EFFECTS OF SULPIRIDE-INDUCED D2 DOPAMINE RECEPTOR BLOCKADE ON IMMUNE RESPONSIVENESS OF RATS

LUCIAN G. HRITCU [1*], CALIN L. MANIU[1]

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Abstract: The involvement of catecholamine receptors (D2 dopamine) was investigated in restraint stress, influence immune system, with concomitant changes in immune response. 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 protein number, antibody titer, lymphocyte number and albumin/globulin ratio. In summary, we provide that D2 dopamine receptor blockade impaired immune responsiveness in restraint stress.

INTRODUCTION

Dopamine is one of the principal neurotransmitters in the central nervous system (CNC), and its neuronal pathways are involved in several key functions such as behavior (Hefco et al., 2003a; Hefco et al., 2003b), control of movement, endocrine regulation, immune response (Fiserova et al., 2002; Levite et al., 2001), and cardiovascular function. Dopamine has at least five G-protein, coupled receptor subtypes, D1-D5, each arising from a different gene (Sibley et al., 1993). Traditionally, these receptors have been classified into D1-like (the D1 and D5) and D2-like (D2, D3 and D4) receptors subtypes, primarily according to their ability to stimulate or inhibit adenylate cyclase, respectively, and to their pharmacological characteristics (Seeman et al., 1993). Receptors for dopamine (particulary of D2 subclass) are the primary therapeutic target in a number in a number of neuropathological disorders including schizophrenia, Parkinson’s disease and Huntington’s chorea (Seeman et al., 1987). Neither dopamine by itself, nor dopaminergic agonists by themselves, has been shown to activate T cell function. Nevertheless, lymphocytes are most probably exposed to dopamine since the primary and secondary lymphoid organs of various mammals are markedly innervated, and contain nerve fibers which stain for tyrosine hydroxylase (Weihe et al., 1991), the enzyme responsible for dopamine synthesis. Moreover, cathecolamines and their metabolites are present in single lymphocytes and in extracts of T and B cell clones, and pharmacological inhibition of tyrosine hydroxylase reduces cathecolamine levels, suggesting cathecolamine synthesis by lymphocytes (Bergquist et al., 1994). 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 important neurological disease like Parkinson’s disease and schizophrenia with hipo- and hyperactivity (Birtwistle et al., 1988) of central dopamine system are well-correlated with severe abnormalities of immune functions (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 total protein number, antibody titer and albumin/globulin ratio we used the Weichselbaum method (biuret test for proteins and the technique for albumins). The leukocyte formula was determined after 18 days postimmunization from blood smear stained with May Grünwald-Giemsa. The leukocyte formula was expressed as the percentage of different types of leukocyte.

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 sulpiride D2 dopamine receptor blockade on immune responsiveness of rats

Experimental data were registered 18 days after LPS and sulpiride administration. D2 dopamine receptor blockade in restraint stress by means of sulpiride impaired more significantly the total number of serum proteins ($p<0.03$) (Figure 1.), antibody titer against LPS (endotoxin lipopolysaccharide from Escherichia coli) ($p<0.05$) (Figure 2.) compared with non-stressed controls and enhanced albumin/globulin ratio ($p<0.05$) (Figure 3.) tested 18 days after immunization. The total number of leukocytes tested 18 days after immunization and sulpiride administration in restraint stress significantly decrease in restraint stress ($p<0.05$) (Figure 4.).

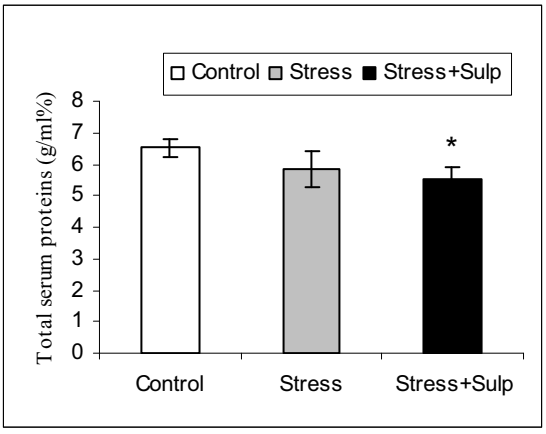

Figure 1. Changes of the total serum proteins (g/ml %) tested 18 days after immunization and sulpiride administration in restraint stress. Values are means ± SEM (n=16 per group). *$p<0.03$ vs. non-stressed Control.

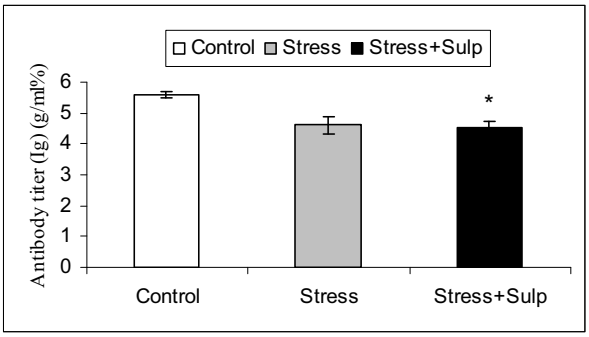

Figure 2. The effect of sulpiride on antibody titer against LPS on 18th day after immunization in restraint stress. Values are means ± SEM (n=16 per group). *p<0.05 vs. non-stressed Control.

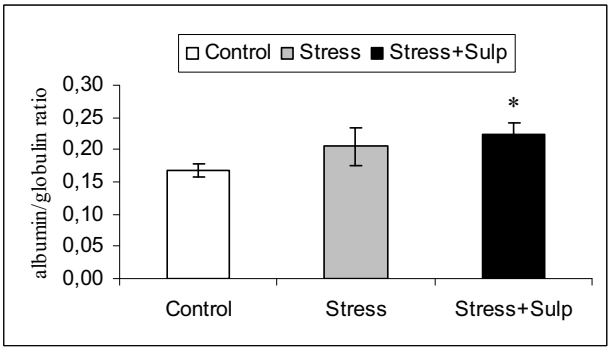

Figure 3. Albumin/globulin ratio on 18th day after immunization and sulpiride administration in restraint stress. Values are means ± SEM (n=16 per group). *p<0.05 vs. non-stressed Control.

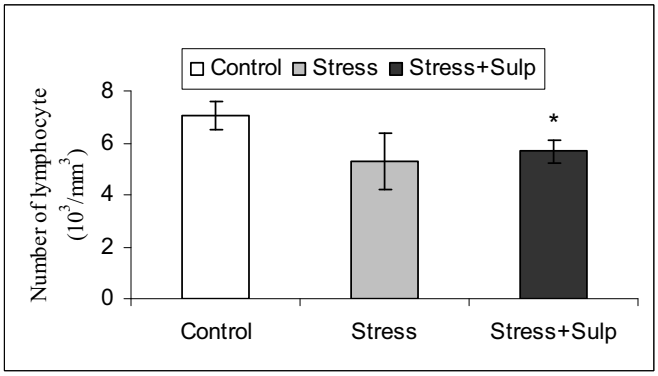

Figure 4. Changes of total number of circulating leukocytes on 18th day after immunization and sulpiride administration in restraint stress. Values are means ± SEM (n=16 per group). *p<0.05 vs. non-stressed Control.

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 considerably decrease of immunity registered on 18^{th} day after LPS and sulpiride administration, tested by antibody titer, albumin/globulin ratio and circulatinf leukocytes number. It is thought that the effect of stress on the immune system is mediated in part by an activation of the hypothalamic-pituitary-adrenal axis and thereby resulting in elevated level of ACTH and thus glucocorticoid secretion. However, some factors other than adrenal corticoids may contribute in stress-related immune suppression. For example, it was shown that adrenalectomized rats demonstrated altered immunity in response to stress (Olariu Ana et al., 1995).

Catecholamines, dopamine, epinephrine and norepinephrine are among the first molecules involved in the response to stressors. Specific receptors expressed on lymphoid cell membranes enabling the regulation of these cells. In case of stress, high levels of produced catecholamines, evoked increased expression of appropriate (adrenergic, dopaminergic) receptors on neural and lymphoid cells (Cabib et al., 1998). Moreover, increase content of dopamine and norepinephrine induces apoptosis of lymphocytes (Bergquist et al., 1997) and our results are in agreement with these data. The inhibitory effects of sulpiride for D2 dopamine receptor are in contrast with dopamine action in normal condition, mainly suppressing immune functions. Our results indicate that in stress conditions the immune functions are suppressed even with D2 dopamine receptor blockade. In these conditions the inhibitory action of dopamine was made through other dopamine receptor bear by the immune cells surface, maybe D3 dopamine receptor.

At the early stages of prenatal development the D3 receptor may be the dominant D2-type receptor, in contrast to the adult, in which D2 receptor is expressed at significantly higher levels in all tissues where the two receptors are co-located (Fishburn et al., 1996). The D3 receptors were observed to be regulated by the brain-derived neutrophic factor (BDNF), a potent regulator of neuronal functions (Guillin et al., 2001). This may be a relevance to dopamine D3 receptors on T cells, since human T cells were found to secrete BDNF, and bear its specific trkB receptors (Besser et al., 1999).

The possible involvement of T cell D3 receptors in pathological conditions is supported by recent studies showing elevated D3 receptor mRNA levels in lymphocytes from human schizophrenic patients (Kwak et al., 2001). This finding led to the suggestions that mRNA levels of the dopamine D3 receptor in lymphocytes could be used as a diagnostic marker for schizophrenia.

In view to decipher the mechanisms involved in dopamine actions on immune responsiveness 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 impaired immune responsiveness in restraint stress.

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[1] “Alexandru Ioan Cuza “University of Iasi, B-dul Carol I, Nr. 20A, 700506, Iasi-Romania;
*hritcu@uaic.ro