Immunology 1996 **88** 140–146

Catecholamines are synthesized by mouse lymphocytes and regulate function of these cells by induction of apoptosis

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SUMMARY

The immune and the nervous systems are anatomically closely related and interact with each other by molecules common to both systems, such as cytokines and neurotransmitters. The purpose of this study was to investigate the participation of catecholamines in the neuroimmunological network. The ability of immune cells to produce catecholamines was examined by a highly sensitive capillary electrophoresis assay, which permits detection of easily oxidized catecholamines in the zeptomole (10^{-21}) range. In addition, the effects of catecholamines on *in vitro* proliferation, differentiation and apoptosis of lymphocytes were assessed. Mouse spleen cells and macrophages contained on average 7×10^{-17} and 2×10^{-17} mole dopamine per cell, respectively. In the former cell population also norepinephrine was found. Several mouse B- and T-cell hybridomas were also shown to contain endogenously produced dopamine in levels ranging from 7×10^{-20} to 2×10^{-18} mole dopamine per cell. In addition, one of the T-cell hybridomas proved to synthesize norepinephrine. The dopamine production of lymphocytes was blocked by the tyrosine hydroxylase inhibitor α-methyl-*p*-tyrosine, whereas incubation with the precursor L-DOPA increased the dopamine content. Incubation with L-DOPA, dopamine and norepinephrine dose-dependently suppressed mitogen induced proliferation and differentiation of mouse lymphocytes. Even short-time pretreatment of lymphocytes with L-DOPA and dopamine strongly suppressed lymphocyte proliferation and cytokine production. Incubation of lymphoid cells with L-DOPA, dopamine and norepinephrine dose-dependently induced apoptosis which, at least partly, explains the suppressive effects of catecholamines on lymphocyte function. Our results demonstrate that catecholamines: (i) are actively produced by lymphocytes and (ii) have the capacity to act as auto- and/or paracrine regulators of lymphocyte activity through induction of apoptosis.

INTRODUCTION

The participation of sympathetic efferent nerve fibres in the pathogenesis of inflammation and immune responsiveness has recently been brought into focus.[1–3] This participation occurs indirectly, by control of blood vessel tone in the inflammatory areas, but there is also a capacity of a direct regulation of immunocompetent cells through an interaction between sympathetic post-ganglionic transmittors and their receptors on lymphocytes.[4–6] Further support for this neuroimmunological network is a close anatomical relationship between sympathetic nerve endings and lymphocytes in spleen, lymph nodes and chronic inflammatory lesions, such as arthritis.[7,8] During recent years it has been demonstrated that immunocompetent cells do not only receive signals from the neuronal cells but may actually synthesize certain neuropeptides.[9] The aims of our study were (i) to assess the capacity of lymphocytes to produce the catecholamine norepinephrine, the sympathetic post-ganglionic transmittor, and its precursor dopamine; (ii) to investigate if and how catecholamines influence lymphocyte reactivity.

To study the intracellular content of dopamine and norepinephrine in mononuclear cells, the capillary electrophoresis technique was employed. Electrochemical detection is an extremely sensitive method permitting analysis of dopamine and norepinephrine in single cells, the lowest detectable amount in the zeptomole (10^{-21}) range. Using this method we have demonstrated that mouse spleen mononuclear cells, peritoneal macrophages as well as B- and T-cell hybridomas contain catecholamines. In addition, the immunoregulatory properties of catecholamines were studied *in vitro* with regard to both proliferative responses and differentiation of B and T cells. Our results point out a possibility of catecholamines to regulate by an auto/paracrine route lymphoid cells by means of apoptosis.

Received 25 October 1995; revised 28 December 1995; accepted 18 January 1996.

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MATERIALS AND METHODS

Mice

Female DBA/1 mice, 12–19 weeks old were originally purchased from Harlan Olac farm (Bicester, UK). The mice were bred in the animal facility of the Department of Clinical Immunology in Göteborg. The mice were housed 10 in each cage and were fed standard laboratory chow and water *ad libitum* under standard conditions of temperature and light.

Preparation of hybridoma cells and leucocytes for catecholamine analysis

The B-cell hybridomas were raised and subcloned in our laboratory, 6B9E4 is specific for staphylococcal collagen adhesin whereas the specificity of 4A12 is unknown. The T-cell hybridomas were obtained from Dr Richard Holmdahl, Lund, hybridoma HCQ-6 is Vβ 8.1+, HCQ.11 is Vβ 8.2+ and HCQ.9 consists of several clones.

To obtain macrophages 1% of sucrose in phosphate buffered saline (PBS) was injected intraperitoneally after 15 min the mouse was sacrificed, the abdomen massaged and the intraperitoneal fluid aspirated. The cells were centrifuged at $515 \times g$ for 5 min. The pelleted cells were resuspended for 10 min in Tris-buffered 0·83% ammonium chloride to lyse erythrocytes and then washed twice in PBS. Differential counts were performed on May–Grünewald–Giemsa-stained smears and displayed that more than 90% of the cells had the morphology of macrophages.

To obtain mononuclear cells, spleens of mice were teased and passed through a nylon sieve. The cells were suspended in PBS and centrifuged. Erythrocytes were lysed and the cells washed in PBS twice.

Mononuclear cells of either origin mentioned above were washed once in culture medium and counted before the extraction procedure. Extraction of catecholamines from mononuclear cells was performed by addition of 1 μl of 0·1 M perchloric acid with 1 mM sodium ethylene diamine tetraacetic acid (EDTA) and 1 mM sodium sulphite to the cell pellet to a total volume of 15 μl. This was ultrasonicated for 1 min on ice and the extract centrifuged for 30 min ($35\,000 \times g$) at 4°. All samples were prepared and analysed in at least triplicates.

The culture medium used throughout the study was Iscove's medium (Gibco, Paisley, UK) supplemented with 10% fetal calf serum (FCS) (Biological Ind., Beit Haemek, Israel), 2 mM L-glutamine, 50 μg/ml gentamycin and 50 μM mercaptoethanol (complete medium).

Determination of catecholamine levels by capillary electrophoresis

The system used for combining electrochemical detection with capillary electrophoresis in small bore capillaries was somewhat similar to that described earlier,[10] equipped with the modified optimized end-column detection.[11] Briefly, the apparatus consisted of a capillary placed between two buffer reservoirs with high voltage applied at the injection end, and the detection reservoir containing the electrochemical detector was held at ground potential. Fused silica capillaries with 10 μm inner diameter and 65 cm length were obtained from Polymicro Technologies, Phoenix, AZ. Electrokinetic injection was used for all sample introductions, 5 s at 30 kV. The sample volume was approximately 600 pl. The separation potentials were constantly at 30 kV. Detection of the easily oxidized analytes was performed in the amperometric mode with a two-electrode configuration. The 5 μm outer diameter carbon-fibre microelectrode was inserted into an etched funnel structure in the end of the capillary and held at the amperometric detection potential of 0·8 V versus a sodium-saturated calomel reference electrode. The calomel reference electrode was positioned in the detection buffer reservoir as previously described in detail.[11] Injected amounts of analyte reported were corrected for injection discrimination of cations and anions. Analytes were quantified by measuring peak areas and comparing with peak areas of known amounts of standards. Linearity was assessed using standard least-square analysis of the peak area versus amount. A set of standards were run inbetween each set of sample separation. Detection limits were determined using $2{\cdot}0 \times 10^6$ cells and estimated at twice the peak-to-peak noise by extrapolation from plots of peak area versus concentration. In between series of runs, the capillary was flushed with 0·1 M NaOH to refresh the inner capillary surface and to maintain reproducible separation conditions.

The electrophoresis buffer was 25 mM 2-(*N*-morpholino) ethanesulphonic acid (Sigma, St Louis, MO) adjusted to pH 5·65 with NaOH. Calibration standards of 1-dihydroxyphenylalanine (L-DOPA), dopamine, dihydroxyphenylacetic acid (DOPAC), norepinephrine, 3-methoxy-4-hydroxyphenylglycol and uric acid (all from Sigma) were prepared as 10 mM stock solution in perchloric acid and diluted to the desired concentration in operating buffer. To etch the detector end of the capillary hydrogen fluoride was used, obtained as a 40% aqueous solution from Aldrich Chemicals (Steinheim, Germany).

Mitogen stimulation of splenocytes

In proliferation assays spleen cells were incubated at a concentration of 1×10^6 mononuclear cells/ml in 96-well flat-bottomed microtitre plates (Nunc, Roskilde, Denmark) in 0·1 ml complete medium at 37° in 5% CO_2 and 95% humidity. Tyrosine (the precursor of L-DOPA), L-DOPA (the precursor of dopamine), dopamine and norepinephrine (Sigma) in concentrations ranging from 0–500 μM and concanavalin A (Con A) (Miles Yeda, Rehovot, Israel) or lipopolysaccharide (LPS) (Sigma) were incuded in the medium. The cells were cultured for 48–72 hr which was previously found to be the optimal culture time.[12] During the final 8–18 hr of culture, 1 μCi ^{3}H-labelled thymidine (Radiochemical Centre, Amersham, UK) was included in each well. The cultures were harvested into glass fibre filters, processed and counted in a β-counter. The cultures were set up in triplicates and results expressed as mean c.p.m. Preliminary experiments showed that the optimal proliferative responses to Con A and LPS were obtained using 2·5 μg/ml and 10 μg/ml of the stimulants, respectively.

In some experiments spleen cells were preincubated with L-DOPA and catecholamines during 4 hr at 37°, washed twice, recounted and resuspended in complete medium before start of the proliferation or differentiation assay.

To study the immunomodulating effects of short-term exposure of lymphocytes to tyrosine, L-DOPA, dopamine and norepinephrine spleen cells were pre-treated and then stimulated with mitogen in 1 ml Iscove's complete medium at 37°. Upon Con A stimulation supernatants were harvested after 24

or 48 hr, for analysis of interleukin 2 (IL-2) or IL-6 and interferon γ (INF-γ) production, respectively, and stored at $-20°$. The differentiation of LPS stimulated splenocytes was studied after 48 hr stimulation of (a) L-DOPA and catecholamine preincubated cells for analysis of IL-6 content in harvested supernatants or (b) cells concomitantly incubated with catecholamines for analysis of frequencies of immunoglobulin secreting cells using the enzyme-linked immunospot (ELISPOT) assay as previously described.[13,14]

Splenocytes were stimulated with Con A, LPS, 0·1 μg staphylococcal enterotoxin A/ml, 10 μg staphylococcal enterotoxin B/ml, 10 μg toxic shock syndrome toxin-1/ml and 0·25 μg anti-CD-3-Ab/ml for 24 and 48 hr. The cells were then washed, recounted and catecholamines were extracted as described above.

Catecholamine production by HCQ-6 hybridoma cells and splenocytes was blocked by the tyrosine hydroxylase inhibitor α-methyl-*p*-tyrosine as previously described.[10] Cells were incubated for 1 hr with 10 μM of α-methyl-*p*-tyrosine, L-DOPA and dopamine, respectively, in complete medium at 37°. The cells were washed, recounted and the catecholamines were extracted.

In proliferation assays spleen cells were incubated with 10 μM α-methyl-*p*-tyrosine for 1 hr, washed and stimulated with Con A or LPS, or incubated with 10 μM α-methyl-*p*-tyrosine overnight before Con A and LPS was added.

Cytokine assays

IL-2 and IL-6 determinations were performed using the CTLL-2 and B9 cell lines, dependent of IL-2 and IL-6, respectively.[13,15] INF-γ levels in supernatant were determined by enzyme-linked immunosorbent assay (ELISA).[16]

Induction and detection of apoptosis in splenocytes

Splenocytes, 1×10^6 mononuclear cells/well, were incubated in 96-well flat-bottomed microtitre plates with 0–500 μM of L-DOPA, dopamine or norepinephrine in a total volume of 0·1 ml at 37°. The cells were either treated for 24 hr followed by two washes in PBS or pretreated for 4 hr at 37°, washed twice, incubated for 24 hr and rewashed. Cells were stained with propidium iodide in a hypotonic staining solution[17] with 0·1 mg/ml of RNAse added and propidium iodide fluorescence of individual nuclei was measured on a FACSort (FACS, fluorescence-activated cell sorter) flow cytometer with a Lysys II software program (Becton-Dickinson, San José, CA). Data are expressed as per cent apoptotic (i.e. hypodiploid) nuclei, spontaneous apoptosis is subtracted.

Statistics

The level of significance of the differences between groups was calculated using paired Student's two-tailed *t*-test. Values are presented as mean ± standard error of mean ($x \pm$ SEM) unless otherwise indicated.

RESULTS

Catecholamines are produced by lymphoid cells

Using capillary electrophoresis dopamine was detected in peritoneal macrophages and spleen cells in concentrations varying between 2×10^{-17} and 7×10^{-17} mole dopamine/cell (Table 1). In the spleen cells also norepinephrine was detected at a concentration of $(2{\cdot}25 \pm 1{\cdot}17) \times 10^{-17}$ mole nonrepinephrine/cell. Since the presence of catecholamines in lymphocytes could have been due to *in vivo* receptor-mediated uptake from neuronal cells rather than by an endogenous synthesis, long-term clones of *in vitro* cultures lymphocytes were next employed. The B-cell hybridomas and two of three T-cell hybridomas contained dopamine in concentrations varying from 7×10^{-20} to 2×10^{-17} mole dopamine/cell and one T-cell clone contained 3×10^{-19} mole norepinephrine/cell (Table 1). The T-cell line without detectable dopamine contained DOPAC, the metabolite of dopamine (data not shown). Measurable amounts of dopamine or norepinephrine were not detected in the complete culture medium processed by the same extraction procedure as the cells.

Table 1. Catecholamine content in mouse lymphocytes and macrophages

Cell type	Dopamine (mol/cell, ± SEM*)	Norepinephrine (mol/cell, ± SEM)
Spleen cells	$(6{\cdot}5 \pm 5{\cdot}7) \times 10^{-17}$	$(2{\cdot}3 \pm 1{\cdot}2) \times 10^{-17}$
Peritoneal macrophages	$(2{\cdot}0 \pm 1{\cdot}8) \times 10^{-17}$	$< 9{\cdot}8 \times 10^{-20}$
B-cell hybridoma		
6B9E4	$(1{\cdot}7 \pm 1{\cdot}1) \times 10^{-19}$	$< 9{\cdot}8 \times 10^{-20}$
4A12	$(6{\cdot}9 \pm 6{\cdot}9) \times 10^{-20}$	$< 9{\cdot}8 \times 10^{-20}$
T-cell hybridoma		
HCQ6	$(1{\cdot}9 \pm 0{\cdot}7) \times 10^{-18}$	$(2{\cdot}8 \pm 0{\cdot}8) \times 10^{-19}$
HCQ9	$< 1{\cdot}1 \times 10^{-20}$	$< 9{\cdot}8 \times 10^{-20}$
HCQ11	$(7{\cdot}1 \pm 3{\cdot}5) \times 10^{-19}$	$< 9{\cdot}8 \times 10^{-20}$

*SEM denotes variation of catecholamine levels within three independent analytical procedures. Spleen cells and peritoneal macrophages were pooled from two mice.

These results strongly suggest that lymphocytes are able to produce catecholamines. Further support for an active dopamine synthesis by T cells is demonstrated in Fig. 1 displaying that a T-cell hybridoma treated with α-methyl-*p*-tyrosine, a catecholamine synthesis inhibitor, displays a considerably depressed intracellular dopamine level. Conversely, the intracellular dopamine content was increased as a consequence of preincubation of T cells with the precursor L-DOPA. As expected, dopamine preincubation of T cells also increased the dopamine content (Fig. 2).

The dopamine content was measured in spleen cells after 24 and 48 hr of mitogen stimulation with Con A, LPS, staphylococcal enterotoxins A and B, toxic shock syndrome toxin-1 and anti-CD-3-Ab. No mitogen induced a noticeable change in the dopamine level as compared to control cells at neither of the timepoints. All cell cultures displayed considerable diminished dopamine content after 24 and 48 hr of *in vitro* culturing (data not shown).

The effect of catecholamines on lymphocyte proliferation and differentiation

Having demonstrated that lymphoid cells are capable of producing dopamine and norepinephrine we next assayed the effect of catecholamines on lymphocyte proliferation and differentiation *in vitro*. Con A was used as a T-cell mitogen

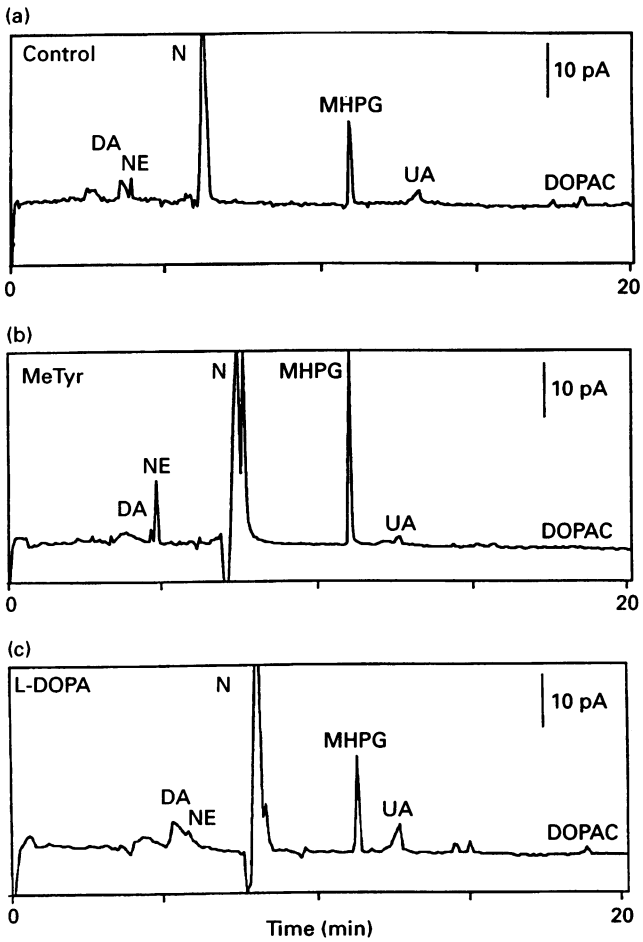

Figure 1. Three representative electropherograms from capillary electrophoresis showing the separation and detection of easily oxidized compounds in T-cell hybridoma HCQ.6 extracts. (a) Electropherogram of non-manipulated HCQ.6 cells. (b) Effect of pretreatment with 10 μM of α-methyl-*p*-tyrosine (MeTyr). (c) Pretreatment with 10 μM of L-DOPA. Electrophoretic mobilities of the major peaks correspond to the calculated electrophoretic mobilities of dopamine (DA), norepinephrine (NE), a neutral compound (N), 3-methoxy-4-hydroxyphenylglycol (MHPG), uric acid (UA) and dihydroxyphenylacetic acid (DOPAC).

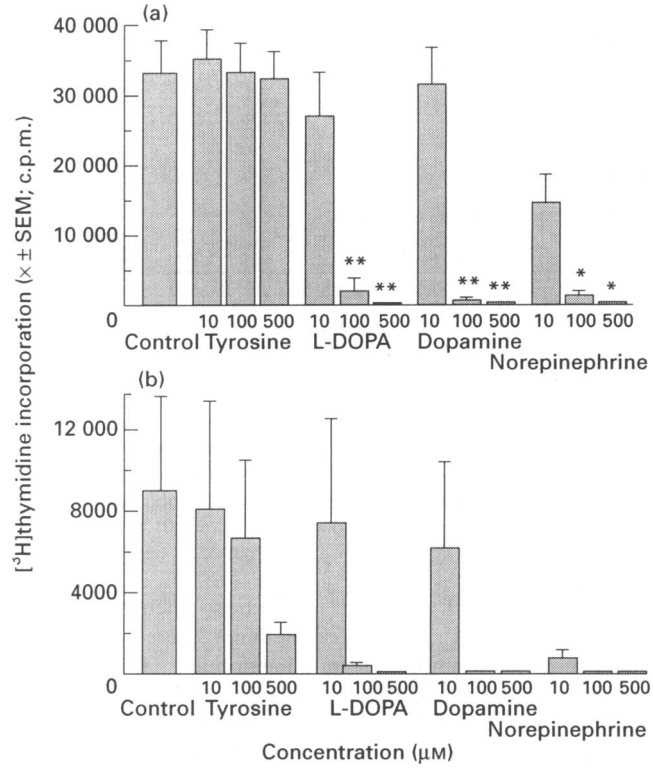

Figure 3. The effect of three days of tyrosine, L-DOPA, dopamine and norepinephrine treatment on the proliferative response of spleen cells to (a) ConA and (b) LPS stimulation. The results represent pooled values from 2–4 separate experiments. $^{*}P < 0{\cdot}05$, $^{**}P \leqslant 0{\cdot}01$.

whereas LPS was employed as a B-cell mitogen. The proliferative responses of spleen mononuclear cells to both Con A and LPS were dose-dependently inhibited by the catecholamines and L-DOPA (Fig. 3a and b). Even short-time exposure (4 hr) of splenocytes to catecholamines before the initiation of the *in vitro* stimulation diminished the proliferative responses (Fig. 4).

The influence of catecholamines on lymphocyte differentiation was studied by means of frequency of immunoglobulin

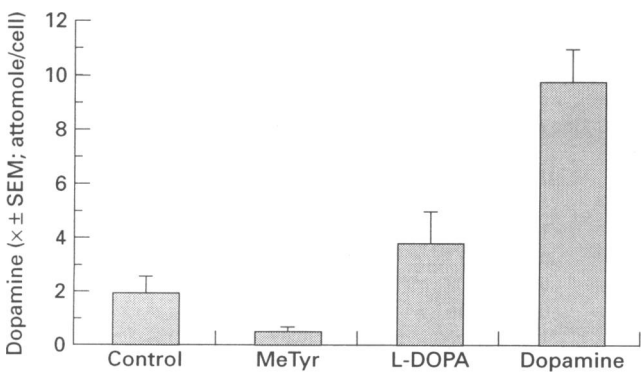

Figure 2. Dopamine content in T-cell hybridoma HCQ.6 after incubation with α-methyl-*p*-tyrosine (MeTyr), a dopamine synthesis inhibitor, L-DOPA, a dopamine precursor, or dopamine. SEM denotes variation of catecholamine levels within three independent analytical procedures.

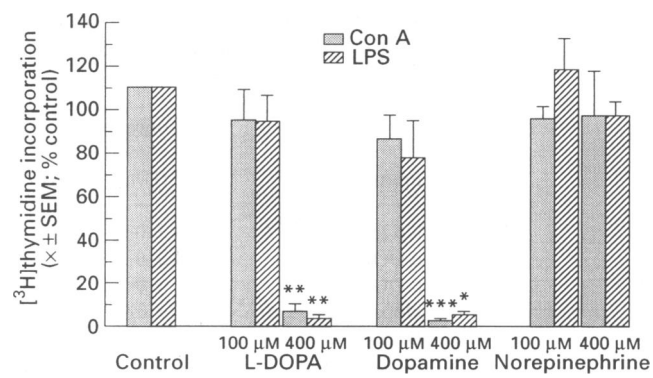

Figure 4. The effect of short-time exposure of splenocytes to L-DOPA, dopamine and norepinephrine on the proliferative response to mitogens. The base-line response to Con A was 30 000 ± 12 900 c.p.m. (three experiments) and to LPS 4800 ± 3600 c.p.m. (two experiments. $^{*}P < 0{\cdot}05$, $^{**}P < 0{\cdot}01$, $^{***}P < 0{\cdot}001$.

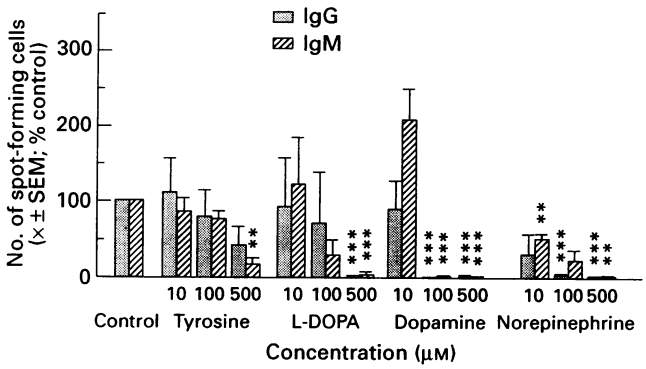

Figure 5. The influence of tyrosine, L-DOPA, dopamine and norepinephrine on the numbers of IgG- and IgM-producing cells in LPS stimulated splenocyte cultures. The base-line numbers of IgG and IgM producing cells were 1200 ± 500 (four independent experiments) and 101 000 ± 41 000 (three independent experiments) spot forming cells per 10^6 mononuclear cells, respectively. $^{**}P < 0{\cdot}01$, $^{***}P < 0{\cdot}001$.

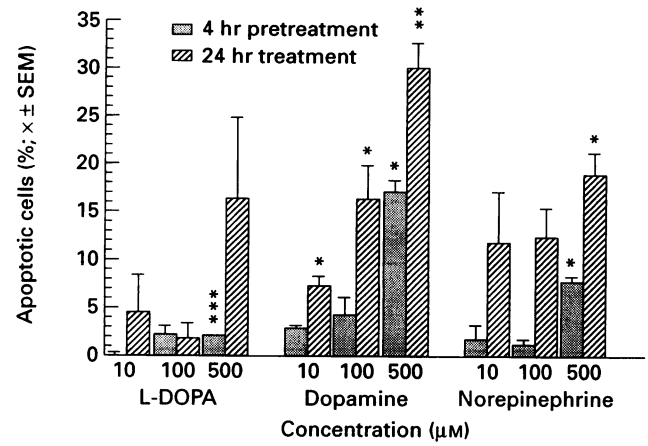

Figure 6. Frequency of apoptotic cells induced *in vitro* in splenocyte cultures by L-DOPA, dopamine and norepinephrine. The results represent mean values ± SEM of 2–3 independent experiments. The levels of significance relate to frequencies of spontaneously apoptotic cells. $^{*}P < 0{\cdot}05$, $^{**}P < 0{\cdot}01$, $^{***}P < 0{\cdot}001$.

secreting cells and cytokine production. Incubation with tyrosine, L-DOPA and catecholamines dose-dependently diminished the number of immunoglobulin G (IgG) and immunoglobulin M (IgM) producing cells in LPS-driven cultures (Fig. 5). Preincubation of splenocytes with high concentrations of dopamine and L-DOPA significantly reduced the production of IL-2, IL-6 and IFN-γ in response to Con A stimulation as compared to control cultures (Table 2). Similarly, LPS-stimulated IL-6 response was suppressed by high concentrations of dopamine and L-DOPA (Table 2). In contrast, pretreatment with norepinephrine did not have a suppressive effect on the cytokine production.

These results suggest that catecholamines produced by immunocompetent cells could act by an auto/paracrine mechanism.

The effect of blocking the catecholamine production on splenocyte proliferation

When catecholamine production by splenocytes was blocked using α-methyl-*p*-tyrosine, the proliferation of the cells in response to Con A or LPS stimulation was not significantly affected as compared to control cells. Even if the mitogen concentrations were suboptimal, the α-methyl-*p*-tyrosine treatment had no clear-cut effect on the proliferative responses (data not shown).

Catecholamines induce apoptosis of splenocytes

How do L-DOPA and catecholamines suppress lymphocyte proliferation and differentiation? The ability of these compounds to induce apoptosis of lymphoid cells was assessed using a previously described and validated technique.[17] Apoptosis was dose-dependently induced in spleen cells by incubation with L-DOPA, dopamine and norepinephrine for 24 hr (Fig. 6). Also, 4 hr exposure of lymphocytes to norepinephrine and dopamine, followed by wash and 24 hr culture period, resulted in increased apoptosis (Fig. 6). These results indicate that the inhibition of lymphocyte function as a result of catecholamine exposure is mediated by triggering of apoptosis.

Table 2. Effects of short-term *in vitro* pretreatment with L-DOPA and catecholamines on cytokine release by mitogen activated lymphocytes

	Concanavalin A-induced cytokine release			LPS-induced cytokine release
Pretreatment	IL-2 (U/ml)	IFN-γ (U/ml)	IL-6 (ng/ml)	IL-6 (ng/ml)
No treatment	2·3 ± 0·2	160 ± 0	1·14 ± 0·29	1·48 ± 0·28
L-DOPA 400 μM	0·4 ± 0·2*	0 ± 0‡	0·52 ± 0·13	0·06 ± 0·06*
L-DOPA 100 μM	2·1 ± 0	130 ± 20	0·97 ± 0·03	2·05 ± 0·38
Dopamine 400 μM	0·3 ± 0†	0 ± 0‡	0·79 ± 0·16	0·18 ± 0·06*
Dopamine 100 μM	1·9 ± 0	140 ± 10	1·35 ± 0·65	2·11 ± 0·09
NE§ 400 μM	2·3 ± 0	200 ± 10	1·15 ± 0·20	2·26 ± 0·36
NE 100 μM	2·5 ± 0·1	200 ± 0*	1·21 ± 0·05	2·94 ± 0·51

Mean values and standard errors of mean from two independent experiments are listed.
$^{*}P < 0{\cdot}05$, †$P \leqslant 0{\cdot}01$, ‡$P \leqslant 0{\cdot}001$ as compared to control.
§NE, norepinephrine.

DISCUSSION

This is the first report demonstrating production of dopamine and norepinephrine by murine cells of lymphocytic origin. In addition, we present data supporting the notion that catecholamines, once produced, might act in an autocrine/paracrine mode to downregulate lymphocyte proliferation and differentiation by induction of apoptosis.

The key issue in the detection of minute levels of catecholamines is the analytical specificity. Use of catecholamine standards in capillary electrophoresis means that one can ascertain adequate evaluation of electropherograms. In addition, use of a specific catecholamine synthesis inhibitor provides further specificity control (*cf.* Fig. 1).

The cellular content of catecholamines varied in a broad range between different cell populations (*cf.* Table 1). In general, cells obtained *ex vivo* displayed higher levels of intracellular dopamine and norepinephrine as compared to cultured lymphocytes. This phenomenon could, at least to some extent, be ascribed to receptor-mediated uptake of dopamine and norepinephrine of neuronal origin. Such a possibility is, however, largely excluded when analysing B- and T-cell hybridomas, since these lymphocyte clones have been cultured long-term *in vitro* without any contact with the nervous system. Interestingly, even within the hybridoma cell clones the amount of dopamine produced displayed a considerable variation indicating that the individual lymphocytes rather than the malignant fusion partner were responsible for the catecholamine production. As a final evidence of catecholamine production by lymphocytes, a tyrosine hydroxylase inhibitor diminished the dopamine content in T hybridoma cells, indicating that an active synthesis of dopamine occurred. This conclusion was further supported by incubation of cell cultures with L-DOPA, the dopamine precursor, which increased the intracellular dopamine content.

What is the role of catecholamine endogenously produced by lymphocytes? It could, as in the case of nerve growth factor of lymphocytic origin,[18] act on conventional target cell populations. More speculatively, the catecholamines could in a paracrine/autocrine way affect lymphocyte function. Lymphocytes express dopamine and nonrepinephrine receptors,[4,6] a prerequisite for such an interaction. Indeed, our data suggest that catecholamines and their precursor are potent and dose-dependent inhibitors of lymphocyte proliferation and differentiation. This is in agreement with an earlier study showing suppression of lymphocyte proliferation by L-DOPA.[19] Even short-time exposure of lymphocytes to L-DOPA and catecholamines exerts a profound decrease of cytokine production. In contrast, *in vitro* blocking of intracellular catecholamine production did not have any enhancing effect on mitogen-driven lymphocyte responses.

Since the action of Con A and LPS on T and B cells, employed in our experimental setup, is macrophage dependent one cannot exclude an effect of catecholamines on the accessory cell population. Indeed, previously it was shown that macrophage activity is suppressed by norepinephrine.[20] However, the proliferative responses of the T-cell clone CTLL-2 and the B-cell lymphoma B9 cells were in a macrophage-free environment strongly suppressed by L-DOPA and catecholamines (data not shown) suggesting that T and B cells may be directly inhibited by these compounds.

One of the key questions are the mechanism(s) whereby catecholamines exert their inhibitory action. We addressed this issue by studying the potential of L-DOPA, dopamine and norepinephrine to trigger programmed cell death. The rationale of this approach was a previously known property of bromocriptine, a dopamine agonist, to induce apoptosis in pituitary adenoma cells.[21] Indeed, *in vitro* exposure of lymphoid cells to L-DOPA and the catecholamines induced dose-dependent apoptosis. Our results do not exclude the possibility that other mechanisms might be operative in the downregulation of lymphocyte reactivity upon exposure to catecholamines and L-DOPA.

What is the physiological *in vivo* role of catecholamine production by, and regulation of, lymphocyte activity? The first point to stress is that the amounts of catecholamines found in the investigated cell subsets and the concentrations of catecholamines and L-DOPA used in the *in vitro* systems are of relevance in the *in vivo* situation. Thus, our results indicate that *ex vivo* obtained splenocytes contain on average 126 μM dopamine. Such a high intracellular concentration might lead to *in vivo* apoptosis, unless the catecholamines were stored in granula. In addition, pharamcological treatment of patients with Parkinson syndrome leads to high serum concentrations of L-DOPA, well in the range of these used in our *in vitro* study.[22] The concentration of norepinephrine outside a sympathetic varicosity could also reach immunosuppressive levels (approximately 40 μM) considering the size of varicosities and their content of norepinephrine, as determined by Dahlström *et al.*[23,24]

We believe that catecholamine producing T and B cells present in the inflammatory lesions could exert downregulatory autocrine effects counteracting the chronicity of the disease. This action might be of importance considering relatively sparse occurrence of sympathetic neurons in the inflamed synovium of patients with rheumatoid arthritis.[25] Indeed, a recent *in vivo* study indicates that rats treated with norepinephrine show decreased T lymphocyte responses.[26]

We conclude that lymphocytes have the capacity to synthesize dopamine. In addition, we provide evidence for catecholamines being potent inhibitors of different stages in T and B lymphocyte activation. These data fully support our previous study using human lymphocytes.[10] Finally, we show that catecholamines are able to induce apoptosis of leucocytes which could explain the inhibition of lymphocyte activity. Having in mind the occurrence of dopamine receptors on lymphocytes our data provide a conceptual framework of autocrine/paracrine mechanisms in the regulation of lymphocyte activities by catecholamines.

ACKNOWLEDGMENTS

We thank Margareta Verdrengh for skilful technical assistance and Dr Alexander Asea for valuable advices. This work was supported by grants from the Swedish Medical Research Council, the Göteborg Medical Society, the Swedish Association against Rheumatism, the University of Göteborg, the Swedish Society for Medical Research, and the Sahlgrenska, the King Gustaf V's 80 Years, the Nanna Svartz, the Börje Dahlin, the Tornspiran, the A-G Crafoord, the Fredrik and Ingrid Thuring and the Wilhelm and Martina Lundgren Foundation.

REFERENCES

1. Levine J.D., Fye K., Heller P., Basbaum A.I. & Whiting-O'keefe Q. (1986) Clinical response to regional intravenous guanethidine in patients with rheumatoid arthritis. *J Rheumatol* **13,** 1040.
2. Levine J.D., Dardick S.J., Roizen M.F., Helms C. & Basbaum A.I. (1986) Contribution of sensory afferents and sympathetic efferents to joint injury in experimental arthritis. *J Neurosci* **6,** 3423.
3. Tarkowski E., Naver H., Wallin G., Blomstrand C. & Tarkowski A. (1995) Lateralization of T-lymphocyte responses in patients with stroke. Effect of sympathetic dysfunction? *Stroke* **26,** 57.
4. Williams L.T., Snyderman R. & Lefkowitz R.J. (1976) Identification of β-adrenergic receptors in human lymphocytes by $(-)^3$H-alprenolol binding. *J Clin Invest* **57,** 149.
5. Griffiths R.S., Chung-A-On K.O., Griffiths K.D., Payne J.W. & Davies J.I. (1992) Sequestration of [^{3}H]-spiperone bile lymphocytes in schizophrenics and their first-degree relatives: a limited vulnerability marker? *J Psychiat Res* **26,** 77.
6. Santambrogio L., Lipartiti M., Bruni A. & Dal Torso R. (1993) Dopamine receptors on human T- and B-lymphocytes. *J Neuroimmunol* **45,** 113.
7. Felten D.L., Felten S.Y., Bellinger D.L. *et al.* (1987) Noradrenergic sympathetic neural interactions with the immune system: Structure and function. *Immunol Rev* **100,** 225.
8. Konttinen Y.T., Kemppinen P., Segerberg M. *et al.* (1994) Peripheral and spinal neural mechanisms in arthritis, with particular reference to treatment of inflammation and pain. *Arthr Rheum* **37,** 965.
9. Blalock J.E. (1989) A molecular basis for bidirectional communication between the immune and neuroendocrine systems. *Physiol Rev* **69,** 1.
10. 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* **91,** 12912.
11. Sloss S. & Ewing A.G. (1993) Improved method for end-column amperometric detection for capillary electrophoresis. *Anal Chem* **65,** 577.
12. Tarkowski A., Jonsson R., Sanchez R. Klareskog L. & Koopman W.J. (1988) Features of renal vasculitis in autoimmune MRL lpr/lpr mice: phenotypes and functional properties of infiltrating cells. *Clin Exp Immunol* **72,** 91.
13. Josefsson E., Månsson J.-E., Blennow K. & Tarkowski A. (1994) Immunomodulating and anti-inflammatory properties of the sympathetic compound 6-hydroxydopamine. *J Neuroimmunol* **55,** 161.
14. Czerkinsky C., Nilsson L.-Å., Nygren H., Ouchterlony Ö & Tarkowski A. (1983) A solid phase enzyme-linked immunospot (ELISPOT) assay for enumeration of specific antibody secreting cells. *J Immunol Meth* **65,** 109.
15. Bremell T., Abdelnour A. & Tarkowski A. (1992) Histopathological and serological progression of experimental *Staphylococcus aureus* arthritis. *Infect Immun* **60,** 2976.
16. Zhao Y.-X., Abdelnour A., Holmdahl R. & Tarkowski A. (1995) Mice with the Xid B cell defect are less susceptible to developing *Staphylococcus aureus*-induced arthritis. *J Immunol* **155,** 2067.
17. Nicoletti I., Migliorati G., Pagliacci M.C., Grignani F. & Riccardi C. (1991) A rapid and simple method for measuring thymocyte apoptosis by propidium iodide staining and flow cytometry. *J Immunol Methods* **139,** 271.
18. Santambrogio L., Benedetti M., Chao M.V. *et al.* (1994) Nerve growth factor production by lymphocytes. *J Immunol* **153,** 4488.
19. Slominski A. & Goodman-Snitkoff G. (1992) DOPA inhibits induced proliferative activity of murine and human lymphocytes. *Anticancer Res* **12,** 753.
20. Van Der Poll T., Jansen J., Endert E., Sauerwein H.P. & Van Deventer S.J.H. (1994) Noradrenaline inhibits lipopolysaccharide-induced tumor necrosis factor and interleukin 6 production in human whole blood. *Infect Immun* **62,** 2046.
21. Yin D., Kondo S., Takeuchi J. & Morimura T. (1994) Induction of apoptosis in murine ACTH-secreting pituitary adenoma cells by bromocriptine. *FEBS Lett* **339,** 73.
22. Grahnén A., Eckernäs S.-Å., Collin C., Ling-Andersson A., Tiger G. & Nilsson M. (1992) Comparative multiple-dose pharmacokinetics of controlled-release levodopa products. *Eur Neurol* **32,** 343.
23. Dahlström A. & Häggendal J. (1966) Some quantitative studies on the noradrenaline content in the cell bodies and terminals of a sympathetic adrenergic neuron system. *Acta Physiol Scand* **67,** 271.
24. Dahlström A., Häggendal J. & Hökfelt T. (1966) The noradrenaline content of the varicosities of sympathetic adrenerig nerve terminals in the rat. *Acta Physiol Scand* **67,** 289.
25. Mapp P.I., Kidd B.L., Gibson S.J. *et al.* (1990) Substance P-, calcitonin gene-related peptide- and C-flanking peptide of neuropeptide Y-immunoreactive fibres are present in normal synovium but depleted in patients with rheumatoid arthritis. *Neuroscience* **37,** 143.
26. Felsner P., Hofer D., Rinner I. *et al.* (1992) Continuous *in vivo* treatment with catecholamines suppresses in vitro reactivity of rat peripheral blood T-lymphocytes via α-mediated mechanisms. *J Neuroimmunol* **37,** 47.