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Effects of glucocorticoids on polyamine metabolism in liver and spleen of guinea pig during sensitization

G. Bjelakovic, D. Pavlovic, I. Stojanovic, T. Jevtovic, J. Nikolic, and **G. Kocic**

Institute of Biochemistry, Faculty of Medicine, University of Niš, Niš, Serbia and Montenegro

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Summary. Glucocorticoids are potent anti-inflammatory and immunosuppressive agents. As endogenous inhibitors of cytokine synthesis, glucocorticoids suppress immune activation and uncontrolled overproduction of cytokines, preventing tissue injury. Also, polyamine spermine is endogenous inhibitor of cytokine production (inhibiting IL-1, IL-6 and TNF synthesis). The idea of our work was to examine dexamethasone effects on the metabolism of polyamines, spermine, spermidine and putrescine and polyamine oxidase activity in liver and spleen during sensitization of guinea pigs. Sensitization was done by application of bovine serum albumin with addition of complete Freund's adjuvant. Our results indicate that polyamine amounts and polyamine oxidase activity increase during immunogenesis in liver and spleen. Dexamethasone application to sensitized and unsensitized guinea pigs causes depletion of polyamines in liver and spleen. Dexamethasone decreases polyamine oxidase activity in liver and spleen of sensitized guinea pigs, increasing at the same time PAO activity in tissues of unsensitized animals.

Keywords: Polyamines – Polyamine oxidase – Dexamethasone – Sensitization – Liver – Spleen – Guinea pigs

Introduction

Glucocorticoids are potent anti-inflammatory and immunosuppressive agents (Coleman, 1972; Thompson, 1999). A large body of evidence implicates spermine as an inhibitor of immune response; spermine down-regulates human neutrophil locomotion (Ferrante, 1985) and is immunosuppressive to T cells (Thompson, 1999). It has been suggested that the accumulation of spermine and products of its oxidative metabolism via polyamine oxidase, mediate anti-inflammatory activity found in many inflammatory and immunological diseases (Zhang et al., 1997). Spermine mediates in suppressing the innate immune response (Suzuki, 2005).

As endogenous inhibitors of cytokine synthesis, which are produced during stress response, glucocorticoids suppress immune activation and uncontrolled overproduction and release of cytokines preventing tissue injury. Beside glucocorticoids, polyamine spermine is endogenous inhibitor of cytokine production (Zhang et al., 1997). Spermine inhibits the synthesis of interleukin-1 (IL-1), IL-6, tumor necrosis factor (TNF). The inhibition of cytokine synthesis by spermine is specific and reversible (Smith, 2001).

Polyamines, spermine (Sp), spermidine (Spd) and putrescine (Put) are aliphatic cations widely distributed in nature as normal constituents of prokaryotic, eukaryotic, plant and animal cells (Tabor and Tabor, 1984; Pegg, 1988; Moinard et al., 2005). Metabolism of polyamines is intimately associated with growth and differentiation of mammalians. Direct binding of polyamines to DNA and their ability to modulate DNA-protein interactions appear to be important in the molecular mechanisms of polyamine action in cell proliferation (Thomas and Thomas, 2001).

It has been already known that spermine concentrations are significantly elevated in tissues during infection, malignant and inflammatory diseases, proposing a direct role of spermine in control of these processes (Wallace, 2003; Bachrach, 2004; Peulen et al., 2004; Moinard et al., 2005). Up to now there is no data about interrelationship between polyamines and the gut immune system (Peulen, 2004). Also, there is no insight about the effects of spermine on immune system of spleen, an organ playing an important role in immune response (Jolois et al., 2002), as well as about changes in polyamine oxidase activity (PAO), which is a key enzyme in polyamine catabolism (Holtta, 1977; Vujic, 2002; Seiler, 2004).

The aim of our present study was to establish the effects of dexamethasone, a synthetic glucocorticoid, on

polyamine amounts and polyamine oxidase activity in spleen and liver of sensitized guinea pigs.

Materials and methods

Male guinea pigs weighing 400–600 g were used for this study. The animals were divided into three groups: first control – unsensitized group (15 animals), second – sensitized (60 animals) and third – sensitized and treated with dexamethasone (60 animals). Sensitization of guinea pigs was done by application of bovine serum albumin mixed with complete Freund's adjuvant at the plantar parts of all four legs (10 mg of bovine serum albumin was dissolved in 0.5 ml of 0.9% NaCl; 0.5 ml of complete Freund's adjuvant, purchased from "Torlac-Belgrade", was added slowly to this solution. The sensitized animals were divided in two parts: the animals that received dexamethasone intraperitoneally in a single daily dose of 10.0 mg/kg body weight and those that received 0.9% NaCl or physiological saline or 155 mmol/l NaCl instead of the hormone. The animals were sacrificed at different time intervals (at 6^{th} – 15 sensitized animals + 15 sensitized and treated with dexamethasone, 14^{th} – 15 sensitized animals + 15 sensitized and treated with dexamethasone, 21^{st} – 15 sensitized animals + 15 sensitized and treated with dexamethasone and 27^{th} day after antigen application – 15 sensitized animals + 15 sensitized and treated with dexamethasone. The effect of dexamethasone was examined at various time intervals during sensitization. The subgroup of sensitized guinea pigs sacrificed on the 6^{th} day after receiving antigen started to receive hormone immediately after application of the antigen. The subgroup of sensitized guinea pigs sacrificed on the 14^{th} day after receiving antigen started receiving hormone on the 7^{th} day after sensitization. The hormone was given for the next 7 days, as well as 1 hour before sacrificing on the 14^{th} day. In the group of animals sacrificed on the 21^{st} day of sensitization the effects of dexamethasone were examined by the administration of this drug to sensitized guinea pigs beginning from day 14 after antigen application. Dexamethasone was given continually every day up to 21^{st} day when the last dose was given 1 hour before sacrificing.

The investigation at the end of four week of sensitization (27^{th} day): the application of dexamethasone began on the 14^{th} day after antigen application to guinea-pigs; the drug was given every day until 27^{th} as well as 1 hour before sacrificing. Dexamethasone was applicated in the morning between 8 and 9 hours AM, as well as 1 hour before sacrificing.

In order to better understand the effects of glucocorticoids on polyamine metabolism we have done experiments with unsensibilized guinea pigs. For this purpose a part of unsensitized guinea pigs (9 animals) received dexamethasone in a single daily dose of 10.0 mg/kg of body weight during the period of six days. The animals were killed by decapitation at 6^{th} day one hour after application of the last, seventh dose of hormone. Control group of animals (9 animals) received 0.9% NaCl instead of the hormone.

At the end of mentioned time intervals the animals received ketalar anesthesia (50 mg/kg intra muscularly) and sacrificing was carried out by decapitation. The liver and spleen were removed quickly (excess blood was removed by blotting), rinsed in saline (0.9% NaCl) and frozen at −70 °C for up to one week until they were analyzed.

Examination of polyamine concentrations was done with butanol extraction followed by ninhydrine identification of separated polyamines by electrophoresis (Russell et al., 1970). For the estimation of PAO activity liver and spleen tissue was cut in small pieces and homogenized in ice-cold water. The homogenates (10% without w/v) were centrifuged at 1500 × g for 10 min at 4 °C. The resulting supernatants were used for investigation of enzyme activities and protein content. Activity of polyamine oxidase (spermine tetrahydrochloride was used as a substrate) was measured by modified spectrophotometric method of Bachrach and Reches (1966) based on the determination of the formed amount of amino aldehydes (Quash et al., 1979). One unit of enzyme activity was defined as an increase in optical density of 0.100 at 660 nm. Proteins were determined according to Lowry's method (Lowry et al., 1951).

The obtained results were statistically analyzed with Student's t-test by using statistical computer program SPSS. Statistic significance was marked as follows: $a^{***}p < 0.001$; $^{**}p < 0.01$; $^{*}p < 0.05$ vs control; $b^{ooo}p < 0.001$; $b^{oo}p < 0.01$; $b^{o}p < 0.05$ vs values in sensitized animals.

Results

Polyamine levels in liver and spleen tissue during sensitization

Sensitization of guinea pigs with bovine serum albumin in the complex with the complete Freund's adjuvant causes augmentation of polyamine amounts in liver and spleen (Figs. 1, 2). In liver the highest amount of spermine was observed at the 21^{st} day of sensitization; the amounts of spermidine and putrescine were the highest at the 14^{th} day after antigen application. At the end of the third and

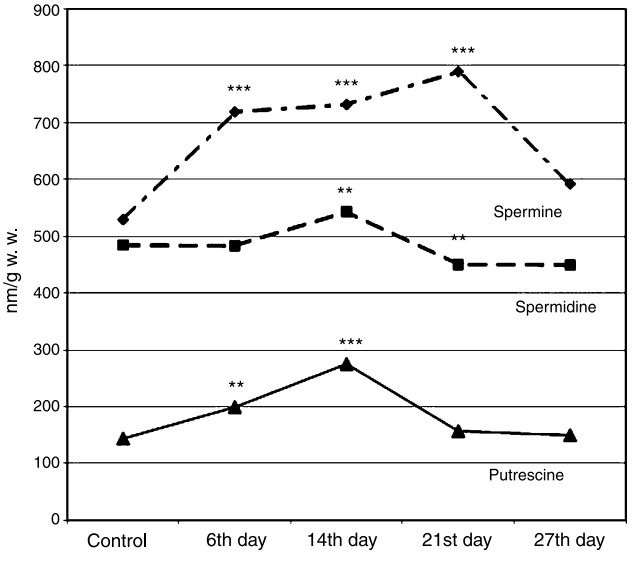

Fig. 1. Polyamine levels in guinea pig liver tissue during sensitization

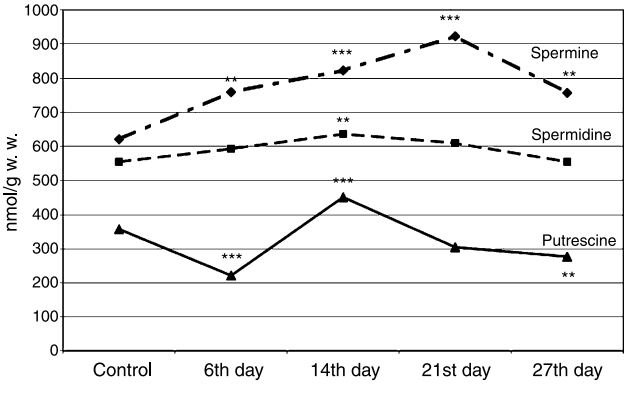

Fig. 2. Polyamine levels in spleen of sensitized guinea pigs

fourth week of the sensitization concentration of spermidine diminished, while putrescine level returned to the control values (Fig. 1).

In the spleen the highest value of spermine was present at the 21st day after antigen application; the highest value of spermidine was observed at the end of the second week (14th day) after antigen application; at the end of the fourth week of sensitization (27th day) the amount of spermidine returned to the control value. Putrescine amount decreased significantly at the end of the first week after antigen application; after this decrease the amount of putrescine raised to the highest value at the end of the second week of sensitization period (14th day), followed by decrease during the third and fourth week (Fig. 2).

Dexamethasone effects on polyamine levels in liver and spleen

Application of dexamethasone caused decrease of Sp, Spd and Put in liver of sensibilized guinea pigs (Fig. 3).

Dexamethasone moderately changed the polyamine levels in spleen: after the initial decrease of Sp and Spd levels, observed at the 6th day, dexamethasone caused the highest augmentation of spermidine amount at 14th day and spermine at 21st day after antigen application. The putrescine concentration followed the values observed at the 6th and 14th day during sensitization; at the 21st and 27th day the amount of Put was significantly higher in comparison with sensitized animals (Fig. 4).

Polyamine oxidase activity in liver and spleen tissue during sensitization

Polyamine oxidase activity increased during sensitization period, especially in the spleen tissue (Figs. 5, 6). The maximum activity was observed on the 21st day after antigen application.

Dexamethasone effects on polyamine oxidase activity

Dexamethasone application to sensitized animals caused decrease of PAO activity in liver and spleen (Figs. 5, 6) more pronounced in spleen.

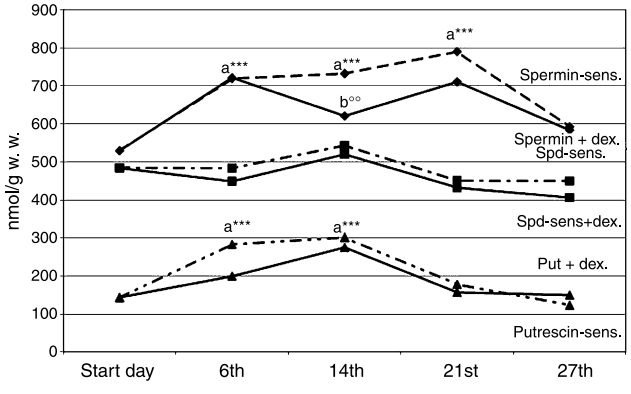

Fig. 3. Dexamethasone effect on polyamine amounts in guinea-pig liver during sensitization

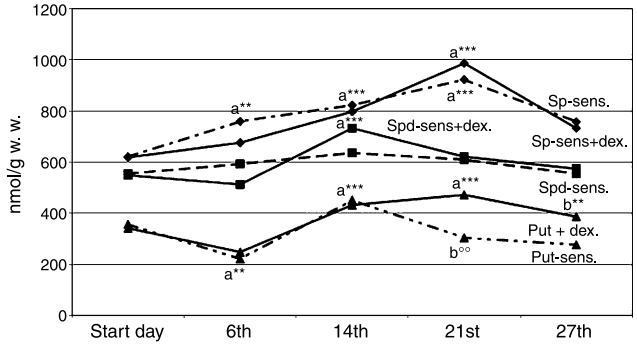

Fig. 4. Dexamethasone effect on polyamine levels in spleen during sensitization

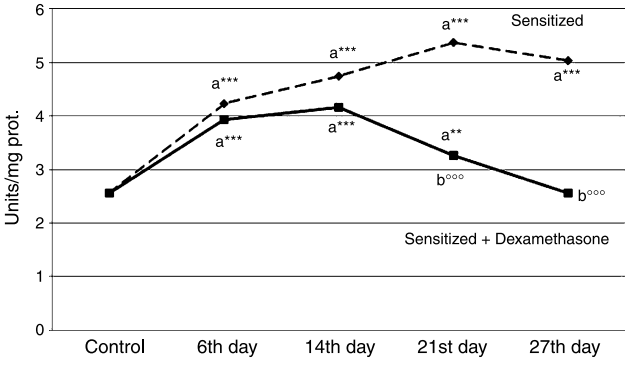

Fig. 5. Dexamethasone effect on polyamine oxidase activity in liver tissue during sensitization

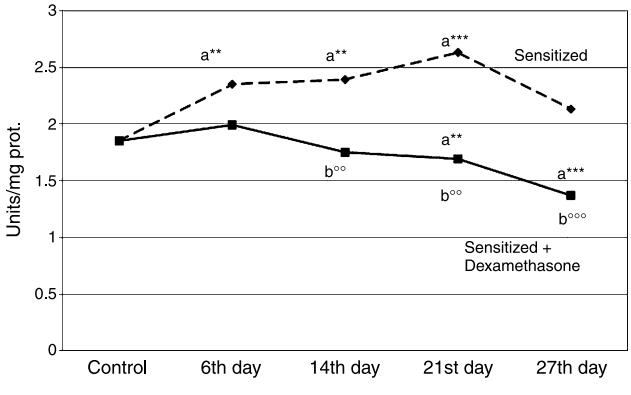

Fig. 6. Dexamethasone effect on polyamine oxidase activity in spleen of guinea pigs

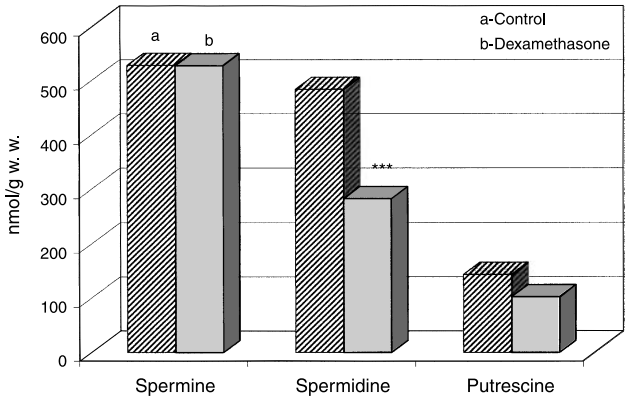

Fig. 7. Effect of dexamethasone to polyamine levels in liver tissue of unsensitized guinea pigs

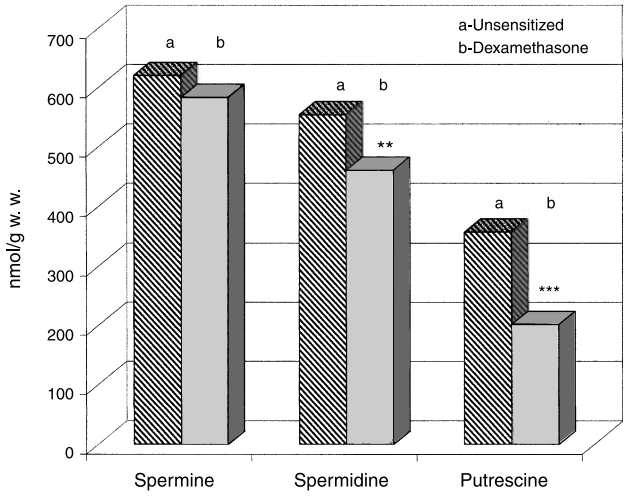

Fig. 8. Effect of dexamethasone on polyamine levels in spleen tissue of unsensitized guinea pigs

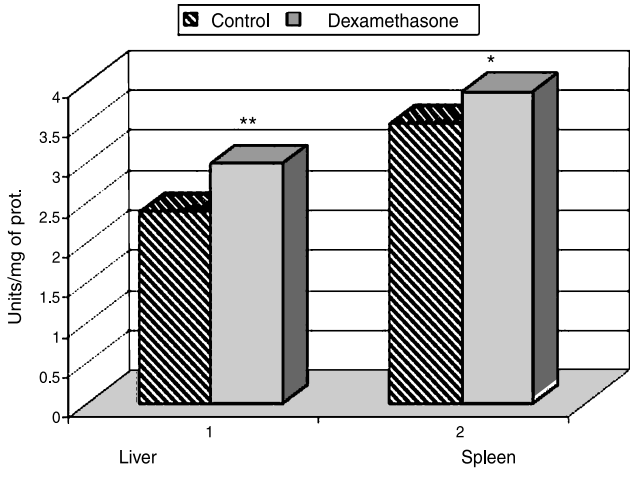

Fig. 9. Effect of dexamethasone on polyamine oxidase activity in liver and spleen tissue of unsensitized guinea pigs

Dexamethasone effects on polyamine amount and PAO activity in liver and spleen of unsensitized guinea pigs

In experiment with unsensitized guinea pigs application of dexamethasone during period of six days caused decrease of Spd and Put in liver and decrease of all three polyamines in spleen tissue (Figs. 7, 8). At the same time, dexamethasone increased PAO activity in both tissues (Fig. 9).

Discussion

At present there is a widespread usage of glucocorticoids in medicine, presumably in treatment of immunological and malignant diseases.

It is known that spermine concentrations are significantly elevated in tissues during infection, neoplastic and inflammatory diseases proposing a direct role of spermine in control of these processes (Pegg, 1988; Hegardt et al., 2000; Thomas and Thomas, 2001; Moinard et al., 2005). During the early immune response to infection (or injury) macrophages synthesize proinflammatory cytokines. The uncontrolled release of larger amounts of cytokines signals the onset of tissue injury. This unwanted effect is normally prevented by endogenous counterregulatory mechanisms that involve inhibition of cytokine overproduction. Glucocorticoids and polyamine spermine are endogenous inhibitors of cytokine production (Zhang et al., 1997; Smith et al., 2001).

A large body of evidence implicates spermine as an inhibitor of immune response (Seiler and Atanassov, 1994; Zhang et al., 1997; Soda et al., 2005). Spermine mediates in suppressing the innate immune response (Suzuki, 2005) and effectively suppresses the synthesis of proinflammatory cytokines in human white blood cells (Soda, 2005). The mechanism of cytokine inhibition by spermine is post-transcriptional, reversible, specific, and independent of polyamine oxidase activity (Zhang et al., 1997; Desiderio et al., 1998; Hegardt et al., 2000; Smith et al., 2001). It has been suggested that the accumulation of spermine, and products of its oxidative metabolism via polyamine oxidase, mediate anti-inflammatory activity found in many inflammatory and immunological diseases (Zhang et al., 1997; Hegardt et al., 2000; Xu et al., 2004).

The increase of polyamines during sensitization in our experiment is in agreement with the mentioned function of spermine; after antigen stimulation of guinea pigs with bovine serum albumin polyamines are synthesized more rapidly in order to prevent overproduction of cytokines. These results are also in agreement with research which proves that cytokines induce ODC activity in liver and spleen (Seiler and Atanassov, 1994).

Glucocorticoids are the most important among the steroid hormones of adrenal cortex. At present, there is a widespread usage of glucocorticoids in different metabolic diseases in medicine, presumably in immunological and malignant diseases (Thomson, 1979). In excess, glucocorticoids suppress inflammatory and immunology response (Litwack and Schmidt, 1997; Rhen and Cidlowsky, 2005).

Glucocorticoids inhibit antigen processing, T cells function, synthesis of cellular mediators of the inflammatory response (interleukins, lymphokines, etc.), cellular migration and action at sites of inflammation (Zhang et al., 1997). Some populations of lymphocytes are killed by glucocorticoids; this explains the efficacy of these steroids in treating certain leukemias such as acute lymphoblast leukemia of childhood (Abbott and Bird, 1983; Miller et al., 2002; Soda et al., 2005).

Glucocorticoids are well known pro-apoptotic agents in certain classes of lymphoid cells (Abbott and Bird, 1983; Desiderio, 1995; Hegardt et al., 2000). They induce apoptosis by inter-nucleosomal DNA cleavage, while spermine could inhibit DNA fragmentation (LaVoie and Witorsch, 1995; Ferioli et al., 1999; Hegardt et al., 2001, 2003; Miller et al., 2002). Studies have shown that spermine prevents cytochrome c release from mitochondria in glucocorticoid-induced apoptosis (Hegardt at al., 2003). Spermidine and putrescine can also inhibit apoptosis but that occurs at much higher concentration than when using spermine (Hegardt at al., 2003).

Glucocorticoids exert catabolic effects on lymphoid tissues in which they cause programmed cell death by depletion of polyamines and by progression of apoptotic program. Depletion of polyamines occurs in lymphoid tissues of glucocorticoid-treated rats, due to inhibition of polyamine biosynthesis and the induction of catabolic pathway followed by production of hydrogen peroxide, product responsible for apoptotic process (Bjelakovic and Pavlovic, 1987; Desiderio, 1998; Ferioli et al., 1999; Hegardt et al., 2000).

The application of dexamethasone during sensitization caused decrease of polyamine concentrations in liver. Dexamethasone moderately changed polyamine levels in spleen causing the initial decrease of Sp, Spd and Put. This decrease is in agreement with literature data, which point out the inhibitory effect of dexamethasone on the enzyme ornithine decarboxylase (ODC) by the induction of ODC-antizyme synthesis (Bishop, 1985). From the 14^{th} day dexamethasone caused the augmentation of polyamines in spleen which could be explained in the context of opposite effects of polyamines to apoptosis induced by dexamethasone (Hegardt et al., 2001).

Homeostasis of polyamine levels in eukaryotic cells is very complex process related to many important metabolic pathways (Tabor and Tabor, 1984). Polyamines can protect cells from programmed cell death (apoptosis); also, they have biological significance as they trigger apoptosis by the production of hydrogen peroxide and amino-dialdehyde, intermediary products in polyamine catabolism by polyamine oxidase (PAO) action (Holtta, 1979; Seiler, 1995, 2004; Rao et al., 1999). The catabolic process of polyamine is initiated by the enzyme spermidine/spermine N1-acetyltransferase (SSAT). By the action of this enzyme spermine or spermidine is metabolized to acetylspermine or acetylspermidine, respectively, the substrates for polyamine oxidase (PAO) (Vujic, 2002; Seiler, 2004). Stefanelli et al. (1987) reported that dexamethasone caused an increase of the activity of cytosolic spermine N^1-acetyl transferase and decrease of ornithine decarboxylase activity in spleen and thymus. The enzyme SSAT has been named APAO to denote acetyl PAO. More recently, an additional enzyme, PAOh1, was cloned in human cells (Vujic, 2003), and the same enzyme was later named spermine oxidase since it directly and specifically converts spermine to spermidine without the intermediate acetylation step (Seiler, 2004).

In the current study, we investigated the polyamine oxidase activity which was increased during sensitization, especially in spleen. Up to now, there is no literature about this enzyme activity during immunogenesis. The increase of PAO activity follows the increase of polyamine levels in liver and spleen during sensitization. These data support the idea that PAO activity does not play an important role in the regulation of polyamine levels.

Dexamethasone decreased polyamine oxidase activity in liver and spleen of sensitized guinea pigs, increasing at the same time PAO activity in tissues of unsensitized animals. The PAO responses observed in sensitized and unsensitized tissues of guinea pigs may represent an important event, suggesting a role of the enzyme in the metabolic effects of glucocorticoids.

Our results are in accordance with published research and may suggest that levels of polyamines might be dependent on a complex mechanism involving the enzymes of the biosynthetic and catabolic pathways (Desiderio, 1995, 1998; Ferioli et al., 1999; Hegardt et al., 2000).

Our results confirm and extent the statement of Rao et al. (1999): ''The discoveries of physiological functions of polyamines are great, but many questions remain to be answered''. The same could be said for the use of glicocorticoides, indicating the necessity of further future investigations in human biology.

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Authors' address: G. Bjelakovic, Institute of Biochemistry, Faculty of Medicine, University of Niš, 18000 Niš, Serbia and Montenegro, Fax: +38118238770, E-mail: bjelakovic@junis.ni.ac.yu