RIGHT-UNILATERAL LESION OF SUBSTANTIA NIGRA-INDUCE CHANGES OF HEMATOLOGICAL PARAMETERS IN WISTAR RATS

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

Keywords: substantia nigra, 6-OHDA, hematological parameters

Abstract: Male Wistar rats, pretreated with desipramine (25 mg/kg b.w., i.p., Sigma), 30 minutes before the 6-hydroxydopamine infusion to protect noradrenergic projections, were used. Specific lesions of the dopaminergic neurons located in substantia nigra pars reticulata were produced with 6-hydroxydopamine (6-OHDA) (8μg/4μl i.c.v., Sigma), a noradrenergic-selective neurotoxin. We assessed the hematological parameters (the total number of erythrocyte, hematocrit and hemoglobin), 1 week after the neurosurgery. Briefly, we observed that electrolytic lesion of substantia nigra induce sever abnormalities of hematopoesis regulation.

INTRODUCTION

It has been known that brain can communicate with the immune system through either the hypothalamic pituitary (HP) axis or the sympathetic nervous system (SNS) (Perez and Lysle, 1995; Elenkov et al., 2000). The possible roles of these two major pathways in regulation the hematopoesis processes was examined by using pharmacological agents such as desipramine and 6-hydroxydopamine (6-OHDA) in order to determine their effects on the hematological parameters. The fact that this two systems are both under the influence of cathecolamines led us to further consider the roles of cathecolamines in regulating the immunity as well as the hematopoesis. Catecholamines and their corresponding receptors are widely distributed in both the central and peripheral nervous system. Besides their vasoactive effect (Siarakas et al., 1997), catecholamines have been known to be involved in different forms of learning and memory (Spreng et al., 2001; Bemelmans et al., 2003; Hefco et al., 2003). Norepinephrine (NE) particularly at the locus coeruleus (LC) area not only can regulate the hormone release from the HP axis but also the activity of SNS (Dent et al., 2001). Immune cell types associated with innate immunity such as NK cells, neutrophils, and macrophages, are the potential subjects to be regulated by catecholamines because these cells express functional β2- and/or α-adrenergic receptors (Rice et al., 2002). In summary, the primary goal of this study was to confirm that hematopoesis processes are regulated by catecholamines and to explore the mechanisms responsible.

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). Rats were acclimated to the new housing conditions for at least 1 week prior to drug treatment.

Neurosurgery and drug administration

The rats were anesthetized with sodium pentobarbital (45 mg/kg b.w. i.p.). The substantia nigra pars reticulata was lesioned right-unilateral by stereotaxic microinjections of eight migrograms (free base) 6-hydroxydopamine, dissolved in 4 μl physiological saline containing 0.1% ascorbic acid, administered through the Hamilton syringe over 4.50 min. The syringe was left in place for 5 min after injection before being slowly removed. The rats were pretreated 30 min before the 6-hydroxydopamine infusion with 25 mg/kg i.p. desipramine (Sigma) to protect noradrenergic projections. Sham-operated rats received an injection of desipramine, followed by vehicle only in the substantia nigra. The following coordinates were used: 5.5 mm posterior to bregma; 2.0 mm lateral to the midline; 7.4 mm ventral to the surface of the cortex (Paxinos and Watson, 1998). The hematological tests were began 1 week after the operation.

After 1 week, whole heparinized blood was collected. To determine the total number of erythrocyte, hematocrit and hemoglobin level a COULTER® Ac·T™ 5diff CP were used.

Histological control

The rats were killed with an overdose of sodium pentobarbital (100 mg/kg i.p.) followed by a transcardial infusion of 0.9% saline and a 10% formalin solution. The brains were removed and placed in a 30% sucrose/formalin solution. The brains were frozen and cut into coronal sections (50 μm) using a freezing microtome and stained with cresyl violet for verification of the point of the syringe needle. Only experimental data from lesions correctly located in the substantia nigra were used for statistical analysis.

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. Effect of the chemical simpathectomy on hematological parameters

Experimental data were registered 1 week after the 6-OHDA administration. 6-OHDA treatment impaired significantly the total number of erythrocyte ($p<0.01$) (Figure 1.), hematocrit value ($p<0.01$) (Figure 2.) and hemoglobin level ($p<0.02$) (Figure 3.) compared with sham-operated groups.

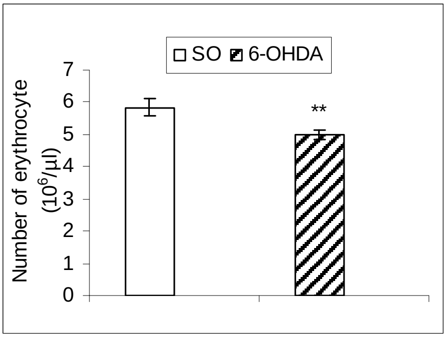

Figure 1. Changes of the total number of erythrocyte tested 1 week after chemical sympathectomy. Values are means ± SEM (n=6 per group). *$p<0.01$ vs. control group.

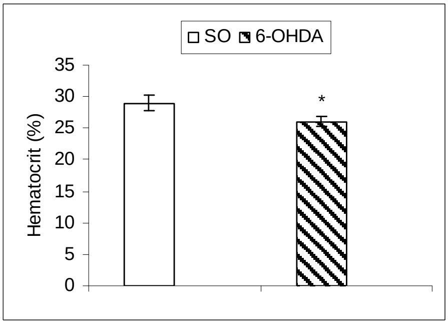

Figure 2. The effect of the chemical sympathectomy on hematocrit value tested 1 week after the neurosurgery. Values are means ± SEM (n=6 per group). *$p<0.01$ vs. control group.

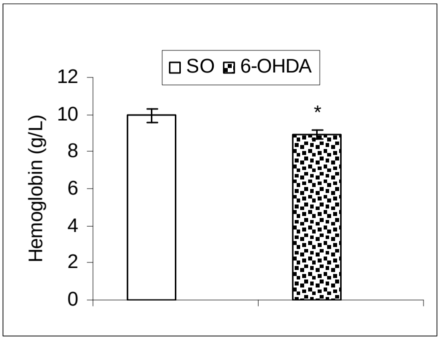

Figure 3. The effect of the chemical sympathectomy on hemoglobin level tested 1 week after the neurosurgery. Values are means ± SEM (n=6 per group). *p<0.02 vs. control group.

We have reported that the central dopaminergic system have a crucial role in regulation of the immune processes as well as hematopoesis (Hritcu et al., 2006; Maniu et al., 2006). In our experiments we used a procedure of chemical simpathectomy by lesioning substantia nigra pars reticulate with 6-OHDA. By means of this particularly electrolytic lesion we observed considerably decrease of hematological parameters registered 1 week after 6-OHDA administration, tested by the total number of erythrocyte, hematocrit value and hemoglobin level.

The regulation of hematopoietic system is achieved at three steps: 1) at the cellular level of bone marrow stroma, 2) at the humoral level by cytokines and 3) by catecholamines and other neuroendocrine factors. Sympathetic nerve endings and bone marrow cells are the main source of bone marrow catecholamines (Maestroni,1998). Among the catecholamines, substantial amount of dopamine was detected in bone marrow (Marino et al., 1997). The direct evidence of involvement of this monoamine in hematopoetic regulation has also been reported. Exogenous administration of dopamine or its analog, 3,4-dihydroxybenzylamine, stimulated erythropoiesis and platelet production in both normal and tumor-bearing mice (Lahiri et al., 1990). In accordance to these findings, in our present study we observed severe abnormalities of hematopoesis after electrolytic lesion of the central dopamine neurons from substantia nigra. Based on these findings we provide the important role of dopamine in hematopoesis.

CONCLUSIONS

On the basis of our results obtained by 6-OHDA administration, we can conclude that in the 6-OHDA treated rats, was observed a sever impairment of hematopoesis processes. The normal functions of hematopoesis processes in linked to the normal activity of the central dopamine system.

REFERENCES

1. Bemelmans, K.J., Goekoop, J.G., de Rijk, R., van Kempen, G.M.J., 2003, *Biol. Psychol.* 62, 1-15.
2. Dent, G.W., Smith, M.A., Levine, S., 2001, *Dev. Brain Res.* 127, 23-30.
3. Elenkov, I.J., Wilder, R.L., Chrousos, G.P., Vizi, E.S., 2000, *Pharmacol. Rev.* 53, 595-638.
4. Hefco, V., Yamada, K., Hefco A., Hritcu, L., Tiron, A., Olariu A., Nabeshima, T., 2003, *Europ. J. Pharmacol.*, 474, 227-232
5. Hritcu, L., Maniu, C., Cimpeanu, C., Clicinaschi Monica, 2006, *The 2nd International Congress of Cellular and Molecular Biology*, 38, abstract book.
6. Lahiri, T., Banerjee, S., Dasgupta, P.S., Ray, M.R., 1990, *Neoplasma* 37, 387–393.
7. Maestroni, G.J., 1998, *Histol. Histopathol.* 13, 271–274.

8. Maniu, C., Hritcu, L., 2006, *Ann. Univ. "Al.I.Cuza"* (in press)
9. Marino, F., Cosentino, M.F., Bombelli, R., Ferrari, M., Lecchini, S., Frigo, G., 1999, *Exp. Hematol.* 27, 489-495.
10. Paxinos, G., Watson, C., 1998, The Rat Brain in Stereotaxic Coordinates. *Academic Press*, New York.
11. Perez, L., Lysle, D.T., 1995, *J. Neuroimmunol*. 63, 1 –8.
12. Rice, P.A., Boehm, G.W., Moynihan, J.A., Bellinger, D.L., Stevens, S.Y., 2002, *Brain Behav. Immun.* 16, 62-73.
13. Siarakas, S., Damas, E., Murrell, W.G., 1997, Pathology 29, 278-285.
14. Spreng, M., Cotecchia, S., Schenk, F., 2001, Neurobiol. Learn. Mem. 75, 214-229.

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

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