

# Catecholamine levels in peripheral blood lymphocytes from multiple sclerosis patients

Cecilia Rajda<sup>a,b,1</sup>, Krisztina Bencsik<sup>a,1</sup>, László Vécsei L<sup>a,1</sup>, Jonas Bergquist<sup>b,c,\*</sup>

<sup>a</sup>Department of Neurology, University of Szeged, Semmelweis u. 6, H-6725 Szeged, Hungary

<sup>b</sup>Department of Psychiatry and Neurochemistry, Institute of Clinical Neuroscience, Göteborg University, Sahlgrenska University Hospital Mölndal, SE-431 80 Mölndal, Sweden

<sup>c</sup>Department of Analytical Chemistry, Institute of Chemistry, Uppsala University, P.O. Box 531, SE-751 21 Uppsala, Sweden

Received 3 July 2001; received in revised form 4 December 2001; accepted 19 December 2001

## Abstract

Circumstantial evidence suggests the involvement of sympathoadrenergic mechanisms in the progress of multiple sclerosis (MS). We studied peripheral blood lymphocytes from MS patients. The levels of dopamine (DA), norepinephrine (NE), epinephrine (E) and their metabolites in extracts of lymphocytes from 58 MS patients and 19 healthy controls were measured by using capillary electrophoresis. The MS patients were divided into clinical subgroups: a laboratory-supported definitive (first-attack) MS group, and a relapsing–remitting (RR) group in remission. The peripheral blood lymphocyte level of epinephrine was significantly higher in the first-attack MS patients ( $p=0.028$ ) than in the controls. However, the norepinephrine levels were significantly ( $p=0.027$ ) lower in the RR patients in remission. The catecholamines are known to be able to affect the lymphocyte activity, both by stimulation and by immunosuppression. Our results suggest that the catecholamines are important regulators of lymphocyte activation in MS, and of potential importance as concerns new diagnostic and therapeutic methods. © 2002 Elsevier Science B.V. All rights reserved.

**Keywords:** Capillary electrophoresis; Catecholamine; Dopamine; Epinephrine; Lymphocytes; Multiple sclerosis; Norepinephrine; Peripheral blood mononuclear cells

## 1. Introduction

Multiple sclerosis (MS) is an inflammatory demyelinating disease of the central nervous system (CNS). Current hypotheses on the pathogenesis of MS suggest that the primary peripheral activation of autoreactive T helper-1 lymphocytes precedes the recognition of CNS auto-antigens. These T cells proliferate, secrete cytokines and cross the blood–brain barrier (BBB) to find their antigens in the CNS where they cause further inflammatory damage. It has been hypothesized that relapsing–remitting (RR) MS is

driven by a systemic antigen presentation and that chronic progressive MS depends on the CNS presentation of antigens (Hafler, 1999).

Studies involving experimental models of MS demonstrate the importance of lymphocytes and sympathoadrenergic mechanisms (Anderton et al., 1999). Thus, in experimental autoimmune encephalomyelitis (EAE) lymphocytes crossing the BBB undergo a transformation that is involved in the progress of the disease (Wekerle, 1993). Experimentally induced hypercatecholaminemia in rats seems to protect the lymphocytes from the immunosuppressing effects of other endogenous stress hormones, but causes suppression of peripheral blood lymphocyte activation if the  $\beta$ -receptors are blocked at the same time. Beta-adrenergic agonists suppress chronic/relapsing EAE (Wiegmann et al., 1995) and decrease the number of  $\beta$ -adrenergic receptors on splenic lymphocytes in Lewis rats (Muthyala et al., 1995).

As an immune privileged site, the brain is not totally separated from the immune system, as thought earlier. The

*Abbreviations:* BBB, blood brain barrier; CNS, central nervous system; CSF, cerebrospinal fluid; DA, dopamine; E, epinephrine; MS, multiple sclerosis; NE, norepinephrine; PBMC, peripheral blood mononuclear cell; RR, relapsing–remitting.

\* Corresponding author. Department of Analytical Chemistry, Institute of Chemistry, Uppsala University, P.O. Box 531, SE-751 21 Uppsala, Sweden. Tel.: +46-18-471-3675; fax: +46-18-471-3692.

*E-mail address:* jonas.bergquist@kemi.uu.se (J. Bergquist).

<sup>1</sup> Tel.: +36-62-545-348.

CNS is connected to the deep cervical lymphatic nodes and shares messengers with the immune system. One group of these common transmitters is the catecholamines. Immuno-competent cells have been shown to contain and produce catecholamines, serotonin (5-HT), melatonin and acetylcholine (Bergquist et al., 1994; Josefsson et al., 1996; Dedenkov et al., 1986; Musso et al., 1996; Rinner et al., 1998), together with many neuropeptides and hormones, and also to express their corresponding receptors (Blalock, 1992; Santambrogio et al., 1993; Felten et al., 1992; Ricci et al., 1995; Felsner et al., 1995; Costa et al., 1995). Catecholamines and their metabolites have been found in the lymphocytes in a number of studies (Bergquist et al., 1994, 1997; Josefsson et al., 1996; Musso et al., 1996, 1998; Bergquist and Silberring, 1998); the amount of intracellular dopamine (DA) is approximately  $10^{-18}$  mol/cell (Bergquist et al., 1994).

Lymphocytes have a cellular uptake mechanism, but are also capable of the endogenous synthesis of DA and norepinephrine (NE). Additionally, they are also able to store and degrade catecholamines and possibly to regulate their own activity via an autocrine loop. Furthermore, catecholamines have been found inside the nuclear envelope (Bergquist et al., 1998), suggesting a possible direct interaction with the transcription machinery or via an interaction with the nuclear factor- $\kappa$ B (NF- $\kappa$ B) regulatory system (Bergquist et al., 2000). Recent results suggest a crucial role of NF- $\kappa$ B1 in the activation and differentiation of autoreactive T cells. Blocking the NF- $\kappa$ B function can be an effective way to prevent autoimmune encephalomyelitis (Hilliard et al., 1999). Elevated regional levels of catecholamines might lead to suppression and finally apoptosis, which would partly explain the immune privilege of the brain (Bergquist et al., 1994, 1997, 1998).

The catecholamines secreted by the sympathetic nervous system predominantly act on human T cells of the CD8<sup>+</sup>, CD28<sup>-</sup> (suppressor) subset (Karaszewski et al., 1991). This subset has the highest  $\beta$ -adrenergic receptor density. NE stimulates, while norepinephric denervation diminishes the Th1 responses (cellular immunity). Humoral immunity is also affected, perhaps via additional signaling to B cells, NE favoring IgM responses and noradrenergic denervation favoring a shift from IgM to IgG responses (Felten and Felten, 1994).

The discovery of catecholamines in lymphocytes and their functional role involving the control of T and B

lymphocytes (Bergquist et al., 1994) led to many questions being raised about their role in neuroimmunological interactions. The regulation of lymphocyte functions by catecholamines could prove to be an important part of immune deactivation in the nervous system. Studies on human neutrophils and peripheral blood mononuclear cells (PBMCs) demonstrated a catecholamine lifecycle in these cells, suggesting the presence of autoregulatory adrenergic mechanisms (Bergquist et al., 1998; Cosentino et al., 1999; Marino et al., 1999).

In the present study it was hypothesized that the deactivation of the immune system after a MS relapse (remission) could be mediated by catecholamines. Accordingly, the intracellular levels of catecholamines in relapsing–remitting (RR) MS patients in remission and in first-attack MS patients are described.

## 2. Subjects and methods

### 2.1. Patients and controls

A total of 58 patients were examined and were found to have clinically and laboratory-supported definitive MS according to the Poser criteria (Poser et al., 1983); 10 were laboratory-supported definitive (first-attack) patients, and 48 had RR MS. Both the cerebrospinal fluid (CSF) findings (oligoclonal bands on isoelectric focusing electrophoresis) and the MRI findings (several periventricular T2-weighted lesions) of the first-attack patients supported the MS diagnosis. All the RR patients were in remission. None of the patients had received steroid therapy within 30 days and none of them were on tricyclic antidepressants, cardiac drugs or amantadine. The neurological conditions of the patients were expressed on the Kurtzke expanded disability status scale (EDSS) (Kurtzke, 1983). Healthy individuals ( $n=19$ ) served as controls. The study was approved by the ethical committee of Albert Szent-Györgyi Medical School at the University of Szeged (886/1998). For the statistical analysis various MS subgroups were formed, depending on (a) the clinical course of the disease: first-attack (10) or relapsing–remitting (48); (b) the EDSS score: EDSS score <4.0 (49) or >4.0 (9); (c) the duration of the disease: <5 years (30) or >5 years (28); (d) the time to the last relapse: relapse period <6 months (19) or >6 months (39). More data on the patients are provided in Table 1.

Table 1  
Patient's data

Group	No. of subjects	Last relapse (months)	No. of relapses/2 years	Onset (year)	EDSS	Age (year)
First-attack MS	10	14.6 ± 3.0	0.9 ± 0.1	0	0.0	38 ± 2
RR MS	48	16.8 ± 1.8	1.0 ± 0.14	2.5 ± 0.07	2.2 ± 0.05	40 ± 1
Relapse within 6 months	19	3.9 ± 0.4	1.5 ± 0.21	2.4 ± 0.11	2.2 ± 0.08	40 ± 2
Relapse-free for >6 months	39	22.5 ± 0.8	0.8 ± 0.12	2.5 ± 0.08	2.1 ± 0.06	40 ± 1
Controls	19	–	–	–	–	30 ± 2

## 2.2. Preparation of lymphocytes

Peripheral vein blood samples (12 ml) were prepared by centrifugation at  $2500 \times g$  for 10 min. Lymphocytes were isolated by centrifugation on a Lymphoprep® (Nycomed Pharma, Oslo, Norway) density gradient and, after washing and centrifugation steps, kept at  $-80^\circ\text{C}$  until analysis. The lymphocytes were extracted by adding  $25 \mu\text{l}$  perchloric acid (containing 1 mM NaEDTA and 1 mM  $\text{Na}_2\text{SO}_3$ ) to the pellet, followed by ultrasonication on ice for 2 min using a MSE Soniprep 150 probe. After centrifugation (30 min,  $4^\circ\text{C}$ ,  $35000 \times g$ ) the supernatant was frozen and stored at  $-80^\circ\text{C}$  until analysis. The pellet was used for spectrophotometric protein quantitation, using bicinchoninic acid protein assay reagent (BCA, Pierce Chemical, Rockford, USA).

## 2.3. Capillary electrophoresis with electrochemical detection

The capillary electrophoretic system used was described in detail earlier (Bergquist et al., 1994, 1998; Josefsson et al., 1996). Briefly, a buffer-filled fused silica capillary (Polymicro Technologies, Phoenix, USA) measuring 10  $\mu\text{m}$  in i.d. and 65 cm in length was placed between two buffer reservoirs. High voltage was applied at the injection end, and the reservoir containing the detector end was held at ground potential. Electrokinetic injection was used for all sample introductions, 5 s at 30 kV; the sample volume was approximately 600  $\mu\text{l}$ . The easily oxidized analytes were detected in the amperometric mode with a two-electrode configuration, using optimized end-column detection (Bergquist et al., 1997). A carbon-fiber microelectrode was inserted into the end of the electrophoresis capillary and

held at 0.8 V versus a sodium-saturated calomel electrode. Reagents: 2-(*N*-morpholino)ethanesulfonic acid (MES), 5-HT, NE, epinephrine (E), DA, *L*-dihydroxyphenylalanine (*L*-DOPA), vanilmandelic acid (VMA), methoxyhydroxyphenyl glycol (MHPG), homovanilic acid (HVA) and dihydroxyphenylacetic acid (DOPAC) were obtained from Sigma (St. Louis, USA) and used in the form received. The electrophoresis buffer was 25 mM MES adjusted to pH 5.65 with NaOH. Calibration standards were prepared as 10 mM stock solutions in perchloric acid and diluted to the desired concentration in electrophoresis buffer. Hydrofluoric acid was obtained as a 40% aqueous solution from Aldrich, Milwaukee, USA), and was used for the etching of the detector end of the capillary.

The catecholamine levels of the lymphocytes were quantified by direct comparison with the standard electropherograms run before and after the patients' samples. The catecholamine contents of the lymphocytes are given in fmol/ $\mu\text{g}$  protein. Detection limits were determined (for DA, NE 0.13 fmol/ $\mu\text{g}$  protein, for E 0.37 fmol/ $\mu\text{g}$  protein, and for DOPAC 0.11 fmol/ $\mu\text{g}$  protein) and estimated at twice the peak-to-peak noise level by extrapolation from plots of peak area versus concentration. Between the series of runs, the capillary was flushed with 0.1 M NaOH to refresh the inner capillary surface and to maintain reproducible separation conditions. For a more detailed description of the method, see Bergquist et al. (1994).

## 2.4. Statistical analysis

The Kruskal–Wallis test (SPSS 7.5 for Windows) was performed for statistical analysis to compare the catecholamine levels in the healthy controls and the subgroups of

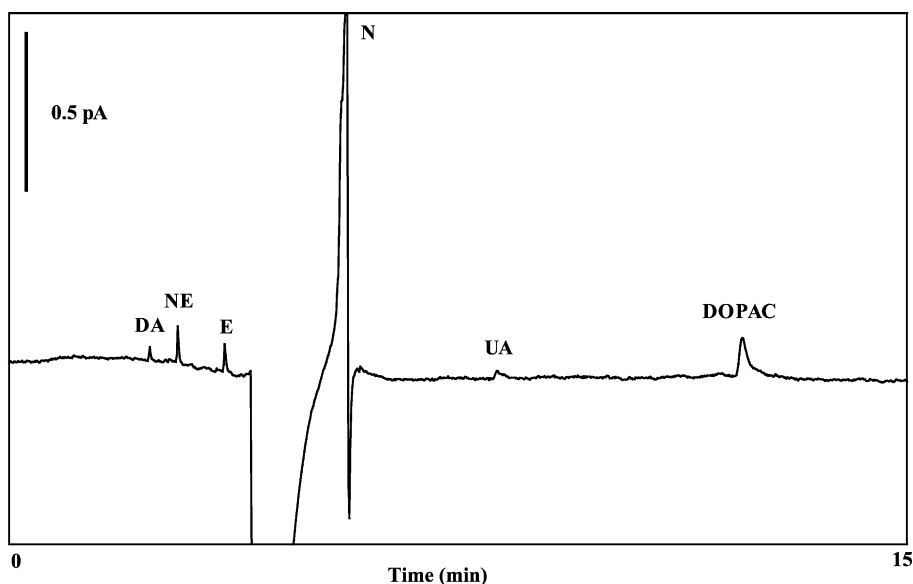


Fig. 1. A representative electropherogram of catecholamines extracted from human peripheral blood lymphocytes, showing the peaks of dopamine (DA), norepinephrine (NE), epinephrine (E), neutral species (N), uric acid (UA) and dihydroxyphenylacetic acid (DOPAC).

MS patients, followed by the Mann–Whitney *U*-test for pairwise comparisons to assess the differences between the patients and the healthy controls. The Kruskal–Wallis test was also used for the statistical analysis of the differences between the healthy controls and the different MS subgroups regarding EDSS score, medication, and duration.

### 3. Results

The electrophoretic mobilities of the major peaks in the electropherogram corresponded to the calculated electrophoretic mobilities of DA, NE, E, uric acid (UA), and DOPAC (Fig. 1). We excluded the 5-HT, MHPG, VMA and ascorbic acid data because their levels were often under the detection limit (MHPG was detectable in 7/19 controls and in 19/58 MS patients, and VMA in 5/19 controls and 27/58 MS patients). We also excluded L-DOPA since it is a neutral molecule and has the same electrophoretic mobility as all other neutrals, therefore leading to difficulties with the quantification. The levels of the catecholamines are presented in Table 2.

#### 3.1. Healthy controls versus first-attack and RR MS patients

When the MS patient subgroups and the healthy individuals were compared, significantly lower levels of NE (Kruskal–Wallis test,  $p=0.027$ ) and higher levels of E (Kruskal–Wallis test,  $p=0.028$ ) were found in the lymphocytes. Pairwise comparisons with the Mann–Whitney *U*-test showed that the RR MS patients ( $p=0.017$ ) and the first-attack MS patients ( $p=0.035$ ) had lower levels of intracellular NE than healthy controls (Table 2). The E content of the lymphocytes in first-attack MS patients was higher as compared to either the RR MS group ( $p=0.008$ ) or the controls ( $p=0.056$ ).

#### 3.2. Differences between healthy controls and MS subgroups regarding EDSS scores, duration of disease and medication

Both the MS patients with shorter disease duration ( $n=30$ , mean  $\pm$  SEM:  $378 \pm 90$  fmol/ $\mu$ g) and those with

longer disease duration ( $n=28$ , mean  $\pm$  SEM:  $453 \pm 154$  fmol/ $\mu$ g) displayed lower intracellular NE levels (Mann–Whitney *U*-test,  $p=0.033$ ) as compared with the control group ( $n=19$ , mean  $\pm$  SEM:  $1594 \pm 599$  fmol/ $\mu$ g). The lymphocytes of both the patients in a better neurological condition ( $n=49$ , mean  $\pm$  SEM:  $368 \pm 64$  fmol/ $\mu$ g) and those with an EDSS score  $>4$  ( $n=9$ , mean  $\pm$  SEM:  $807 \pm 516$  fmol/ $\mu$ g) contained less NE ( $p=0.036$ ) than the cells of the controls ( $n=19$ , mean  $\pm$  SEM:  $1594 \pm 599$  fmol/ $\mu$ g). The administration of anxiolytics did not exert any significant effect on the catecholamine levels of the lymphocytes. Slight, nonsignificant differences in the NE contents of the lymphocytes were found between the group without immunomodulating medication ( $n=42$ , mean  $\pm$  SEM:  $332 \pm 56$  fmol/ $\mu$ g), those receiving interferon- $\beta$ -1b treatment ( $n=9$ , mean  $\pm$  SEM:  $450 \pm 235$  fmol/ $\mu$ g), those receiving glatiramer acetate treatment ( $n=7$ , mean  $\pm$  SEM:  $1039 \pm 649$  fmol/ $\mu$ g) and the controls ( $n=19$ , mean  $\pm$  SEM:  $1594 \pm 599$  fmol/ $\mu$ g).

### 4. Discussion

Modern analytical tools such as capillary electrophoresis techniques allow the detection of intracellular catecholamine levels and give an insight into their regulation of lymphocyte differentiation, proliferation and apoptosis. The increased beta-adrenergic receptor density on the lymphocytes of MS patients in relapse suggests an involvement of lymphocytes and catecholamines in the pathogenesis of the disease. A general problem in MS research is that the phenomena observed can either be secondary to the disease progress with no causality, or reflect mechanisms of importance for the disease.

Scattered reports suggest a role for low molecular weight neurotransmitters in the pathogenesis of MS. Elevation of the levels of NE by using antidepressants and L-DOPA has been found to affect the symptoms of MS (Berne-Fromell et al., 1987). Maprotilin and lofepramin enhance the levels of NE in the synapses (Baumann and Maitre, 1979). Seventy-five percent of MS patients treated with L-DOPA experienced an improvement after 1–2 months (Berne-Fromell et al., 1987). Numerous

Table 2

Catecholamine contents of peripheral blood lymphocytes in healthy individuals and various subgroups of MS patients

Test group	No. of subjects	DA	NE		E		DOPAC
Healthy individuals	19	$1.39 \pm 0.32$	$1.59 \pm 0.60$	* 50.0	$0.06 \pm 0.02$	* 43.8	$2.02 \pm 1.41$
RR (first-attack MS)	10	$1.89 \pm 0.85$	$0.24 \pm 0.10^a$	* 30.0 <sup>b</sup>	$0.18 \pm 0.08^c$	* 62.9 <sup>d</sup>	$2.14 \pm 2.08$
RR (MS in remission)	48	$1.68 \pm 0.33$	$0.48 \pm 0.11^a$	* 36.0 <sup>c</sup>	$0.23 \pm 0.14^c$	* 40.0	$6.29 \pm 2.71$

Values given as mean  $\pm$  SEM fmol/ $\mu$ g protein and as \* mean ranks.

RR = relapsing–remitting, DA = dopamine, NE = norepinephrine, E = epinephrine; DOPAC = dihydroxyphenylacetic acid.

<sup>a</sup> Significant difference between the first-attack and RR MS patients and healthy controls with Kruskal–Wallis test,  $p=0.027$ .

<sup>b</sup> Significant difference between first-attack MS patients and healthy controls with Mann–Whitney *U*-test,  $p=0.035$ .

<sup>c</sup> Significant difference between the first-attack and RR MS patients and healthy controls with Kruskal–Wallis test,  $p=0.028$ .

<sup>d</sup> Significant difference between first-attack and RR MS patients with Mann–Whitney *U*-test,  $p=0.008$ .

<sup>e</sup> Significant difference between RR MS patients and healthy controls with Mann–Whitney *U*-test,  $p=0.017$ .

studies have revealed that NE may regulate early immune events such as antigen localization, presentation, B cell activation, inhibition of T suppressor cell activation and the functions of both Th1 and Th2 cell function (Sanders, 1998; Madden and Livnat, 1991). NE may also suppress the normal immune response (Bergquist et al., 1998). Elevated levels of NE have been observed in the CSF, but not in the blood of MS patients (Barkhatova et al., 1997). It has been hypothesized that there is a deficiency of NE in the nerve terminals in MS, similar to the DA deficiency in Parkinson disease patients. This hypothesis is supported by the fact that near the fourth ventricle lies the locus ceruleus, a NE-mediated part of the brain regarded as a “stress center”. Lower levels of NE in MS could possibly explain the reduced awareness and memory function, the difficulties with micturition and the cerebellar symptoms, which are the opposite of the “fight or flight” reactions (Berne-Fromell et al., 1987). Recent MRI and neuropathological findings suggest early axonal damage in MS that could be prognostic for the further disease progression (Ferguson et al., 1997; Trapp et al., 1998; Raine et al., 1999; Lovas et al., 2000). If the neurons are damaged, there could be an uncontrolled release of catecholamines and high local concentrations in the area of the lesion. The lymphocytes in the region may be exposed to these high concentrations causing high intracellular levels by an initial uptake (as may be possible in first-attack).

We found changed intracellular catecholamine levels in the PBMCs of the MS patients. The analyzed changes reflect the whole PBMC population and probably only a small proportion of them are directly involved in the CNS pathogenesis. However, if the effect of immune regulation in MS is more systemic, this could be measured in the periphery. Normally just a few leukocytes are present in the CSF and the collection of these cells would be very difficult and demand single cell analysis. After considering these problems, we concentrated on collecting PBMCs. The inclusion criteria for first-attack patients were several T2-weighted lesions on the brain MRI and positive CSF findings (oligoclonal bands, elevated IgG levels in the CSF, and a positive IgG immune blot). In the city of Szeged, the incidence of MS in 1996 was 7/100.000 (Bencsik et al., 1998). Because of the low number of first-attack MS patients, it was difficult to add more data to this group.

Catecholamines also affect the natural killer cell function through  $\beta$ -adrenergic receptors (Takamoto et al., 1991). Activated lymphocytes have increased numbers of muscarinic and nicotinic receptors (Besedovsky and DelRey, 1996). A number of reports suggest involvement of the catecholaminergic system in MS. A two-fold increase in  $\beta$ -receptor density was found on the PBMCs during relapse in RR MS patients and in secondary chronic progression MS, while the levels of NE and E in the plasma were similar to the control levels (Zoukos et al., 1992). From patients with

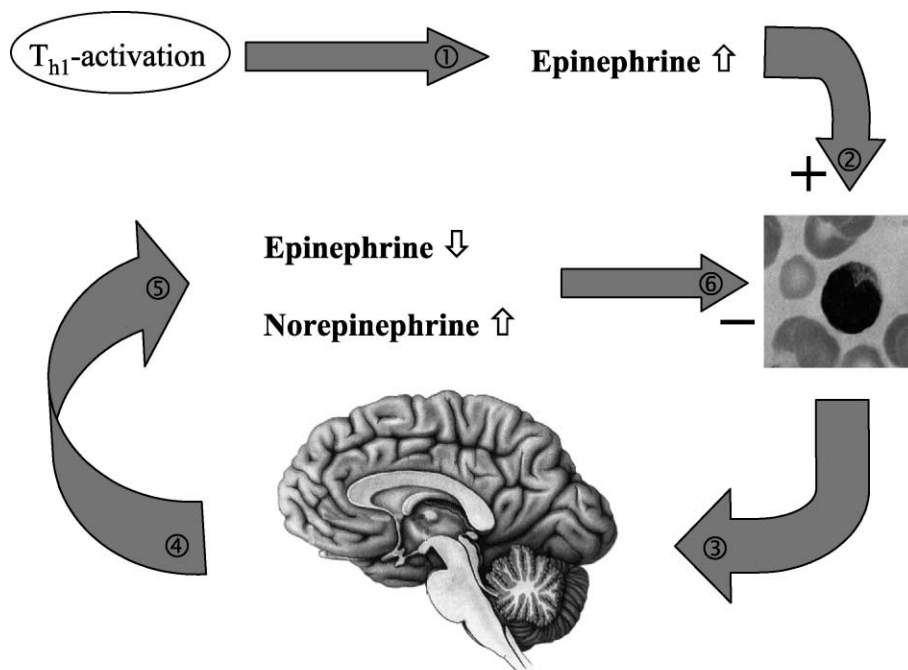


Fig. 2. The hypothesized role of the catecholamines in the pathogenesis of MS. ① Th1 T cell activation takes place in the periphery. ② Increased epinephrine levels activating the lymphocytes augment the entrance of lymphocytes through the BBB. ③ Well inside the CNS, the lymphocytes find their antigens and are activated. ④ After the activation of the lymphocytes, a feedback process is initiated. ⑤ This feedback loop leads to lymphocyte deactivation and the epinephrine content of the lymphocytes is decreased. This epinephrine decrease is then followed by an increase in norepinephrine, causing a down-regulation of the lymphocytes ⑥, and leading to the steady state of remission.

chronic progressive MS, an increased number of  $\beta$ -adrenergic receptors was found on the CD8+ T cells. In contrast, patients with stable MS and those with relapsing–remitting disease before, during or after attacks had unchanged receptor densities (Karaszewski et al., 1991). The plasma E levels in samples drawn from patients in supine and upright positions were similar in chronic progressive MS to those for normal individuals, but the supine plasma NE levels were higher in chronic progressive MS (Karaszewski et al., 1993).

In a recent study, the percentages of T and B cells in the peripheral blood from MS patients in relapse, with viral inflammatory or with noninflammatory neurological disease were similar (Oreja-Guevara et al., 1998). Various cell surface molecules on the peripheral blood CD4+ T cells and the disease activity (by MRI examination) were monitored in relapse and in remission, but no differences and no correlation to disease activity could be found (Stuber et al., 1996).

No differences in plasma dopamine- $\beta$ -hydroxylase activity have been reported between healthy individuals and MS patients (either in relapse or in remission) (Markianos et al., 1991). The synthesis of catecholamines in lymphocytes is under nicotinic control and acetylcholine might regulate catecholamine synthesis through activation of the rate-limiting enzyme tyrosine hydroxylase (Musso et al., 1997). We have not encountered any other data on differences in enzyme activity related to the catecholamine metabolism in the lymphocytes or peripheral blood of MS patients.

We observed higher intracellular levels of epinephrine in first-attack MS patients, and the lymphocytes express primarily  $\beta$ -adrenergic receptors. Thus, we can propose the following hypothesis, presented schematically in Fig. 2. An increased level of E activates the lymphocytes; they cross the BBB and find their antigens. This process is followed by the production of cytokines, which either result in an inflammatory process or act as the major compartment in the relapse process. A relapse-increased  $\beta$ -receptor density on the lymphocytes has been described, lending support to our hypothesis (Zoukos et al., 1992). It is not clear whether the lymphocytes merely mirror the state of the disease, reflecting the altered hypothalamus–pituitary gland–adrenal medulla (HPA) axis function and drain the catecholamines from the plasma, or are active participants, eliminating the catecholamines by uptake and degradation or releasing them into the MS plaque. The lower level of NE in the peripheral blood lymphocytes of RR MS patients in remission could be due to the  $\beta$ -adrenergic receptor down-regulation after a bout or to the degradation of the catecholamines. Remission may be due to a general down-regulation of the immune response by immunologically nonspecific mechanisms, such as the endogenous secretion of corticosteroids. Later in the disease process, a negative feedback suppresses the production of the catecholamines, resulting in a decreased catecholamine content of the peripheral blood lymphocytes during remission. This may explain why RR

MS patients in remission may have lower levels of catecholamines such as NE and also account for the neuroimmunological entity of the relapse.

Higher catecholamine levels in the peripheral blood lymphocytes might prevent relapses. Catecholamines have a relatively short duration of action, which could be triggered by widespread activation, except when the levels are chronically changed. One of the risk factors for autoimmunity is the low NE level in MS patients, which reflects the hypoactivity of the HPA axis.

Relapses can be induced by infection, stress, or an elevated level of E, which activates the lymphocytes, resulting in turn to activation of the disease. After nicotinic activation of the lymphocytes, intracellular NE and L-DOPA production occurs (Musso et al., 1997). The catecholamine levels may play an important regulatory role, especially in RR MS patients, when the  $\beta$ -receptors on the lymphocytes are increased. This needs to be further investigated before any strong conclusions may be drawn.

MS patients have a significantly lower NE content in their peripheral blood lymphocytes than that for healthy individuals, but in the early stage of the disease, and hence in first-attack patients, the E content is higher. With regard to the fact that the lymphocytes in relapse have a higher  $\beta$ -receptor density, new means of early intervention in the pathogenesis of MS at the lymphocyte level may be possible. These data suggest a connection between the peripheral blood lymphocyte catecholamine content and the course of the disease, and may contribute to a better understanding of the pathogenesis of MS. They may also suggest a new therapeutic approach through recognition of the role played by lymphocytes in this disease.

## Acknowledgements

We thank Erika Seres, MD for her contribution to this work, Éva Nagy-László and Klára Szűcs-Péter for their skillful technical assistance, Krisztina Boda, PhD for her help with the statistical analysis, Margit Török for her help in collecting blood samples, and Rita Persson for technical advice and valuable support. We are also grateful to Professor Tomas Olsson (Center for Molecular Medicine, Karolinska Hospital, Stockholm, Sweden) for providing helpful comments and discussions during the preparation of this manuscript. This study was supported by Swedish Institute stipend grant No. 504/1998, the Fredrik and Ingrid Thuring Foundation, the Wilhelm and Martina Lundgren Foundation, the Magnus Bergvall Foundation, the Swedish Alzheimer Foundation, the Gamla Tjänarinnor Foundation, the Swedish Lundbeck Foundation, the Swedish Society for Medical Research, Swedish Natural Science Research Council (NFR) grant K-AA/Ku 12003-300, and Swedish Medical Research Council (MFR) grant 13123. JB has a senior research position at the Swedish Research Council (VR).

## References

- Anderton, S., Burkhart, C., Metzler, B., Wraith, D., 1999. Mechanisms of central and peripheral T-cell tolerance: lessons from experimental models of multiple sclerosis. *Immunol. Rev.* 169, 123–137.
- Barkhatova, V.P., Zavalishin, I.A., Askarova, L.S., Shavratskii, V.K., Demina, E.G., 1997. Neurotransmitter changes in multiple sclerosis. *Zh. Nevropatol. Psikhiatr. im. S. S. Korsakova* 97, 7–10.
- Baumann, P.A., Maitre, L., 1979. Neurobiochemical aspects of Maprotilin (Ludiomil) action. *J. Int. Med. Res.* 7, 391–400.
- Bencsik, K., Rajda, C., Klivényi, P., Járdánházy, T., Vécsei, L., 1998. The prevalence of multiple sclerosis in the Hungarian city of Szeged. *Acta Neurol. Scand.* 97, 315–319.
- Bergquist, J., Silberring, J., 1998. Identification of catecholamines in the immune system by electrospray ionization mass spectrometry. *Rapid Commun. Mass Spectrom.* 12, 683–688.
- Bergquist, J., Tarkowski, A., Ekman, R., Ewing, A., 1994. Discovery of endogenous catecholamines in lymphocytes and evidence for catecholamine regulation of lymphocyte function via an autocrine loop. *Proc. Natl. Acad. Sci. U. S. A.* 91, 12912–12916.
- Bergquist, J., Josefsson, E., Tarkowski, A., Ewing, A., Ekman, R., 1997. Measurements of catecholamine-mediated apoptosis of immunocompetent cells. *Electrophoresis* 18, 1760–1766.
- Bergquist, J., Tarkowski, A., Ewing, A., Ekman, R., 1998. Catecholaminergic suppression of immunocompetent cells. *Immunol. Today* 19, 562–567.
- Bergquist, J., Ohlsson, B., Tarkowski, A., 2000. Nuclear factor-kB is involved in the catecholaminergic suppression of immunocompetent cells. *Ann. N. Y. Acad. Sci.* 917, 281–289.
- Berne-Fromell, K., Fromell, H., Lundkvist, S., Lundkvist, P., 1987. Is multiple sclerosis the equivalent of Parkinson's disease for noradrenaline? *Med. Hypotheses* 23, 409–415.
- Besedovsky, H.O., DelRey, A., 1996. Immune–neuro-endocrine interactions: facts and hypotheses. *Endocr. Rev.* 17, 64–102.
- Blalock, J.E., 1992. Production of peptide hormones and neurotransmitters by the immune system. In: Blalock, J.E. (Ed.), *Neuroimmunoendocrinology*. Karger, Basel, pp. 1–24.
- Cosentino, M., Marino, F., Bombelli, R., Ferrari, M., Lecchini, S., Frigo, G., 1999. Endogenous catecholamine synthesis, metabolism, storage and uptake in human neutrophils. *Life Sci.* 64, 975–981.
- Costa, P., Auger, C.B., Traver, D.J., Costa, L.G., 1995. Identification of m3, m4 and m5 subtypes of muscarinic receptor mRNA in human blood mononuclear cells. *J. Neuroimmunol.* 60, 45–51.
- Dedenkov, A.N., Rakhlin, N.T., Kvetno, I.M., Kurilets, E.S., Balmasova, I.P., 1986. Immunohistochemical and electron-microscopic identification of serotonin, melatonin, and  $\beta$ -endorphin in granules of natural killer cells. *B. Eksperim. Biol. Med.* 102, 491–493.
- Felsner, P., Hofer, D., Rinner, I., Porta, S., Korsatko, W., Schauenstein, K., 1995. Adrenergic suppression of peripheral blood T-cell reactivity in the rat is due to activation of peripheral  $\alpha$ 2-receptors. *J. Neuroimmunol.* 57, 27–34.
- Felten, S.Y., Felten, D.L., 1994. Neural–immune interactions. In: Bloom, F.E. (Ed.), *Progress in Brain Research. Neuroscience: From the Molecular to the Cognitive*. Elsevier, Amsterdam, pp. 157–162.
- Felten, S.Y., Felten, D.L., Bellinger, D.L., Olschowka, J.A., 1992. Noradrenergic and peptidergic innervation of lymphoid organs. In: Blalock, J.E. (Ed.), *Neuroimmunoendocrinology*. Karger, Basel, pp. 25–48.
- Ferguson, B., Matyszak, M.K., Esiri, M.M., Perry, V.H., 1997. Axonal damage in acute multiple sclerosis lesions. *Brain* 120, 393–399.
- Hafner, D.A., 1999. The distinction blurs between an autoimmune versus microbial hypothesis in multiple sclerosis. *J. Clin. Invest.* 104, 527–529.
- Hilliard, B., Samoilo, E.B., Liu, T.S., Rostami, A., Chen, Y., 1999. Experimental autoimmune encephalomyelitis in NF-kappa B-deficient mice: roles of NF-kappa B in the activation and differentiation of autoreactive T cells. *J. Immunol.* 163, 2937–2943.
- Josefsson, E., Bergquist, J., Ekman, R., Tarkowski, A., 1996. Catecholamines are synthesized by mouse lymphocytes and regulate function of these cells by induction of apoptosis. *Immunology* 88, 140–146.
- Karaszewski, J.W., Reder, A.T., Anlar, B., Kim, W.C., Arnason, B.G.W., 1991. Increased lymphocyte beta-adrenergic receptor density in progressive multiple sclerosis is specific for the CD8+, CD28 – suppressor cell. *Ann. Neurol.* 30, 42–47.
- Karaszewski, J.W., Reder, A.T., Anlar, B., Arnason, B.G.W., 1993. Increased high-affinity beta-adrenergic receptor densities and cyclic AMP responses of CD8 cells in multiple sclerosis. *J. Neuroimmunol.* 43, 1–8.
- Kurtzke, J.F., 1983. Rating neurologic impairment in multiple sclerosis: an expanded disability status scale (EDSS). *Neurology* 43, 655–661.
- Lovas, G., Szilagyi, N., Majtenyi, K., Palkovits, M., Komoly, S., 2000. Axonal changes in chronic demyelinated cervical spinal cord plaques. *Brain* 123, 308–317.
- Madden, K.S., Livnat, S., 1991. Catecholamine action and immunologic reactivity. In: Ader, R., Felten, N.L., Cohen, N. (Eds.), *Psychoneuroimmunology*. Academic Press, New York, pp. 283–310.
- Marino, F., Cosentino, M., Bombelli, R., Ferrari, M., Lecchini, S., Frigo, G., 1999. Endogenous catecholamine synthesis, metabolism, storage and uptake in human peripheral mononuclear cells. *Exp. Hematol.* 27, 489–495.
- Markianos, M., Sfagos, C., Bistolaki, E., 1991. Platelet monoamine oxidase and plasma dopamine-beta-hydroxylase activities in patients with multiple sclerosis. *Acta Neurol. Scand.* 84, 531–533.
- Musso, N.R., Brenci, S., Setti, M., Indivieri, F., Lotti, G., 1996. Catecholamine content and in vitro catecholamine synthesis in peripheral human lymphocytes. *J. Clin. Endocrinol. Metab.* 81, 3553–3557.
- Musso, N.R., Brenci, S., Indivieri, F., Lotti, G., 1997. L-Tyrosine and nicotine induce synthesis of L-DOPA and norepinephrine in human lymphocytes. *J. Neuroimmunol.* 74, 117–120.
- Musso, N.R., Brenci, S., Indivieri, F., Lotti, G., 1998. Acetylcholine-induced, calcium-dependent norepinephrine outflow from human peripheral lymphocytes. *J. Neuroimmunol.* 87, 82–87.
- Muthyala, S., Wiegmann, K., Kim, D.H., Arnason, B.G.W., Chelmik-Schorr, E., 1995. Experimental allergic encephalomyelitis, beta-adrenergic receptors and interferon gamma-secreting cells in beta-adrenergic agonist treated rats. *Int. J. Immunopharmacol.* 17, 895–901.
- Oreja-Guevara, C., Sindern, E., Raulf-Heimsoth, M., Malin, J.P., 1998. Analysis of lymphocyte subpopulations in cerebrospinal fluid and peripheral blood in patients with multiple sclerosis and inflammatory diseases of the nervous system. *Acta Neurol. Scand.* 98, 310–313.
- Poser, C.M., Paty, D.W., Scheinberg, L., 1983. New diagnostic criteria for multiple sclerosis: guidelines for research protocols. *Ann. Neurol.* 13, 227–231.
- Raine, C., Cannella, B., Hauser, S., Genain, C., 1999. Demyelination in primate autoimmune encephalomyelitis and acute multiple sclerosis lesions: a case of antigen-specific antibody mediation. *Ann. Neurol.* 46, 144–160.
- Ricci, A., Vega, J.A., Zaccheo, D., Amenta, F., 1995. Dopamine D<sub>1</sub>-like receptors in the thymus of aged rats: a radioligand binding and autoradiographic study. *J. Neuroimmunol.* 56, 155–160.
- Rinner, I., Felsner, P., Liebmann, P., Hofer, D., Wolfler, A., Globerson, A., Schauenstein, K., 1998. Adrenergic/cholinergic immunomodulation in the rat model—in vivo veritas? *Dev. Immunol.* 6, 245–252.
- Sanders, V.M., 1998. The role of norepinephrine and beta-2-adrenergic receptor stimulation in the modulation of Th1, Th2, and B lymphocyte function. *Adv. Exp. Med. Biol.* 437, 269–278.
- Santambrogio, L., Lipartiti, M., Bruni, A., Toso, R.D., 1993. Dopamine receptors on human T- and B-lymphocytes. *J. Neuroimmunol.* 45, 113–120.
- Stuber, A., Martin, R., Stone, L.A., Maloni, H., McFarland, H.F., 1996. Expression pattern of activation and adhesion molecules on peripheral blood CD4+ T-lymphocytes in relapsing–remitting multiple sclerosis patients: a serial analysis. *J. Neuroimmunol.* 66, 147–151.
- Takamoto, T., Hori, Y., Koga, Y., Toshima, H., Hara, A., Yokoyama, M.M.,

1991. Norepinephrine inhibits human natural killer cell activity in vitro. *Int. J. Neurosci.* 58, 127–131.
- Trapp, B.D., Peterson, J., Ransohoff, R.M., Rudick, R., Mork, S., Bo, L., 1998. Axonal transection in the lesions of multiple sclerosis. *N. Engl. J. Med.* 338, 278–285.
- Wekerle, H., 1993. Lymphocyte traffic to the brain. In: Partridge, W.M. (Ed.), *The Blood–Brain Barrier*. Raven Press, New York, pp. 67–85.
- Wiegmann, K., Muthyala, S., Kim, D.H., Arnason, B.G.W., Chelmika-Schorr, E., 1995. Beta-adrenergic agonists suppress chronic/relapsing experimental allergic encephalomyelitis (CREAE) in Lewis rats. *J. Neuroimmunol.* 56, 201–206.
- Zoukos, Y., Leonard, J.P., Thomaidis, T., Thompson, A.J., Cuzner, M.L., 1992. Beta-adrenergic receptor density and function of peripheral blood mononuclear cells are increased in multiple sclerosis: a regulatory role for cortisol and interleukin-1. *Ann. Neurol.* 31, 657–662.