Chronic α1-adrenoreceptor blockade produces age-dependent changes in rat thymus structure and thymocyte differentiation

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Summary. In order to examine the influence of chronic α1-adrenergic receptor (α1-AR) blockade on the thymus structure and T-cell maturation, peripubertal and adult male rats were treated with urapidil (0.20 mg/kg BW/d; s.c.) over 15 consecutive days. Thymic structure and phenotypic characteristics of the thymocytes were assessed by stereological and flow cytometry analysis, respectively. In immature rats, treatment with urapidil reduced the body weight gain and, affecting the volume of cortical compartment and its cellularity decreased the organ size and the total number of thymocytes compared to age-matched saline-injected controls. The percentage of CD4+8- single positive (SP) thymocytes was decreased, while that of CD4-8+ was increased suggesting, most likely, a disregulation in final steps of the positively selected cells maturation. However, α1-AR blockade in adult rats increased the thymus weight as a consequence of increase in the cortical size and cellularity. The increased percentage of most immature CD4-8- double negative (DN) cells associated with decreased percentage of immature CD4+8+ double positive (DP) thymocytes suggests a decelerated transition from DN to DP stage of T-cell development. As in immature rats, the treatment in adult rats evoked changes in the relative numbers of SP cells, but contrary to immature animals, favoring the maturation of CD4+8- over CD4-8+ thymocytes. These results demonstrate that: i) chronic blockade of α1-ARs affects both the thymus structure and thymocyte differentiation, ii) these effects are age-dependent, pointing out to pharmacological manipulation of α1-AR-mediated signaling as potential means for modulation of the intrathymic T-cell maturation.

Key words: α1-adrenoceptor blockade, Urapidil, Rat, Thymus structure, Thymocyte development

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Introduction

Throughout intrathymic differentiation T cells actively migrate between stratified stromal regions, so that each thymopoietic stage is localized in anatomically and functionally distinct subregions (Shortman et al., 1990; Lind et al., 2001). The bone-marrow-derived precursors enter the thymus near the cortico-medullary junction and then they migrate toward the subcapsular epithelium where TCRß rearrangement and assembly of ß/pTα complex, leading to expression of coreceptor CD4 and CD8 molecules, and rearrangement of TCR α locus occur. As thymocytes enter this stage, they travel back through the cortex. On this route CD4+8+ double positive (DP) thymocytes are subjected to selection processes. Positively selected cells differentiate into mature CD4+ or CD8+ single positive (SP) cells, which migrate through the medulla before exiting the thymus (Lind et al., 2001). Thymocytes that are not positively selected die either of "neglect" or as result of negative selection (Chan et al., 1993).

The catecholamines norepinephrine and epinephrine are believed to be important modulators of immune functions, especially following exposure to stress when they act in concert with activation of the hypothalamic pituitary axis (Cunnick et al., 1990). *In vitro*, catecholamines have been shown to modulate a range of immune cell functions including cell proliferation, cytokine and antibody production, lytic action and migration (Madden, 2003). It has been hypothesized that *in vivo* catecholamines influence immune functions by altering the responsiveness of individual immune cells (Madden, 2003) and/or by changing the number and/or subset proportion of cells that participate in a given immune response (Benschop et al., 1996). Furthermore, data from *in vitro* and *in vivo* studies have demonstrated that pharmacological manipulation of ß-adrenoceptor (AR)-mediated catecholamine action may affect the thymus structure and T-cell maturation and that these effects are age-dependent (Singh and Owen, 1976; Singh

et al., 1979; Leposavić et al., 2000, Madden and Felten, 2001; Rauški et al., 2003; Plećaš-Solarović et al., 2004).

Although it is reckoned that catecholamines exert the effects on the immune system mainly via ß$_2$-ARs (Elenkov et al., 2000; Sanders and Straub, 2002; Madden, 2003), it should be pointed out that α_2-AR expression has been demonstrated in rat lymph node and spleen cells (Fernández-López and Pazos, 1994), as well as in macrophages (Spengler at al., 1990). In addition, functional studies clearly implicated α_2-ARs not only in the modulation of mature lymphocyte functions (Sanders and Munson, 1984; Spengler et al., 1990; Felsner et al., 1995), but also in the regulation of thymocyte apoptosis (Ćupić et al., 2003) suggesting their possible role in the fine tuning of T-cell maturation.

More recently, it has been shown that rat quiescent lymphocytes express the genes, not only for α_2-ARs, but also for α_1-ARs (Schauenstein et al., 2000). However, according to the study of Rouppe and her collaborators (2002) α_1-ARs are not detected in peripheral blood mononuclear cells of healthy humans, but human primary lymphoid organs do express mRNA encoding these receptors (Kavelaars, 2002). Therefore, it has been assumed that the human lymphocytes express α_1-ARs at certain stages of development, but that their expression is not detectable on the mature cells entering the peripheral circulation (Kavelaars, 2002). Moreover, it has been reported that the expression of α_1-ARs by immune system cells can be modulated by glucocorticoid, ß-AR agonist (Rouppe et al., 1999) and cytokine TNF-α and IL-1ß (Heijnen et al., 2002) action.

Very little is known on the role of α_1-ARs in the modulation of the immune response. There is evidence to suggest that α_1-ARs are involved in the regulation of hematopoiesis (Maestroni and Conti, 1994), and dendritic cell maturation and migration (Maestroni, 2000). Moreover, it has been shown that the treatment of fetal thymic organ culture with phenylephrine, an α_1-AR agonist, evokes an increase in the thymocyte proliferative activity, and consequently, in cell yield (Singh, 1979).

Since there are data indicating the possible role of α_1-AR signalling in the regulation of T-cell maturation, in the present study we examined the effects of chronic urapidil-induced α_1-AR blockade on the rat thymus structure and T-cell development during the peripubertal period, when the thymus in rats peaks in size and begins to involute (Marchetti et al., 1990; Morale et al., 1991), and during the early adult period of life. Urapidil is known as a postsynaptic α_1-AR blocker with a pharmacodynamic profile similar to prazosin (Langtry et al., 1989). Structural characteristics of the thymus after chronic urapidil treatment were followed by sterological analysis and its influence on thymocyte differentiation was assessed by determining the relative proportion of main thymocyte subsets delineated by expression of CD4/CD8 coreceptor molecules and TCRαß by two-colour and one-colour flow cytometric analysis, respectively.

Material and methods

Animals and treatment

Male Wistar rats aged either 21 (BW: 70-80 g) or 75 days (BW: 250-300 g) at the beginning of experiment were maintained in our animal room under a standard 12-h photoperiod, at 21±2°C, with free access to food and water. The animals from both age groups were assigned randomly (n=5/group) to receive urapidil or saline. Over 15 consecutive days, they were injected subcutaneously (s.c.) with 0.20 mg/kg BW/day of urapidil (Ebrantil, Byk Gulden, Germany) diluted in saline or an equivalent volume (1 ml/kg BW/day) of saline. The dose chosen was based on doses reported to be effective at blocking other α_1-adrenergic induced effects *in vivo* (Plećaš et al., 1996; Ittner et al., 2002). One hour after the final injection the animals were individually removed from their cages and anaesthesia was induced in bell-jar using diethyl ether (LEK, Slovenia). After decapitation, the thymus was removed, dissected free of parathymic lymph nodes and adherent membranous tissue and two lobes were divided and individually weighed. The right lobus was used for analysis of phenotypic characteristics of thymocytes, while the left lobus was processed for stereological analysis.

Preparation of thymic cell suspensions

The thymic lobes were excised and placed in individual Petri dishes containing ice-cold phosphate-buffered saline (PBS, pH 7.3). The thymocyte suspension was prepared by grinding the thymic tissue between the frosted ends of microscope slides and passing the resultant suspension through a fine nylon mesh. Thus obtained single-cell suspension was washed three times in ice-cold PBS containing 2% fetal calf serum (Gibco, Grand Island, N.Y., USA) and 0.01% sodium azide (PS medium). The cells were then counted in an improved Neubauer haemacytometer and cell density was adjusted to 10^7 cells/ml by addition of PS medium. The viability of such cell preparations, as determined by Trypan blue exclusion, was routinely greater than 95%.

Flow cytometry (FCA)

Immunofluorescence staining of thymocytes was performed using two independent systems, including (a) direct two-colour staining with fluorescein isothicyanate FITC-conjugated anti CD4 (clone W3/25, Serotec, Oxford, UK) and phycoerythrin (PE)-conjugated anti-CD8 (clone MRC OX-8, Serotec) monoclonal antibodies (mAbs) and (b) indirect one-colour staining with biotin-conjugated mAb, directed at a constant determinant of the rat TCR (clone R73, Serotec), as first-step reagent, followed by FITC-conjugated streptavidin (Becton Dickinson, Mountain View, Calif. USA), as second-step

reagent. The immunostaining was carried out as it has been previously reported (Leposavić et al., 2000).

All samples were analyzed on the same day on a FACScan flow cytometer (Becton Dickinson). Dead cells and debris were excluded from analysis by selective gating based on anterior and right-angle scatter. 10^4 flow cytometric events for the two-colour and 5×10^3 flow cytometric events for the one-colour FCA were analyzed. The analyses were carried out with respect to appropriate isotypic and fluorochrome-matched controls, with Consort 30 and Lysis software (Becton Dickinson).

Stereological analysis

After fixation in Bouin's solution, dehydratation in a graded series of ethyl alcohol and embedding in paraffin, the thymic tissue was serially cut at 5 µm thick sections and the sections were stained with haematoxylin and eosin. Every 40th section (20 sections per organ) was subjected to analysis of different stereological parameters.

Stereological measurements were performed by point and intersection counting method (Weibel, 1979; Karapetrović et al., 1995; Pejčić-Karapetrović et al., 2001; Plećaš-Solarović et al., 2004) using image analysis software (Micro Image Version 4.0, OLYMPUS). The test areas were randomly chosen, and each image, acquired using a digital camera, was saved, overlaid with the corresponding grid and analyzed.

Absolute volumes of the thymic cortex and medulla were estimated from the volume of the processed and embedded organ and volume density (Vv) of corresponding compartment. The volume of fixed thymic tissue was calculated from the fresh tissue weight, specific gravity (Casley-Smith, 1988) and the percentage of tissue shrinkage (approximately 34%), which was determined stereologically (Plećaš-Solarović et al., 2004). Thus, all morphometrical data refer to fixed thymic tissue. Vv of each thymic compartment was determined under magnification of x40, using an orthogonal test grid with 100 points, and according to the equation: Vv=Pf/Pt, where Pf is the number of test points hitting the analyzed structure and Pt is total number of test points falling on the organ. Total number of analyzed test-areas was 100 per animal.

The total number of thymocytes in the thymic compartment was calculated from numerical density (Nv) of thymocytes and the absolute volume of that compartment. The Nv of thymocytes, representing the number of cells per unite volume, was estimated under immersion magnification, using a grid that corresponds to the multipurpose M42 test-system (Weibel, 1979). The test grid was placed randomly, but positioned parallel to, and just touching, the capsule for the outer cortex and the cortico-medullary junction for the deep cortex analysis, respectively (Kendall and Al-Shawaf, 1991). For estimating Nv of medullary thymocytes, the grid was placed randomly throughout the medulla. For each thymic compartment 60 test-areas per animal were measured. Nv was calculated according to the equation: Nv=NA/(D+Go) where NA, as the number of thymocytes per surface unit of test area, was estimated from the relation: NA=N/At. N is the number of cells per test area and At is actual size of the test area. D, the mean caliper diameter of thymocytes, was calculated as: D=6Vv/Sv. Vv in this equation refers to volume density of thymocytes, while Sv is the surface density, calculated as: Sv=2If/Lt. If is the number of intersections of the test lines with the plasma membrane of thymocytes and Lt is the total length of test lines. The depth sharpness (Go) was determined from the equation: $Go=\lambda/(n+Na)^2$, λ being the wavelength of light, n is the coefficient of diffraction of the immersion oil and Na, the numerical aperture of the objective lens.

Statistical analysis

The results are expressed as means ± SEM. Group differences in the stereological parameters of the thymus as well as in the relative proportion of thymocyte subpopulations were analyzed by the nonparametric Wilcoxon test, using Statistical Package for Social Science (SPSS) Version 7.5. Significance was set at $p<0.05$.

Results

Immature rats

Thymus weight and total thymus cellularity

In rats treated with urapidil for 15 consecutive days, from 21st day of life onward, a significant ($p<0.05$) reduction in the body weight was observed (Table 1). This decrease was followed by a significant ($p<0.05$) and proportional decrease in the thymus weight, thus the relative thymus weight remained unchanged (Table 1).

Table 1. Absolute and relative weights of the non-processed thymuses and total number of thymocytes in sexually immature (36-day-old) and young adult (90-day-old) male rats treated with urapidil for 15 consecutive days.

	BODY WEIGHT (g)	THYMUS WEIGHT (mg)	RELATIVE THYMUS WEIGHT (mg/100 g BW)	TYMOCYTE (cells x10⁶)
Sexually immature rats				
Saline	168±8	665±33	397±18	1109±51
Urapidil	126±7*	526±41*	416±24	726±46**
Adult rats				
Saline	340±10	570±31	168±7	659±54
Urapidil	306±13	706±39*	232±13**	890±113

Values are means ± SEM for 5 animals in each group. * p<0.05 and **p<0.01 vs. saline-injected control of the same age.

The decrease in thymus size in urapidil-treated immature rats reflected a significant (p<0.01) reduction in total thymocyte number (Fig. 1A and Table 1).

Volumes of the thymus compartments, their cellularity and thymocyte size

In urapidil-treated rats the volume of the cortex (p<0.05) (Fig. 1B), as well as the total number (p<0.01) of cortical thymocytes (Fig. 2B) were significantly decreased, compared to corresponding values in age-matched saline-injected rats. The mean thymocyte size was changed only in the outer part of the cortex, thus the mean diameter of these cells was significantly (p<0.05)

smaller in rats subjected to chronic α_1-AR blockade than in appropriate controls (Fig. 2A).

Neither the size of medullary compartment nor its cellularity was affected in rats treated with urapidil (Figs. 1C, 2B). The mean thymocyte diameter also remained unaltered in these rats (Fig. 2A).

A cursory histological examination revealed slightly enlarged medullary areas relative to cortex and expanded interlobular septa in urapidil-treated rats (Fig. 3).
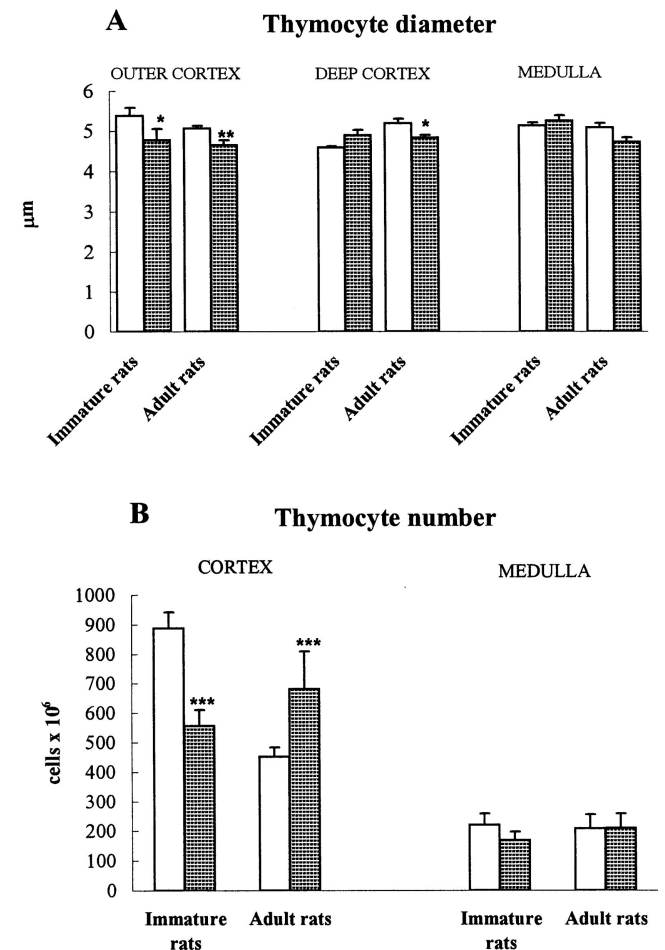
Expression of CD4/8 and TCRαß on thymocytes

The long lasting α_1-AR blockade in immature rats produced a significant (p<0.01) decrease in the frequency of CD4+8- SP cells followed by an increase (p<0.01) in that of CD4-8+ SP cells (Fig. 5, C and D). According to the expression of TCRαß molecules,
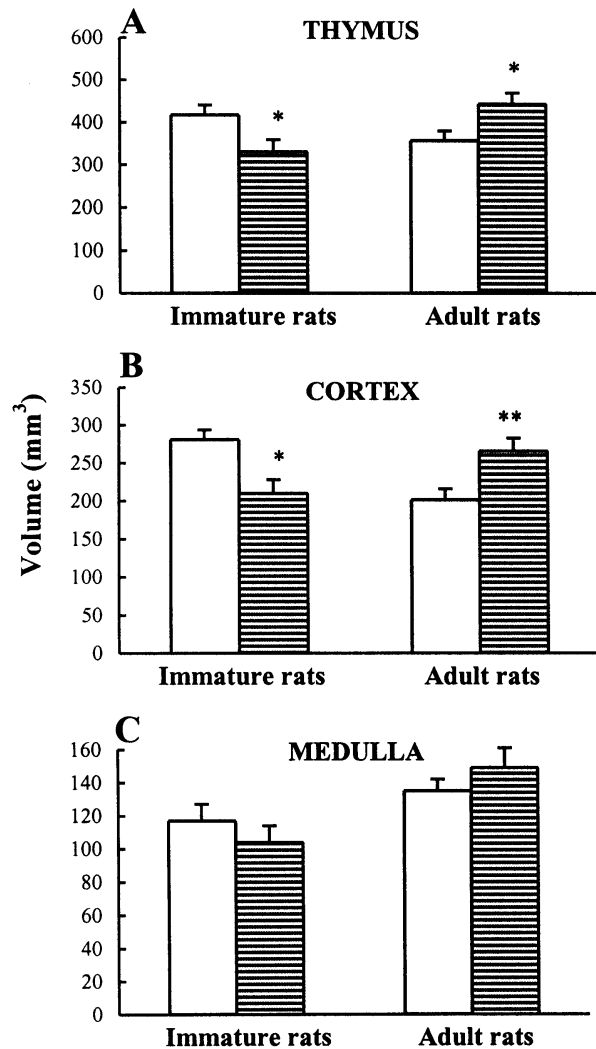
**Fig. 1.** Volumes of the thymus **(A)**, thymic cortex **(B)** and medulla **(C)** in sexually immature and adult rats injected with urapidil (stripped bars) or saline (open bars) over 15 consecutive days. Results are means ± SEM for 5 animals in each group. * p<0.05 and **p<0.01 vs. saline-injected controls of the same age.


Fig. 2. Mean diameter of thymocytes in the cortex (outer and deep) and medulla **(A)** and total number of thymocytes in the cortex and medulla **(B)** of thymuses from sexually immature and adult rats injected with urapidil (shaded bars) or saline (open bars) over 15 consecutive days. Results are means ± SEM for 5 animals in each group. * p<0.05, ** p<0.025 and *** p<0.01 vs. saline-injected controls of the same age.
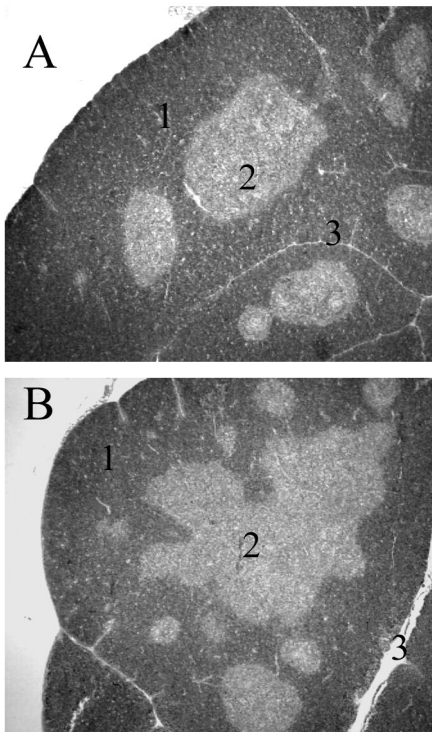
Fig. 3. Histological structure of the thymuses from sexually immature rats treated with urapidil over 15 days **(B)** and saline-injected age-matched control **(A)**. After urapidil-treatment medullary compartment seems slightly more pronounced and interlobular septum expanded. 1: cortex; 2: medulla; 3: interlobular septum. H-E staining. x 40
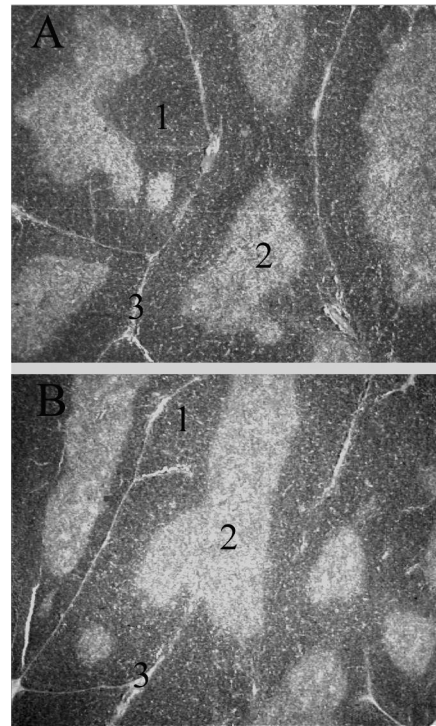
Fig. 4. Thymuses from adult rats: control **(A)** and treated with urapidil over 15 days **(B)**. No appreciable qualitative histological alterations are obvious in urapidil-treated rat compared with control animal. 1: cortex; 2: medulla; 3: interlobular septum. H-E staining. x 40
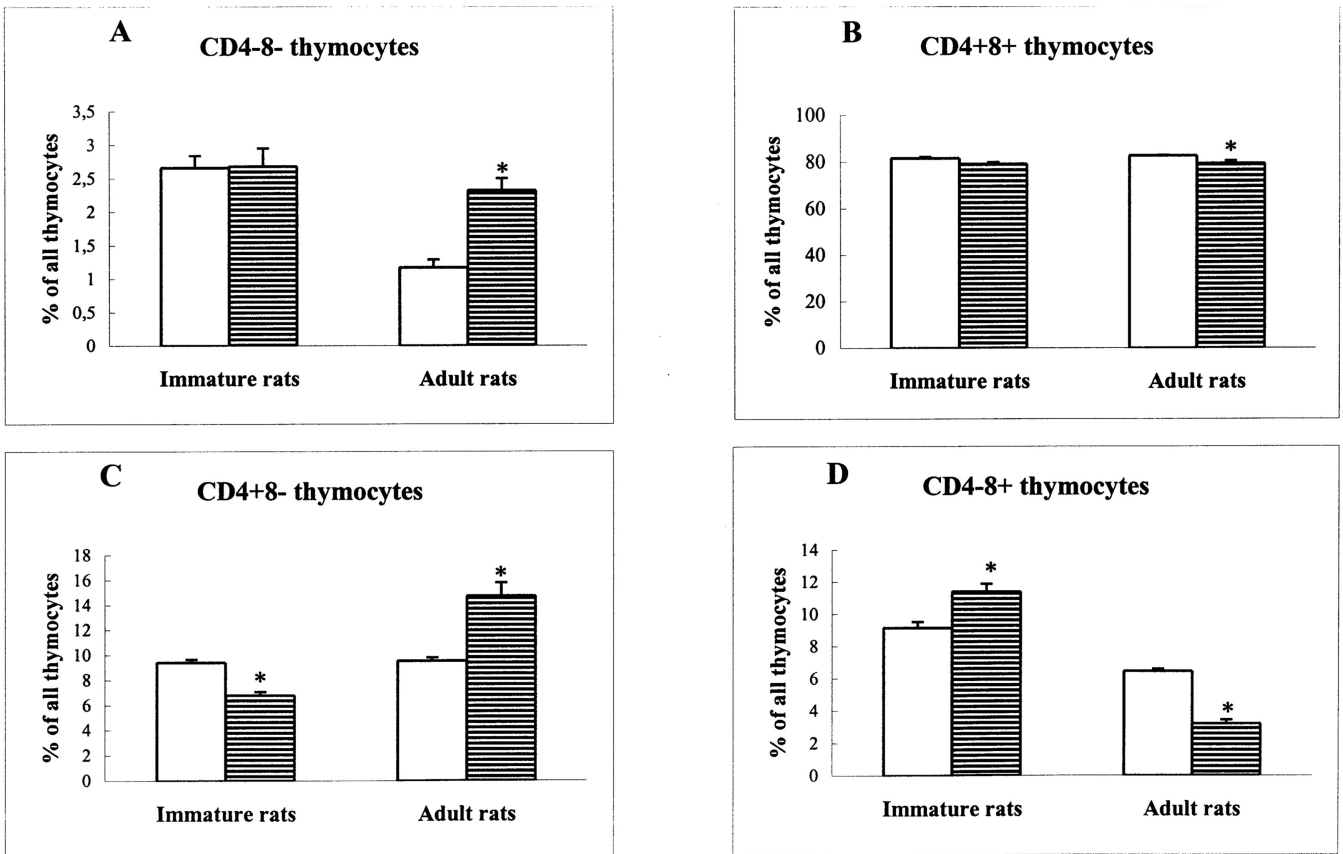
Fig. 5. Relative proportions of CD4-8- **(A)**, CD4+8+ **(B)**, CD4+8- **(C)** and CD4-8+ **(C)** thymocytes in sexually immature and adult rats injected with urapidil (striped bars) or saline (open bars) for 15 consecutive days. Results are means ± SEM for 5 animals in each group. Error bar less than 0.03% is omitted. * p<0.01 vs. saline-injected controls of the same age.

subsets of thymocytes were delineated as: 1) cells with low level of TCRαß expression (TCRαß^{low}), 2) cells with high level of TCRαß expression (TCRαß^{high}) and 3) cells expressing TCRαß at not detectable level (TCRαß$^-$) (Fig. 6). In immature rats urapidil significantly influenced neither the percentage of TCRαß$^-$ cells nor the relative proportions of the cells expressing detectable levels of TCRα (TCRαßl^{ow} and TCRαß^{high} cells) (Fig. 7).

Adult rats

Thymus weight and total thymus cellularity
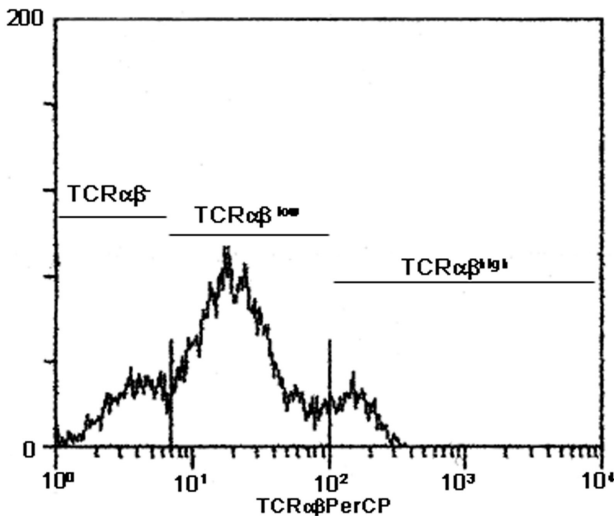
In contrast to immature rats, the 15-day-long

Fig. 6. Representative single parameter histogram of TCRαß expression in adult control rat.

treatment with urapidil in adult rats did not affect the body weight, but significantly ($p<0.05$) increased the thymus size, thus the relative organ weight was also significantly ($p<0.01$) increased compared with that in age-matched control rats (Table 1).

Although the total number of thymocytes was increased in urapidil-treated rats, this increase did not reach statistical significance (Table 1).

Volumes of the thymus compartments, their cellularity and thymocyte size

Fifteen-day-long urapidil treatment significantly increased ($p<0.01$) the volume of thymus cortex (Fig. 1B), as well as the total number of cortical thymocytes (Fig. 2B). In both outer and deep parts of their cortex the mean diameter of thymocytes was smaller ($p<0.01$ and $p<0.05$, respectively) relative to control values (Fig. 2A). Similarly to immature rats, urapidil affected neither the volume of the thymic medulla nor the size of its thymocyte population (Fig. 1C, 2B). The mean diameter of medullary thymocytes also remains unaltered (Fig. 2A).

Qualitative histology revealed no obvious alterations in the thymus of rats treated with urapidil relative to age-matched controls (Fig. 4).

Expression of CD4/8 and TCRαß on thymocytes

Analysis of thymocyte phenotypic profile revealed that urapidil in adult rats significantly influenced the relative proportions of all four major thymocyte subpopulations (Fig. 5). The frequencies of CD4-8- DN (Fig. 5A) and CD4+8- SP (Fig. 5C) thymocytes were increased ($p<0.01$), while those of CD4+8+ DP (Fig. 5B) and CD4-8+ SP (Fig. 5D) cells were significantly ($p<0.01$) diminished in these rats.

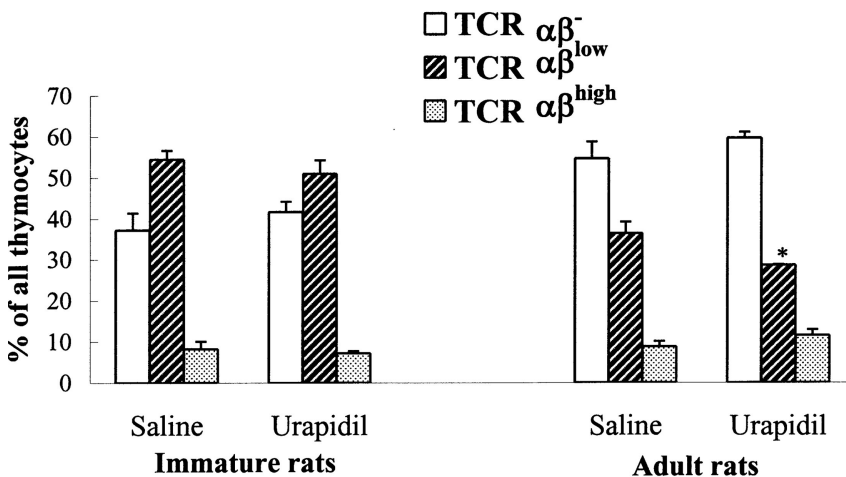Analysis of the TCRαß expression showed in adult rats treated with urapidil a slight, but significant

Fig. 7. Relative numbers of thymocytes expressing TCRαß at low (TCRαß^{low}) and high (TCRαß^{high}) density and those bearing no TCRαß molecules (TCRαß$^-$) in sexually immature and adult rats injected with urapidil or saline for 15 consecutive days. Results are means ± SEM for 5 animals in each group. Error bar less than 0.30% is omitted. * $p<0.01$ vs. saline-injected controls of the same age.

reduction (p<0.01) in the percentage of TCRαß^{low} cells, while the relative numbers of TCRαß^{high} and TCRαß$^-$ cells remained unaltered (Fig. 7).

Discussion

The results of this study show that chronic α_1-AR blockade in male rats affects the thymus structure and T-cell maturation in age-dependant manner.

The long-lasting blockade of α_1-AR signalling in sexually immature rats within the peripubertal period, when the thymic involution normally begins (Marchetti et al., 1990; Morale et al., 1991), evoked a significant reduction in the thymic weight reflecting the reduction in the size of its cortex as a result of the thymocyte depletion, suggesting an accelerated initial thymus involution rate. In the same line are the present data indicating the reduced size of thymocytes in the outer cortex where the thymocytes with pronounced blastogenic potential are expected to be located (Morale et al., 1991). This finding is consistent with the data showing that phenilephrine, an α_1-AR agonist, in fetal thymus explants stimulates thymocyte proliferation (Singh, 1979). To support previous hypothesis are findings that in mice at young age the earliest cellular aspect of thymic involution is associated with diminished subcapsular proliferation and a decrease in thymocyte size (Li et al., 2003). A markedly decreased body weight in urapidil-treated immature rats may suggest involvement of systemic mediators of growth and/or differentiation in the induction of urapidil-induced thymus involution, as well. Since α_1-AR stimulation inhibits growth hormone (GH) secretion in rat (Müller et al., 1999), it does not seem likely that the observed effect is mediated via changes in GH secretion. On the other hand, as it has been shown that chronic treatment with prazosin, a selective α_1-AR antagonist, reduces body weight and activate hypothalamo-pituitary-adrenal axis in rats (Stachowiak and Malendowicz, 1993), on one side, and as glucocorticoids are known to decrease the body weight and thymus size (Latta et al., 2002), on the other side, the reduction of the thymic weight and cellularity in urapidil-treated rats can be, at least partly, ascribed to an altered glucocorticoid secretion and to higher susceptibility of thymocytes to apoptosis. The urapidil treatment did not influence the relative proportions of thymocyte subsets expressing different levels of surface TCRαß. However, the thymocyte phenotypic profile defined by expression of CD4/CD8 coreceptor molecules was altered, thus the decrease in the frequency of CD4+8- SP cells was paralleled by an increase in that of CD4-8+ SP cells. Since SP thymocytes, except a few of them, belong to TCRαß mature subset (Tsuchida et al., 1994), it seems that absence of α_1AR signalling skews T-cell maturation toward CD4-8+ cell-line.

In contrast to immature rats, urapidil-treatment in adult rats produced the increase in thymus weight reflecting an enlargement in the volume of thymus cortex. The enlargement of cortical volume in these animals was due to increased cellularity of this compartment. The augmented cellularity of thymus cortex may be the result of 1) increased entrance of pro-thymocytes and/or thymocyte proliferation and 2) decreased apoptosis and/or decelerated cortical differentiation. The data indicating that α_1-ARs are involved in regulation of hematopoiesis (Maestroni and Conti, 1994) may suggest an altered T-cell precursor immigration into the thymus of animals subjected to α_1-AR blockade. The reduced size of the cortical thymocytes does not speak in favour of an increased thymocyte proliferation rate. As it has been described that chronic exposure of adult rats to norepinephrine enhanced thymocyte apoptosis via α-AR (Stevenson et al., 2001), it may be assumed that a reduced apoptosis is, at least partly, responsible for the increased thymus size and cellularity in adult urapidil-treated rats.

Furthermore, the treatment with α_1-AR blocker substantially affected the distribution of the main thymocyte subsets delineated by expression of CD4/8. The over-representation of the CD4-8- DN subpopulation and the under-representation of the CD4+8+ DP subpopulation suggest the occurrence of suppression in thymocyte transition from DN to DP stage of development. In accordance with this assumption is a reduction of the percentage of TCRαß^{low} thymocytes that are shown to be mainly CD4+8+ DP cells ready to enter into the positive selection process (Tscuchida et al., 1994). Additionally, it has been shown that the treatment, compared with immature rats, in adult rats evoked the opposite effect on the relative proportion of SP cells, most likely, favouring the maturation of CD4+8- SP cells over CD4-8+ thymocytes. This finding is consistent with the hypothesis that mature CD4+ cells, whether newly generated in the thymus or re-entrants from periphery, exert a negative feed-back on the CD4-8- DN to CD4+8+ DP thymocyte transition and positive feed-back on the maturation of DP cells toward CD4+8- SP cells (Mehr et al., 1997).

Differential effect of the treatment on the thymus structure and distribution of main thymocyte subsets in peripubertal and adult rats may be related to the substantial differences in the morphometric characteristics of thymuses and phenotypic profile of thymocytes observed in intact rats of these ages (Leposavić et al., 1996; Plećaš-Solarović et al., 2004).

Furthermore, as autoradiographic studies demonstrating that, not only density of ß-adrenoceptors, but also their distribution within the thymus, significantly changes with sexual maturation (Marchetti et al., 1990), it may be speculated that density and/or distribution of α_1-adrenoceptors are subjected to developmentally-induced changes which, in turn, may be responsible for differential effects of α_1-signaling in sexually immature and mature rats. Finally, the present findings are consistent with data showing: a) differential concentration of the hormones influencing thymic structure and function (i.e., gonadal steroids,

gonadotropins, GnRH) in peripubertal and adult rats and ii) differential effects of these hormones on the morphometric characteristics of the thymus and on the thymopoiesis in these two groups of rats (Karapetrović et al., 1995; Leposavić et al., 1996, 2005).

These are the first data to show that systemic α_1-AR blockade affects the structure of the rat thymus and thymocyte differentiation, the effects being age-dependent and, most likely, mediated through different mechanisms in immature and adult animals. A more detailed assessment of additional thymocyte differentiation markers in combination with functional markers of proliferation and apoptosis will help further characterisation of the α_1-AR blockade-induced age-dependent changes in the thymus. These data may be of clinical importance as there are circumstances under which increased naïve T-cell generation by thymus is necessary for successful recovery of the host, such as following iatrogenic and disease-induced T-cell depletion (McFarland et al., 2001).

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