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MEASUREMENT OF CATECHOLAMINES IN MOUSE BONE MARROW BY MEANS OF HPLC WITH ELECTROCHEMICAL DETECTION

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ABSTRACT

Background and Objective. Noradrenergic innervation is present in the bone marrow and adrenergic agents can modulate hematopoiesis. However, since no data are available concerning endogenous catecholamines at this level, we investigated their presence and origin.

Methods. Using a high performance liquid chromatographic method, we have and measured endogenous catecholamines in bone marrow from normal, 6-OHDA-treated and pargyline-treated mice.

Results. Noradrenaline, adrenaline and dopamine levels were, respectively, 2806.74±408.85, 803.37±87.66 and 274.47±51.54 pg/g of tissue. Noradrenaline levels were lower after 6-OHDA (1130.47±142.73 pg/g of tissue, $p < 0.01$ vs. control values) and higher after pargyline (4122.62±509.54 pg/g of tissue, $p < 0.05$). None of these treatments significantly affected adrenaline or dopamine content.

Interpretation and Conclusions. Noradrenaline in the bone marrow originates mainly from sympathetic nerve endings and is metabolized through specific enzymatic pathways. Adrenaline and dopamine may originate from other sources, such as the systemic circulation.

Key words: noradrenaline, adrenaline, dopamine, bone marrow, HPLC

Anatomical studies point towards the existence of sympathetic noradrenergic nerves in both primary and secondary lymphoid organs.[1] In particular, in the bone marrow varicose noradrenergic nerve fibers enter as arteriolar plexuses and are distributed in the surrounding parenchyma, ending among hematopoietic and lymphopoietic cells.[1-3] The presence of noradrenergic innervation in the bone marrow may thus represent an important mechanism by which the nervous system can modulate hemato- and lymphopoietic processes.

Indeed experimental evidence indicates that bone marrow cells are endowed with adrenergic receptors.[4] Moreover, adrenergic agents are able to modulate hematopoiesis in different animal models[4-7] and, at least *in vitro*, in human bone marrow as well.[8] However, in this regard the role of endogenous catecholamines is still unclear.

Although indirect evidence has been given for the presence of noradrenaline of neurogenic origin in the bone marrow,[2,3,9] to the best of our knowledge direct measurement of endogenous catecholamines at this level has never been performed. Thus, in the present paper, we applied a high performance liquid chromatographic method to detect and measure endogenous catecholamines in the bone marrow. Then, with this same method, we studied the effects of chemical sympathetic denervation and monoamine oxidase (MAO) inhibition on bone marrow catecholamine content, in order to obtain functional evidence of catecholamine release from sympathetic nerve endings and their metabolism through specific enzymatic pathways.

Materials and Methods

Animals

Experiments were performed on female C57BL/6 mice (2 to 6 months old), purchased from Charles River Italia (Calco, CO, Italy) and kept at 21±1°C under a 12-hour light:dark cycle with free access to food and water. Animals were sacrificed by cervical dislocation.

Bone marrow preparation

Bone marrow was flushed from the long bones with 2 mL of 0.4 N $HClO_4$ solution containing 0.84 mg/mL disodium EDTA, 1 drop of 4% sodium pyrosulfite solution. Recovered tissue was weighed, samples were centrifuged (14,000 rpm, 4°C, 10

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min), and supernatant was collected and stored at –80°C until assayed.

Pharmacological treatments

6-hydroxydopamine (6-OHDA; 100 mg/kg body weight) was injected intraperitoneally 2 days before sacrifice. Pargyline (100 mg/kg body weight) was injected subcutaneously for 8 days (1 injection per day) until sacrifice.

Catecholamine assay

Catecholamines in the samples were assayed by HPLC with electrochemical detection. The HPLC system consisted of a dual-piston pump (model LC9A, Shimadzu, Kyoto, Japan), a Beckman C_{18} ultrasphere-XL ODS 3 µM (70×4.6 mm) analytical column equipped with an XL ODS 3 µM (5×4.6 mm) guard cartridge (Beckman Instruments, Bioindustrial Business Unit, Fullerton, CA, USA), an autosampler (model SIL9A, Shimadzu), an ESA Coulochem II electrochemical detector with a 5011 analytical cell (ESA, Bedford, MA, USA). The first detector potential was set at +300 mV and the second detector at –300 mV. The chromatograms were collected, stored and processed with a computerized integrator (Model 1022 Personal Integrator, Perkin Elmer). The mobile phase was composed of ultrapure water/acetonitrile (82.0: 18.0, v/v), 20 mM K_2HPO_4, 0.69 mM EDTA, 0.27 mM SDS. The pH was adjusted to 3.0 with H_3PO_4 and the solution was filtered (Millipore, 0.22 µM). The flow rate was 0.9 µL min^{-1}.

Each sample was added with 30 µL of 0.14 M 3,4-dihydroxybenzylamine (DHBA), 500 µL of a molar solution of TRIZMA base and 30 µL of 2.69 mM EDTA and alumina-extracted. Catecholamines were recovered in 150 µL of 0.4 N $HClO_4$ and 30 µL were injected in the HPLC system. Catecholamines were quantitated by using the peak area ratio related to DHBA, the internal standard, to correct for incomplete recovery, and referred to a standard curve generated by injecting catecholamine standards (0.6-200 pg). The values were then normalized for tissue weight.

Data analysis

Values are given as means±s.e. mean, with *n* indicating the number of experiments. Statistical analysis of the data was carried out with the two-tailed Student's t-test, and P values less than 0.05 were regarded as significant.

Drugs and chemicals

(-)-noradrenaline bitartrate, (-)-adrenaline bitartrate, dopamine hydrochloride, 6-OHDA hydrochloride, pargyline hydrochloride, DHBA hydrobromide, L-ascorbic acid, L-tyrosine, alumina, TRIZMA base were purchased from Sigma (St. Louis, MO, USA). All other reagents and solvents were purchased from Merck (Darmstadt, Germany).

Results

Figure 1 shows typical chromatograms obtained after injection of a standard sample containing catecholamines and of samples of bone marrow supernatant. The quantification limit of the method was 0.6 pg of injected noradrenaline, adrenaline or dopamine. The detector response was directly proportional to the amount of catecholamines injected from 0.6 to 200 pg per 30 µL injected.

The amount of recovered bone marrow was 38.1±3.4 mg (n=12) in normal mice, which did not significantly differ from that in mice treated with 6-OHDA (32.6±1.1 mg; n=12) or pargyline (35.1±2.7 mg; n=10). Catecholamines were always detected in the samples. Noradrenaline, adrenaline and dopamine levels in the bone marrow from normal mice were 2806.74±408.85 (n=12), 803.37±87.66 (n=12) and 274.47±51.54 (n=10) pg/g of tissue, respectively. In mice treated with 6-OHDA or pargyline, bone marrow adrenaline and dopamine levels were not significantly different from those of nor-

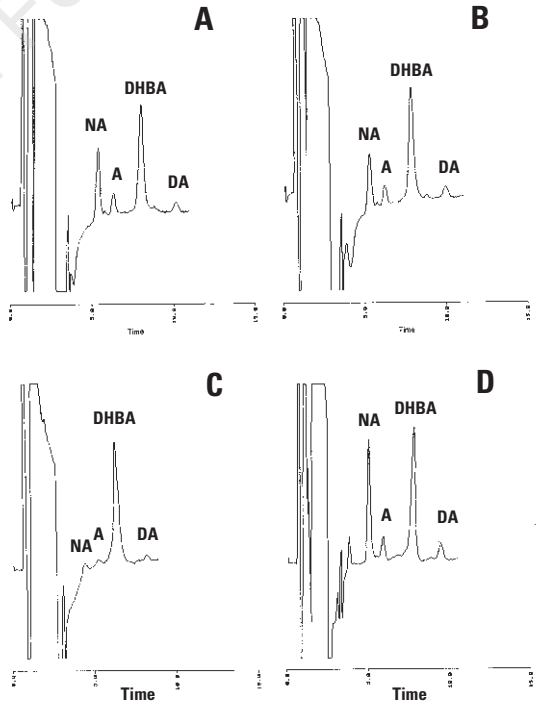

Figure 1. Chromatograms obtained after injecting 30 µL of a standard sample containing noradrenaline (NA, 25 pg; retention time = 5.53), adrenaline (A, 25 pg; retention time = 6.35) and dopamine (DA, 25 pg; retention time = 10.23) dissolved in 0.4 NHcl04 (A), or of samples of bone marrow supernatant obtained from normal (B), 6-OHDA-treated (C) and pargyline-treated (D) animals. Each sample was added with DHBA as internal standard. Time scale in minutes. For further details, see *Materials and Methods*.

mal animals. On the other hand, the amount of noradrenaline was lower after treatment with 6-OHDA (1130.47±142.73 pg/g of tissue, n=12, P<0.01) and higher after treatment with pargyline (4122.62±509.54 pg/g of tissue, n=10, P<0.05) with respect to normal values (Figure 2).

Discussion

The presence of noradrenergic fibers in the bone marrow has been demonstrated using fluorescence histochemical staining for catecholamines.[3] However, to the best of our knowledge, direct measurement of catecholamines in this tissue had never been accomplished before. We now report that mouse bone marrow contains significant amounts of endogenous noradrenaline, adrenaline and dopamine.

The noradrenaline content at this level is about 3 ng/g of tissue. This is not a very high amount, particularly when compared to other lymphoid organs, such as rat thymus and lymph nodes, where noradrenaline is in the range of 47-131 ng/g of tissue.[10] In our experiments, bone marrow noradrenaline is reduced by sympathetic denervation with 6-OHDA and increased after chronic MAO inhibition with pargyline. Thus it seems that noradrenaline in the bone marrow originates mainly from sympathetic nerve endings. Moreover, indirect evidence was found for the presence of specific enzymatic pathways responsible for catecholamines destruction.

Concerning adrenaline and dopamine, their modifications after 6-OHDA or pargyline treatment do not reach statistical significance, although the former at least tends to increase during chronic pargyline administration. Therefore it would seem that only a minor part, if any, of these two catecholamines is of neurogenic origin. In particular, the very low dopamine content in the bone marrow could be consistent with its role as a biosynthetic precursor of noradrenaline.

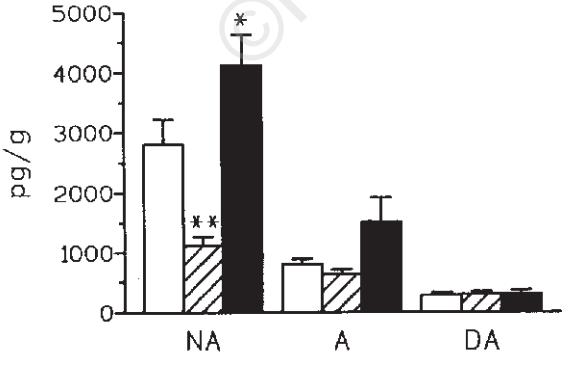

Figure 2. Noradrenaline (NA), adrenaline (A) and dopamine (DA) in bone marrow from normal (empty columns), 6-OHDA-treated (hatched columns) and pargyline-treated (closed columns) animals. Data are the mean of at least 10 observations and are expressed as pg/g of tissue. Vertical bars indicate SEM. *p<0.05 and **p<0.01 vs. normal animals.

After 6-OHDA treatment, a significant amount of catecholamines is still present in the bone marrow and this observation could indicate that at this level these substances may originate not only from sympathoadrenergic fibers, but also from other sources, such as the systemic circulation. It has recently been shown that circulating mouse lymphocytes synthesize catecholamines.[11] This raises the possibility that bone marrow cells also produce catecholamines, which could act as autocrine and/or paracrine regulators of lymphohematopoietic processes.[12] This finding may be relevant to stem cell transplantation.[13,14]

In conclusion, we successfully employed a high performance liquid chromatographic method to directly measure endogenous catecholamines in mouse bone marrow, showing that noradrenaline, adrenaline and dopamine are present at this level. Since circumstantial evidence suggests that the adrenergic system modulates hematopoiesis,[4-8] studying catecholamines in the bone marrow will help to clarify their possible role as endogenous modulators.

References

1. Felten SY, Felten DL, Bellinger DL, et al. Noradrenergic sympathetic innervation of lymphoid organs. Prog Allergy 1988; 43:14-36.
2. Felten DL, Felten SY, Carlson SL, Olschowka JA, Livnat S. Noradrenergic and peptidergic innervation of lymphoid tissue. J Immunol 1985; 135:755s-65s.
3. Bellinger DL, Lorton D, Felten SY, Felten DL. Innervation of lymphoid organs and implications in development, aging, and autoimmunity. Int J Immunopharmacol 1992; 14:329-44.
4. Maestroni GJM, Conti A. Noradrenergic modulation of lymphohematopoiesis. Int J Immunopharmacol 1994; 16:117-22.
5. Dresch C, Minc J, Mary JY. In vivo protection of normal mouse hematopoiesis by β2 blocking agent during S-phase chemotherapy. Cancer Res 1984; 44:493-7.
6. Maestroni GJM, Conti A, Pedrinis E. Effect of adrenergic agents on hematopoiesis after syngeneic bone marrow transplantation in mice. Blood 1992; 80:1178-82.
7. Maestroni GJM, Conti A. Modulation of hematopoiesis via 1-adrenergic receptors on bone marrow cells. Exp Hematol 1994; 22:313-20.
8. Dresch C, Minc J, Poirier O, Bouvet D. Effect of beta adrenergic agonists and beta blocking agents on hemopoiesis in human bone marrow. Biomedicine 1981; 34:93-8.
9. Miller ML, McCuskey RS. Innervation of bone marrow in the rabbit. Scand J Hematol 1973; 10:17-23.
10. Del Rey A, Besedovsky HO, Sorkin E, Da Prada M, Arrenbrecht S. Immunoregulation mediated by the sympathetic nervous system, II. Cell Immunol 1981; 50:329-34.
11. Josefsson E, Bergquist J, Ekman R, Tarkowski A. Catecholamines are synthesized by mouse lymphocytes and regulate function of these cells by induction of apoptosis. Immunology 1996; 88:140-6.
12. Carlo-Stella C, Rizzoli V. Stem cells and stem cell factor(s). Haematologica 1995; 80:1-4.
13. Carlo-Stella C, Cazzola M, De Fabritiis P, et al. CD34-positive cells: biology and clinical relevance. Haematologica 1995; 80:367-87.
14. Carlo-Stella C, Tabilio A. Stem cells and stem cell transplantation. Haematologica 1996; 81:573-87.