ROLE OF D2 DOPAMINE RECEPTOR ON MODULATION OF THE LEUKOCYTE FORMULA IN RESTRAINT STRESSED RATS

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Keywords: sulpiride, restraint stress, D2 dopamine receptor, leukocyte formula

Abstract: Dopamine is a monoamine neurotransmitter of both central and peripheral nervous system. Its role in the neural-immune communication has been discussed in the present study. Results reveal that in vivo blockade of D2 dopamine receptor by means of sulpiride, a selective antagonist for D2 dopamine receptor produce changes in functional activities of the immune effector cells. Adults rats pretreated once with LPS (a bacterial product) (25µg/250µl, i.p.), produce an immune response, were subjected to i.p. injection with sulpiride (4 mg/kg b.w., i.p.), a selective antagonist for D2 dopamine receptors, after 3 days postimmunization. After 18 days later, we assessed the total leukocyte number, neutrophils, eosinophils and basophils number. In summary, we provide that D2 dopamine receptor blockade suppress or enhance the immune effector cells number in restraint stress.

INTRODUCTION

Autonomy is not the final dictum regarding the functional activities of the immune system in higher organisms including human subjects. It is known that immune system is regulated, to a great extent, by central as well as peripheral sympathetic nervous system. This is primarily achieved by several neurotransmitters, neuropeptides, hormones and cytokines which interact with different immune effector cells and thereby ultimately regulate the homeostatic response of an individual disease and other environmental stresses (Weigent and Blalock, 1987). Among these neural mediators of homeostasis, catecholamines play a significant role. Although several reports are now available indicating the role of epinephrine and norepinephrine of the cathecolamine family on the immune system, information regarding the role of the central and peripheral dopamine on immune regulation is scanty and has only recently been known. This functional significance of dopamine on immune system is further strengthened by the presence of its receptors (Basu et al., 1993; Ricci and Amenta, 1994), specific endogenous dopamine transport system in leukocyte (Bondy et al., 1992; Basu et al., 1993) and finally the endogenous synthesis of this monoamine in leukocytes (Bergquist et al., 1994; Cosentino et al., 1999). The existence of putative dopamine receptors of D2, D3, D4 and D5 subtypes on immune cells has been proposed of several authors, primarily on the basis of dopaminergic ligand binding assays and specific mRNA expression as monitored by reverse transcription-PCR. Several experiments evoked the idea of a role for dopamine in modulating, mainly suppressing immune functions (Qui et al., 1994). Animals treated with bromocriptine, a dopamine agonist, also showed suppression of antibody production to SRBC and LPS (Besedovsky and del Ray, 1996) and suppressed activities of lymphocytes in mixed lymphocyte culture (Hiestand et al., 1986).Moreover, the interest regarding the role of dopamine on immune system becomes more relevant when some of the important neurological disease like Parkinson’s disease and schizophrenia with hypo- and hyperactivity (Temlett, 1996; Birtwistle and Baldwin, 1998) of central dopaminergic system are well-correlated with severe abnormalities of immune functions (Fiszer et al., 1991; Muller et al., 1993).

MATERIALS AND METHODS

Animals

The experiments were carried out on male Wistar rats weighing 180-200g at the start of the experiment. They were fed and allowed to drink water at libitum. They were housed under natural day/night conditions (22^0C, 50% umidity) for at least 4 weeks before the stress exposure.

Stress procedure

Male rats were handled for several minutes each day for 1 week prior to the initiation of restraint stress in order to habituate each rat to human contact and to diminish stress due to handling during bleeding, cage changes, and any other contacts that might otherwise have altered stress levels.

Rats were subjected to an established physical restraint protocol. Animals were placed in 1,5l Plexiglas tubes with multiple holes to allow ventilation. Rats were held orizontally in the tubes for a continuous 20 minutes, daily, during 18 days period, without food and water. The non-stressed controls were kept in their original cages with food and water supply.

Drug administration

LPS (endotoxin lipopolysaccharide) (Sigma), produce an immune response, was administrated once (acute administration) in dose of 25µg/250µl, i.p., 3 days prior to the restraint. Sulpiride (4 mg/kg b.w., i.p.) (Sigma) or physiological saline (0,9% NaCl solution), as a vehicle control, were administrated after 3 days postimmunization, in a volume of 0,1 ml/100g per rats i.p., daily, 30 minute prior to the restraint stress procedure. Rats were treated in accordance with institutional guidelines.

After 18 days postimmunization, whole heparinised blood was collected. To determine the count of leukocyte (mm^3), blood sample was taken with an leukocytes pipette and diluted (1/20) with the Türk solution. One drop of hemolized blood was transferred onto Neuberg`s haemocytometer, on the counting area of the haemocytometer and than coverslipped. The blood sample was therefore monolayered in a space of 0.1 mm height. The total number of leukocyte was in a $5mm^2$ area was counted and expressed as the number of leukocyte from 1 mm^3 whole blood.

The blood samples were examined in a light microscope KRÜSS model with a magnification of 400x. The leukocyte formula was determined by means of blood smears stained with May Grünwald-Giemsa. The leukocyte formula was expressed as the percentage of different types of leukocytes.

Statistical analysis

Results were expressed as mean ± S.E.M. The results were analyzed statistically by means of the Student's "t" test. $p<0.05$ was taken as the criterion for significance.

RESULTS AND DISSCUSIONS

1. Effects of the D2 dopamine receptor blockade by means of sulpiride on leukocyte formula in restrained stressed rats

Experimental data were registered 18 days after LPS and sulpiride administration. D2 dopamine receptor blockade in restraint stress by means of sulpiride enhance none significantly the total number of leukocytes (Figure 1.), neutrophils (Figure 2.) and basophils (Figure 4.) and impair significantly the total number of eosinophils ($p<0.05$) (Figure 3.).

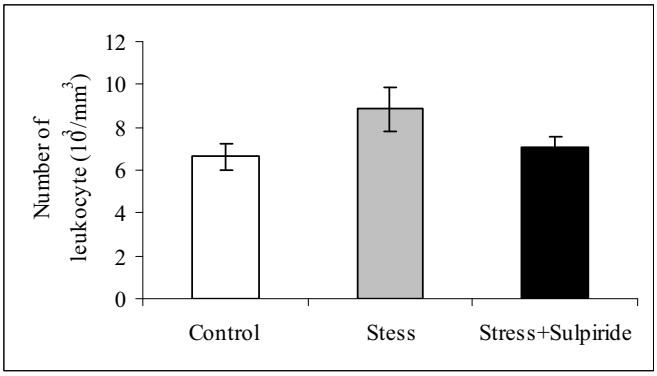

Figure 1. Changes of the total number of leukocyte ($10^3/mm^3$) tested 18 days after immunization and sulpiride administration in restraint stress. Values are means ± SEM (n=16 per group).

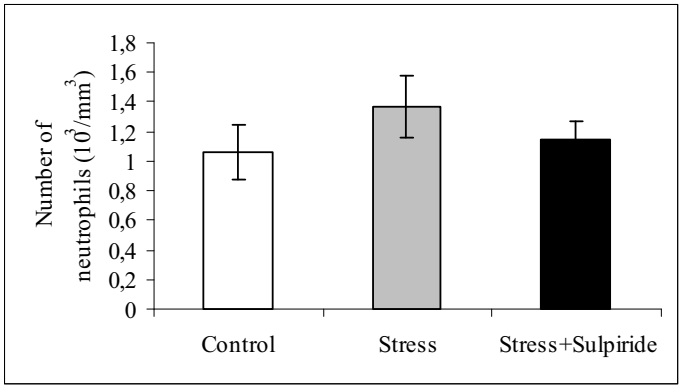

Figure 2. The effect of sulpiride on the neutrophils number on 18th day after immunization in restraint stress. Values are means ± SEM (n=16 per group).

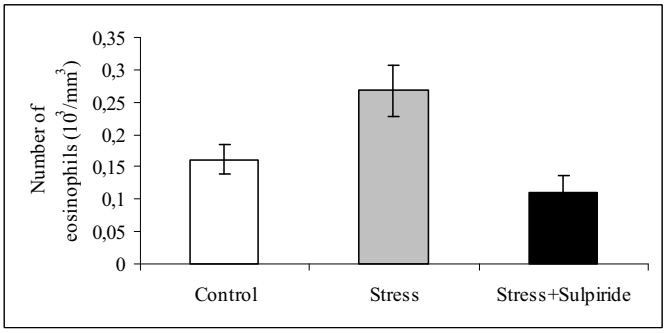

Figure 3. The effect of sulpiride on the eosinophils number on 18th day after immunization in restraint stress. Values are means ± SEM (n=16 per group). *$p<0.05$ vs. non-stressed Control.

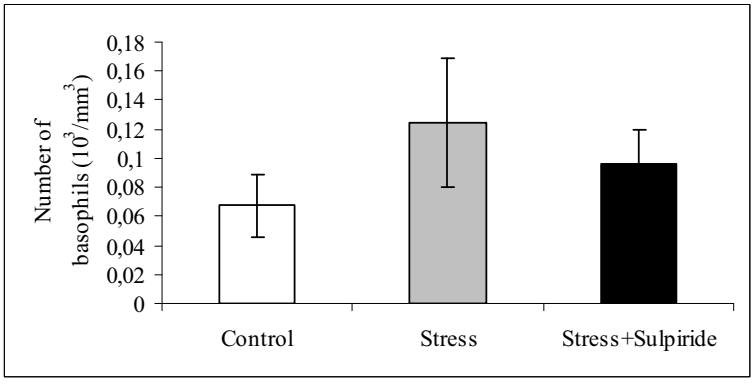

Figure 4. Changes of total number of basophils on 18th day after immunization and sulpiride administration in restraint stress. Values are means ± SEM.

Stress-inducing stimuli have been shown to be immunosuppressive (Hritcu et al., 2004) but the effect of stressors on the immune response depends on the type of the immune response, physical and psychological characteristics of the stressor and timing of the immune event testing (Dantzer et al., 2000). In our experiments we used procedure of restraint by held rats orizontally in the tubes for a continuous 20 minutes, daily, during 18 days period, without food and water, which is particularly stressful. By means of this particularly stressful we observed changes on leukocyte formula number on 18th day after LPS and sulpiride administration, tested by the total leukocyte, neutrophils, eosinophils and basophils number. Results reveal that in restraint stress the D2 dopaminereceptor blockade could enhance or suppress the total number of leukocytes subtypes.

Dopaminergic and adrenergic systems appear to take an important role on immune network. Restraint stress causes release of norepinephrine, epinephrine and dopamine and elevates plasma corticosterone level, with subsequent inhibition on immune response (Hritcu and Maniu, 2006) and decrease resistance to infection or cancer (Iwakabe et al., 1998; Freire-Garabal et al., 1993; Riley, 1981).

The existence of putative dopamine receptors of D2, D3, D4 and D5 subtypes on immune cells has been proposed of several authors, primarily on the basis of dopaminergic ligand binding assays and specific mRNA expression as monitored by reverse transcription-PCR. Several experiments evoked the idea of a role for dopamine in modulating, mainly suppressing immune functions (Qui et al., 1994). Our results indicate that blockade of D2 dopamine receptor by means of sulpiride avoid the suppressive effect of dopamine in normal conditions. The suppressor effect of dopamine in conditions of D2 dopamine receptor blockade could be explained by the presence of other dopamine receptor bear on the immune cell surface, maybe D3 dopamine receptor. In adult phase the D2 and D3 dopamine receptor are co-located (Fishburn et al., 1996).

In view to describe the mechanisms involved in dopamine actions on the leukocytes subtypes of rats, future research is required.

CONCLUSIONS

On the basis of our results obtained by sulpiride D2 dopamine receptor blockade, we can conclude that in the rats, chronic administration of sulpiride enhance or suppress the leukocytes subtypes in restraint stress.

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Acknowledgements. This research was supported by the National Council of Scientific Research and University Education (CNCSIS), Romania.

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