number (from 3.44 × 10$^2$/µl to 5.05 × 10$^2$/µl) in the blood. Thus, the trafficking of NK cells from...
the spleen (or other sites) into the circulation can not be only explanation for AMPH-induced stimulation of NKCC in the peripheral blood.

In contrast to the general dependence of AMPH-induced alteration of splenic immune cells on β-adrenergic mechanism, changes in the peripheral blood were partially related to the adrenergic system. In detail, AMPH-induced lymphopenia and granulocytosis were not affected in rats pretreated with PROP. There are two possible explanations for these effects. First, catecholamines may be unable to influence circulating lymphocytes and granulocytes. In fact, Benschop et al. (20) reported that β-blockade did not inhibit the stress-induced alteration of granulocyte number. Second, AMPH-induced peripheral lymphopenia and granulocytosis may involve some other hormones, e.g. glucocorticoids. It is well established that CORT (and other glucocorticoids) mediates the stress-induced decrease in lymphocytes and increase in granulocyte numbers (e.g. 9). In this study, AMPH injection evoked a marked elevation of plasma CORT level. Thus, in the case of β-adrenergic blockade, glucocorticoids may be responsible for AMPH-induced lymphopenia and granulocytosis.

In conclusion, the present study demonstrates that AMPH-induced enhancement of NK cell number and lytic activity in the peripheral blood is mediated by β-adrenergic mechanism. Despite the increase in NK cell subset in the peripheral blood is accompanied by a decrease in NK cells in the spleen, we suggest that the recirculation of NK cells from the spleen not fully explains AMPH-induced stimulation of peripheral blood NKCC.

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