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Polyamine Levels in Testes and Seminal Vesicles From Adult Golden Hamsters During Gonadal Regression-Recrudescence

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ABSTRACT: The exposure of golden hamsters to short days results in early regression of the reproductive organs and subsequent spontaneous recrudescence characterized by active cellular regeneration and differentiation. Thus, adult male hamsters were subjected to short photoperiod (SP, 6L:18D) for 9, 12, 14, 16, 18, and 22 weeks or maintained under long photoperiod (LP, 14L:10D) for 22 weeks, to assess photoperiodic-related changes in testicular and seminal vesicle (SV) levels of polyamines (PA) that are involved in cell growth and differentiation. During the regression phase, the weights of the organs and the circulating levels of luteinizing hormone (LH), follicle-stimulating hormone (FSH), prolactin, testosterone, dihydrotestosterone, and 5α-androstane-3α, 17β-diol were significantly diminished and, thereafter, during the recrudescence phase, they recovered total or partially their control values. In both tissues, the exposure to SP for 14–16 weeks resulted in an increase of PA concentrations, followed by a return to control levels in the recrudescence period. At the time of maximal tissue involution, the ornithine decarboxylase (ODC) activity (key regulatory enzyme of PA biosynthesis) showed a significant increase in testis, preceding the sharp peak of PA concentration. However, a marked decrease in ODC activity was detected in SV. The concentration of *N*-acetyl PA in SV showed an increment at 16 weeks of SP, while no modifications were detected in testicular concentration. When PA, *N*-acetyl PA, and ODC activity were expressed per testis and per SV, values fell significantly during the involution period, but in the recrudescence phase levels were recovered concomitantly with the restoration of the organ weight and function. In conclusion, the photoperiodic-related changes in PA and their *N*-acetyl derivatives might play a crucial role in regrowth and differentiation of the male sexual organs during the spontaneous recrudescence phase. Additionally, organ-specific regulation of the PA biosynthesis pathway could also take place.

Key words: Polyamines, ornithine decarboxylase, *N*-acetyl polyamines, androgens, testis, seminal vesicles, golden hamster.

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The golden (Syrian) hamster (*Mesocricetus auratus*) is a seasonal breeder under natural conditions and presents photoperiodic-related changes in its reproductive ability (for a review see Bartke, 1985). These cycles of gonadal activity may be reproduced under laboratory conditions by altering the daily photoperiod. Accordingly, the exposure of adult male golden hamsters to less than 12.5 hours of light per day causes a severe involution in the reproductive tract ("regression"), inhibition of spermatogenesis, a decline in serum luteinizing hormone (LH), follicle-stimulating hormone (FSH), and prolactin (PRL) levels (Berndtson and Desjardins, 1974), a decrease in total content of testicular receptors for LH, FSH, and PRL (Bex and Bartke, 1977; Bartke et al, 1980, 1982; Bartke, 1985) and a diminution in the reproductive functions as assessed by reduced blood and testicular concentrations of testosterone (T) (Desjardins et al, 1971; Bex et al, 1978). Atrophy of the reproductive tract involves not only a reduction in size but also profound morphological changes, i.e., seminiferous tubules are greatly reduced containing only Sertoli cells, spermatogonia, and a few primary spermatocytes (Sinha Hikim et al, 1988). The return to more than 12.5 hours of light per day reestablishes the natural gonadal activity ("induced recrudescence") (Berndtson and Desjardins, 1974). However, when adult male golden hamsters are maintained under short photoperiods (SP) for an extended period of time, reproductive function undergoes natural recovery ("spontaneous recrudescence") (Turek et al, 1975). During recrudescence, plasma levels of FSH, LH, and PRL and their testicular receptors are restored, gonadal activity recovered, and consequently, spermatogenesis, a process that involves germ cell proliferation and differentiation, is reestablished.

Polyamines (PA) are polycations ubiquitously present in both eukaryotic and prokaryotic cells. The main natural PA in mammals are putrescine (Pu), spermidine (Sd), and spermine (Sp). Although the physiological function of these amines is still not well understood at the molecular level, they are closely related to cell growth and differentiation (Scalabrini et al, 1991). Ornithine decarboxylase (ODC), the major limiting enzyme in this biosynthetic

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pathway, is the required step for *de novo* synthesis of Pu. This enzyme increases its activity in response to a variety of trophic stimuli such as hormones, drugs, environmental factors, tissue regeneration, and growth factors (Russell, 1981).

The intracellular PA levels are finely tuned by a great variety of regulatory mechanisms, i.e., transcriptional, posttranscriptional, translational, and posttranslational mechanisms that control PA biosynthetic enzymes (Heby et al, 1990). In addition, the PA levels are regulated by catabolic reactions (Pegg and McCann, 1982), interconversion to *N*-acetyl derivatives (Casero and Pegg, 1993), and also by transport into and out of the cells (Seiler and Dezeure, 1990; Kramer et al, 1993). A model of PA homeostasis has already been proposed that integrates these different systems (Shappell et al, 1993).

This study was undertaken to determine whether hamsters undergoing regression and spontaneous recrudescence present modifications of PA levels in the reproductive tract, particularly in testis and seminal vesicles (SV). This last tissue was selected among the accessory glands as SV contain moderate concentrations of PA, and, in contrast to the prostate, they are not engaged in the active secretion of these compounds (Piik et al, 1977). In addition, the levels of related *N*-acetyl derivatives and ODC-specific activity were also analyzed.

Materials and Methods

Animals and Treatment

Male golden hamsters were raised in our animal colony. The animals were maintained from birth in long photoperiod (LP; 14 hours light: 10 hours dark; lights on 7:00–21:00), at constant temperature (20–22°C). Pelleted food and tap water were provided ad libitum.

In the present study, groups of 10 adult hamsters were kept either under LP conditions for 22 weeks or exposed to SP (6 hours light: 18 hours dark; lights on 9:00–15:00) for 9, 12, 14, 16, 18, and 22 weeks. Hamsters were killed by decapitation. Trunk blood was collected and serum separated by centrifugation and stored at −20°C for horrhone determinations.

Testes and SV were removed, and after the SV fluid was gently expressed, both organs were weighed. The tissues were stored at −20°C and, within 3 months, assayed for ODC activity, PA, and *N*-acetyl polyamines (NAPA) levels.

Hormone Assays

Plasma levels of FSH and LH were measured with a validated heterologous radioimmunoassay (RIA) (Chandrashekar et al, 1987) using materials and protocols supplied by the NIDDK (Bethesda, Maryland) Rat Pituitary Hormone Distribution Program. Reference preparation rFSH-RP-2 and rFSH-S-11 antiserum for FSH RIA and, rLH-RP3 standard and rLH-S-10 antiserum for LH RIA were used. Plasma PRL levels were determined by homologous RIA (Soares et al, 1983). A reference preparation AFP-10302E and a specific antiserum, kindly provided by Dr. Parlow and Dr. Talamantes respectively, were used.

The sensitivities of these assays were 60, 10, and 30 pg/tube for FSH, LH, and PRL, respectively. All plasma samples were measured, in duplicate, in the same assay to avoid interassay variance. The intraassay coefficients of variation were less than 4.0% for FSH and LH and less than 6.0% for PRL.

Plasma testosterone (T), dihydrotestosterone (DHT), and 5α-androstane-3α, 17β diol (Diol) concentrations were also measured by RIA after extraction with diethylether, as previously described by Suescun et al (1985). Highly specific antibodies (DHT-1-CMO-BSA and Diol-15-CMO-BSA) were used. T was measured using an antibody (T-7α-butyrate-BSA) that has a 35% cross-reaction with DHT.

Preparation of Tissue for ODC Activity

The frozen tissues from animals exposed to LP or SP were thawed and homogenized individually in ice-cold 10 mmol/L Tris buffer, pH 7.4, containing 1.5 mmol/L ethylenediaminetetraacetic acid (EDTA), 0.5 mmol/L dithiothreitol (DTT), 0.25 mol/L sucrose, and 0.2% sodium azide (10 ml/g tissue) using an Ultraturrax homogenizer (Junk and Kunkel, IKA Werk, Staufer, Germany) at 0–4°C. From each homogenate, an aliquot was separated to determine DNA concentrations. The homogenates were centrifuged for 30 minutes at 20,000 × *g* and the supernatants used to determine, in duplicate, ODC activity and protein concentration.

ODC Assay

ODC activity was measured according to the original method (Pegg et al, 1970), slightly modified by Levy et al (1981) and previously validated in our laboratory for testis (de las Heras and Calandra, 1987) and SV (Gonzalez et al, 1994). Enzyme activity was expressed as pmol of $^{14}CO_2$ released/hour/mg of protein.

Preparation of Tissue Extracts for PA and NAPA Assays

The frozen tissues from animals exposed to LP or SP were thawed and homogenized in 0.2 mol/L perchloric acid. The homogenates were maintained for 2 hours at 4°C, and an aliquot was separated to determine protein and DNA concentrations. Thus, the homogenates were centrifuged for 30 minutes at 20,000 × *g*. Supernatants were used to determine, in duplicate, PA and NAPA tissues levels.

PA Assay

The PA content was assayed according to Seiler (1983), and as validated by Gonzalez (1993) and Gonzalez et al (1991) for testis and SV, respectively. The sensitivity of the method is 0.25 nmol PA in 500 µl. The total PA levels were calculated as the sum of Pu, Sd, and Sp levels.

NAPA Assay

Determination of acetylputrescine (APu), N^1-acetylspermidine (N^1ASd), N^8-acetylspermidine (N^8ASd), and N^1-acetylspermine (N^1ASp) was carried out by the method described by de las Heras et al (1992) and validated for testis and SV (Gonzalez, 1993). The sensitivity of the method is 0.25 nmol PA in 500 µl.

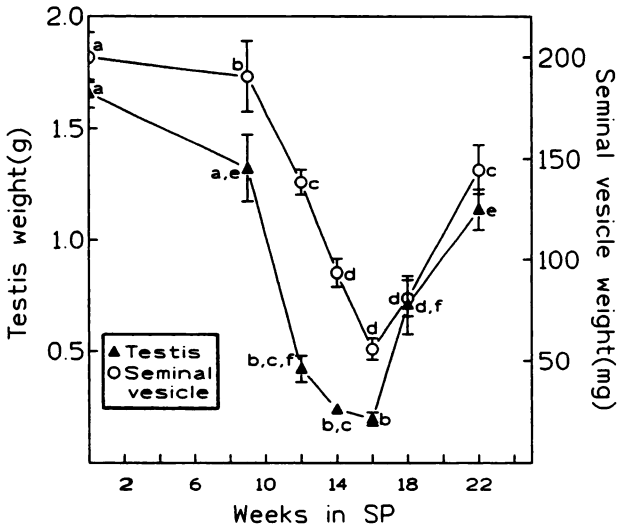

FIG. 1. Testicular weight (▲) and SV weight (○) in adult male golden hamsters exposed to LP or to SP during 9, 12, 14, 16, 18, or 22 weeks. The data represent the mean ± SEM (n = 8–10 animals per group). Where SEM lines are not present, the SEM was encompassed by the area of the symbol. Values without the same letter are significantly different at least at $P < 0.05$.

Other Methods

Protein concentration was measured by the method of Lowry et al (1951), using bovine serum albumin (BSA) as standard. DNA concentration was measured by the method of Labarca and Paigen (1980).

Statistical Analyses

Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Tukey–Kramer's test for multiple comparisons. Data are expressed as mean ± standard error of the mean (SEM). Parameters were correlated using linear regression. The correlation coefficient (r) was calculated and $P < 0.05$ was considered significant.

Results

Organ Weight

Exposure of adult male golden hamsters to SP resulted in the expected atrophy of the reproductive system. The weights of the testis and SV were maximally regressed between 14 and 16 weeks of SP (Fig. 1). Thereafter, organ weights gradually recovered. Body weight did not change during the experiment (data not shown).

Serum Hormone Levels

The patterns of serum FSH, LH, PRL, and androgen concentrations are shown in Table 1. The exposure to SP resulted in a decline in serum LH levels showing a nadir after 14 weeks ($P < 0.05$). LH levels of the animals under prolonged exposure to SP (22 weeks) spontaneously returned to near control values.

Serum levels of FSH were already lower in animals exposed during 9 weeks to SP compared to the control group, and the levels returned to normal values after 18 weeks of restrictive photoperiod.

Serum PRL levels were severely reduced by SP. The decrease in circulating PRL levels was larger at 16 weeks than at 9 weeks of exposure to SP. When animals were kept 22 weeks under SP, PRL levels started their recovery, though at the end of the experiment when compared to control animals, the levels of this hormone remained dramatically low.

The circulating T levels in hamsters exposed to SP for 9 weeks were significantly reduced when compared to the values in animals maintained under LP. The decrease was maximal at 14 weeks (14% of LP). However, as the restrictive conditions continued, a gradual return to the values detected in the control group was observed. At 22 weeks of SP, T was significantly restored but the levels still remained diminished compared to those of control animals (50% of LP). Serum DHT concentration showed a pattern similar to T levels. The minimal values were found at 14–16 weeks of SP. Hamsters exposed to SP during 16 weeks showed a significant decrease ($P < 0.05$) in circulating Diol levels. These hormone levels recovered when SP was maintained over a period of 18 weeks.

Effect of SP on PA and NAPA Testicular Levels

Testicular concentrations (nmol/g of tissue) of Pu, Sd, and Sp are shown in Figure 2 (left, panels a, b, c, respec-

Table 1. *Effect of long photoperiod (LP) and short photoperiod (SP) on FSH, LH, prolactin (PRL), testosterone (T), dihydrotestosterone (DHT), and 5α-androstane-3α, 17β diol (Diol) serum levels (ng/ml) in adult male golden hamsters*

	LH (ng/ml)	FSH (ng/ml)	PRL (ng/ml)	T (ng/ml)	DHT (ng/ml)	DIOL (ng/ml)
LP	3.69 ± 0.35^{a}	2.58 ± 0.10^{a}	17.21 ± 2.73^{a}	5.78 ± 0.26^{a}	1.50 ± 0.16^{a}	1.49 ± 0.10^{a}
9 weeks SP	2.60 ± 0.62^{a,c}	1.63 ± 0.14^{b}	4.63 ± 1.82^{b}	2.60 ± 0.18^{b,c}	—	1.10 ± 0.10^{a,b}
12 weeks SP	1.83 ± 0.29^{a,d}	1.66 ± 0.15^{b}	0.68 ± 0.13^{b}	1.18 ± 0.10^{b,c}	0.42 ± 0.05^{b}	1.02 ± 0.12^{a,b}
14 weeks SP	0.44 ± 0.05^{b,d}	1.64 ± 0.17^{b}	0.42 ± 0.05^{b}	0.81 ± 0.14^{b}	0.23 ± 0.05^{b}	0.86 ± 0.12^{a,b}
16 weeks SP	0.81 ± 0.34^{b,c,d}	—	0.35 ± 0.03^{b}	1.09 ± 0.23^{b,c}	0.25 ± 0.01^{b}	0.75 ± 0.13^{b}
18 weeks SP	0.76 ± 0.15^{b,c,d}	2.61 ± 0.13^{a}	0.45 ± 0.03^{b}	1.75 ± 0.20^{b,c}	0.49 ± 0.04^{b}	1.19 ± 0.27^{a,b}
22 weeks SP	2.32 ± 0.80^{a,d}	2.60 ± 0.15^{a}	1.41 ± 0.50^{b}	2.99 ± 0.62^{c}	0.66 ± 0.25^{b}	1.13 ± 0.10^{a,b}

The data represent the mean ± SEM (n = 8–10 animals per group). Values without the same letter are significantly different at least at $P < 0.05$.

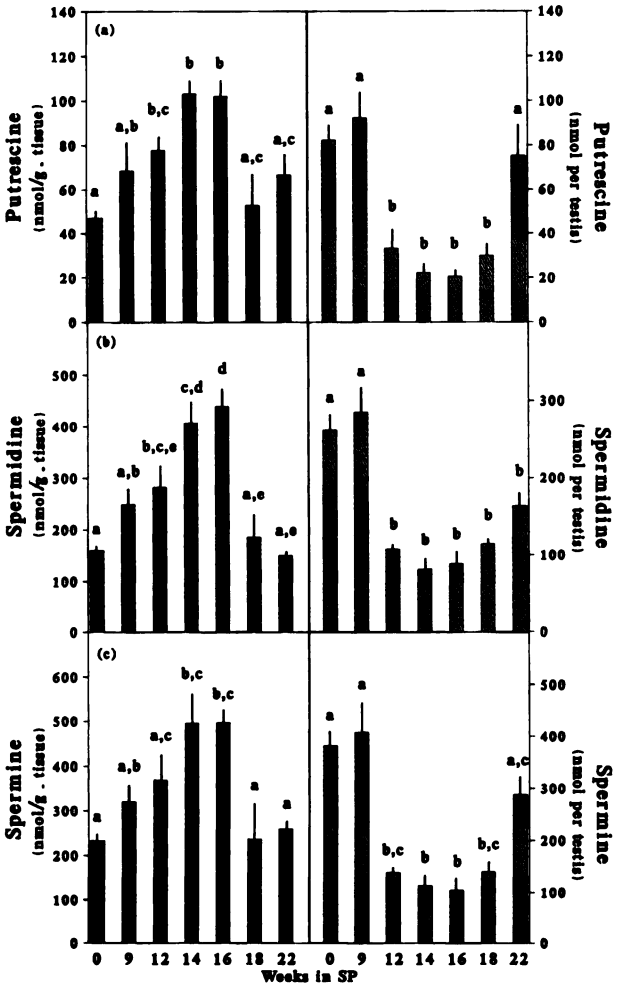

FIG. 2. Concentration (■) and content (■) of Pu (a), Sd (b), and Sp (c) in testis from adult male golden hamsters exposed to LP or to SP during 9, 12, 14, 16, 18, or 22 weeks. The data represent the mean ± SEM (n = 8–10 animals per group). Values without the same letter are significantly different at least at $P < 0.05$.

tively). After 14–16 weeks in SP, testes had a sharp increase in PA concentration. Additionally, if PA concentrations are expressed in terms of nmol/mg of protein, a significant increment during the regression period is also observed (data not shown).

However, when these results were expressed as nmol per testis (Fig. 2, right), Pu, Sd, and Sp (panels a, b, c, respectively) showed a maximum decrease toward 14–16 weeks of SP compared to control animals. In all groups studied the main PA was Sp.

When total testicular PA concentration was compared with gonadal weight, an inverse correlation could be detected between both parameters ($r = -0.81$; $P < 0.001$). PA concentration reached maximal values when testicular growth was nearly arrested. Nevertheless, total testicular PA content showed a direct correlation with gonadal weight ($r = 0.94$; $P < 0.001$).

The concentration of the acetyl derivatives of the PA (nmol/g of tissue) did not show significant modifications as a consequence of the exposure to SP (Table 2). Similarly, if NAPA concentrations are expressed in terms of nmol/mg of protein, values remained unchangeable during the exposure to short days (data not shown). However, at 14–16 weeks of SP a significant diminution in the content (expressed as nmol per testis) of these *N*-acetyl derivatives was observed and, after 18–22 weeks, a slow recovery of the values was detected (Table 2). The main NAPA assayed either in normal or regressed testis was APu.

ODC Activity in Testis

The pattern of ODC activity in testis is shown in Figure 3 (panel a). A sharp and significant increase ($P < 0.05$) in specific ODC activity (pmol/hour/mg of protein) was observed in animals exposed during 12 weeks of SP. At 22 weeks of SP, the levels fell near to control values. A similar rise during the involution stage is observed when specific ODC activity is expressed in terms of pmol/hour/g of tissue (data not shown). The increment in ODC preceded the rise in total PA concentration by 2–4 weeks.

However, if ODC values are expressed per testis, a marked decrease is detected at 14–16 weeks in SP followed by a recovery of the activity during the recrudescence period (data not shown).

Effect of SP on PA and NAPA in Seminal Vesicles

The concentration (nmol/g of tissue) of Pu, Sd, and Sp (Fig. 4, left, panels a, b, c, respectively) in SV increased by 16 weeks in SP. A similar increment in regressed SV was observed when Pu and Sp concentrations are expressed in terms of nmol/mg of protein or nmol/mg of DNA, while Sd showed no significant variations as a result of the exposure to SP (data not shown).

Notwithstanding, when these results were expressed as nmol per SV, a clear decrease was observed toward 14–16 weeks of SP ($P < 0.05$) (Fig. 4, right, panels a, b, c). An inverse correlation was observed between SV weight and total PA concentration ($r = -0.76$, $P < 0.001$), while total PA content and gland weight showed a direct correlation ($r = 0.94$, $P < 0.001$). The concentration (nmol/g of tissue) of APu, N^1ASd, N^8ASd, and N^1ASp in SV is shown in Figure 5 (panels a, b, c, d, respectively). NAPA concentration showed a sharp and significant increase at 16 weeks of SP. Additionally, if APu, N^1ASd, and N^1ASp concentrations are expressed in terms of nmol/mg of protein or nmol/mg of DNA, a marked increase is observed at 16–18 weeks in SP; while N^8ASd did not show significant variations as a result of exposure to SP (data not shown).

When NAPA content was assessed, a diminution was detected toward 16 weeks of SP, and, thereafter, these

Table 2. *Effect of long photoperiod (LP) and short photoperiod (SP) on content (nmol per testis) and concentration (nmol/g tissue) of acetylputrescine (APu), N¹-acetylspermidine (N¹-ASd), N⁸-acetylspermidine (N⁸-ASd), and N¹-acetylspermine (N¹-ASp) in testis from adult male golden hamsters*

	APu	N¹ASd	N⁸ASd	N¹ASp
LP				
nmol per testis	75.38 ± 8.15[a]	13.83 ± 1.29[a]	12.86 ± 1.33[a]	12.28 ± 1.57[a]
nmol/g tissue	43.81 ± 4.81[a]	8.02 ± 0.73[a]	7.43 ± 0.70[a]	7.11 ± 0.87[a]
9 weeks SP				
nmol per testis	58.24 ± 4.67[a,c]	10.38 ± 1.00[a]	10.68 ± 1.57[a,b]	10.94 ± 2.84[a,b]
nmol/g tissue	40.87 ± 2.44[a]	7.39 ± 0.94[a]	7.62 ± 1.29[a]	7.79 ± 2.07[a]
12 weeks SP				
nmol per testis	30.64 ± 9.30[b,d]	2.18 ± 0.32[b]	5.40 ± 2.16[b,c]	5.04 ± 2.12[b,c]
nmol/g tissue	52.16 ± 5.05[a]	10.74 ± 3.38[a]	8.53 ± 1.62[a]	9.11 ± 2.31[a]
14 weeks SP				
nmol per testis	11.98 ± 2.22[b]	2.18 ± 0.43[b]	2.45 ± 0.61[c]	2.05 ± 0.88[c]
nmol/g tissue	49.26 ± 9.21[a]	8.87 ± 1.56[a]	9.13 ± 2.06[a]	7.83 ± 2.66[a]
16 weeks SP				
nmol per testis	18.88 ± 4.23[b,d]	3.52 ± 0.55[b]	3.08 ± 0.52[c]	3.65 ± 1.08[b,c]
nmol/g tissue	45.69 ± 10.10[a]	8.31 ± 1.16[a]	7.16 ± 0.97[a]	9.07 ± 1.17[a]
18 weeks SP				
nmol per testis	23.96 ± 2.76[b,d]	3.52 ± 0.63[b]	3.50 ± 0.45[c]	2.96 ± 0.26[c]
nmol/g tissue	36.65 ± 5.37[a]	5.86 ± 1.68[a]	5.69 ± 1.35[a]	5.02 ± 0.70[a]
22 weeks SP				
nmol per testis	40.38 ± 1.85[c,d]	5.71 ± 0.91[b]	6.30 ± 0.85[b,c]	4.99 ± 0.68[b,c]
nmol/g tissue	40.63 ± 2.98[a]	5.55 ± 0.60[a]	6.39 ± 1.16[a]	5.02 ± 0.78[a]

The data represent the mean ± SEM (n = 8–10 animals per group). Values without the same letter are significantly different at least at $P < 0.05$.

values slowly began to recover (Table 3). The main *N*-acetyl compound detected in all groups studied was APu.

ODC Activity in Seminal Vesicles

The ODC activity (pmol/hour/mg of protein) in SV is shown in Figure 3 (panel b). A marked decrease of this parameter was observed at 14–16 weeks in SP when the total PA concentration reached maximal values. Thereinafter, the specific enzyme activity returned to values similar to those found in control animals.

Similarly, a significant fall in the ODC activity during the regression period is detected if the values are expressed in terms of pmol/hour/g of tissue or pmol/hour/mg of DNA, as well as if ODC activity is expressed per SV (data not shown).

Discussion

In the present study, when adult golden hamsters were continuously exposed to SP, the testis and accessory sexual glands remained regressed for 14–16 weeks and thereinafter underwent a process of recrudescence. These observations are in accordance with previous reports (Gaston and Menaker, 1967; Turek et al, 1975). The results observed for plasma LH, FSH, PRL, and T levels are in good agreement with previously published data (Berndtson and Desjardins, 1974; Turek et al, 1975; Tamarkin et al, 1976; Bex et al, 1978). It is now accepted that there is a degree of similarity, in the serum hormonal milieu and the testicular production of T, between hamsters undergoing gonadal regression and immature animals (Berkowitz and Heindel, 1984). The present work shows that, when the regression was maximal, serum Diol and DHT levels remained low. Nevertheless, metabolic studies performed in immature hamsters have shown a significant increase of 5α-reduced products (Tsuji et al, 1984; Yabumoto et al, 1985). This novel and marked diminution in DHT and Diol serum levels between 14 and 16 weeks is probably due to the reduction in the precursor concentration or to an impairment in the steroidogenic process. The latter could be a consequence of the reduction in gonadotropin secretion by the pituitary gland, as proposed by Chandrashekar and Bartke (1989). However, a modification in the specific activity of the corresponding T metabolizing enzymes could not be excluded because determination of these activities was not performed.

To our knowledge, this is the first report of PA presence in testis and SV of male hamsters under LP and SP conditions. In adult animals, the testicular content of Pu, Sd, and Sp, as well as ODC activity expressed per testis, decreased after 14–16 weeks in SP and thereafter were

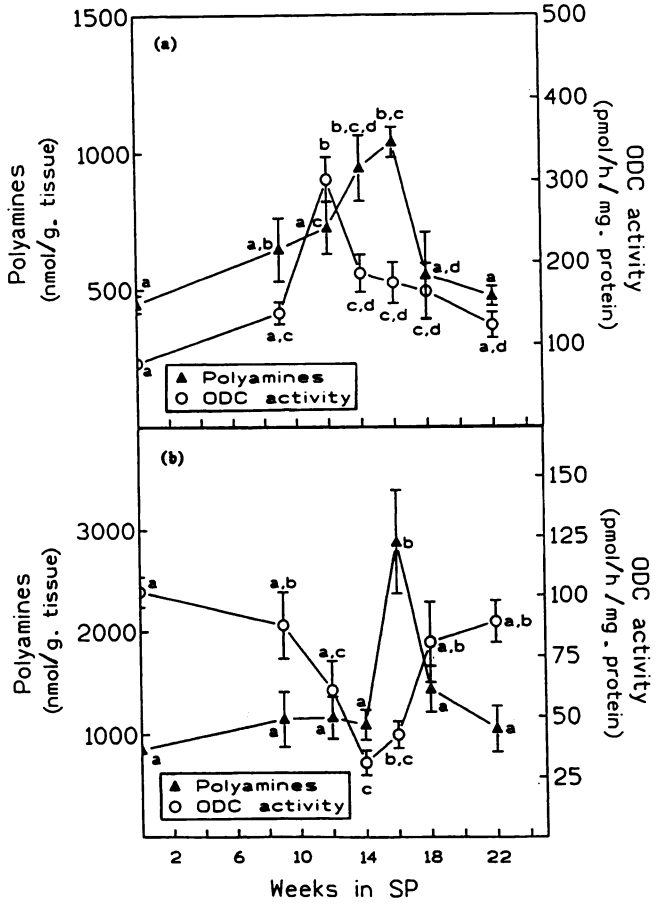

FIG. 3. Total concentration of PA (▲) and ODC activity (○) in testis (a) and SV (b) from adult male golden hamsters exposed to LP or to SP during 9, 12, 14, 16, 18, or 22 weeks. The data represent the mean ± SEM (n = 8–10 animals per group). Where SEM lines are not present, the SEM was encompassed by the area of the symbol. Values without the same letter are significantly different at least at $P < 0.05$.

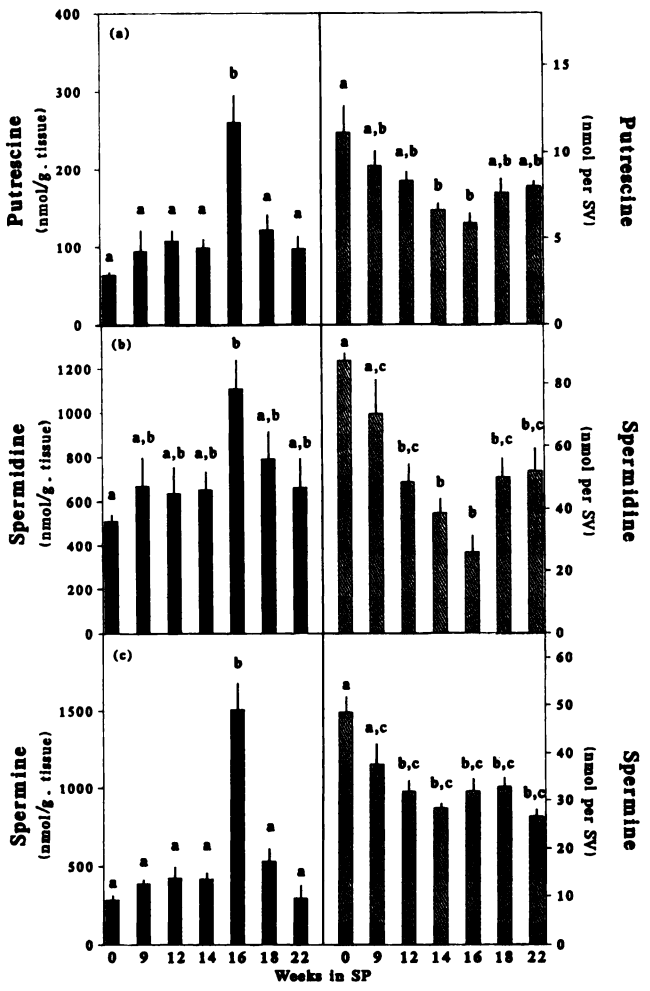

FIG. 4. Concentration (■) and content (□) of Pu (a), Sd (b), and Sp (c) in SV from adult male golden hamsters exposed to LP or to SP during 9, 12, 14, 16, 18, or 22 weeks. The data represent the mean ± SEM (n = 8–10 animals per group). Values without the same letter are significantly different at least at $P < 0.05$.

slowly restored. These modifications of total PA content and ODC activity accompany the variations in testicular weight. In contrast, when testicular PA concentration and ODC activity (expressed as g of tissue or mg of protein) were analyzed, a marked increase was observed in regressed testes. Under LP conditions, testicular cell populations are a mixture between diploid and haploid cells, whereas, in SP condition, only diploid cells remain in the gonad. Therefore, expression of PA concentration and ODC activity in terms of mg of DNA fail to reflect the levels of both parameters present per cell in the control group and, then, does not permit the comparison of the data between LP and SP groups.

There are two possible explanations for the increased PA concentration detected in regressed hamster testes. First, a genuine rise of PA levels due to an increment in the ODC activity, or an intake from systemic circulation, or through PA interconversion via acetyl derivatives. The increase in ODC specific activity detected at 12 weeks of exposure to SP, which preceded by 2–4 weeks the maximal increase of PA concentration, could indicate that these polycations are synthesized *de novo* in testicular tissue. PA have been implicated in cellular proliferation and differentiation as well as in the biosynthesis of protein and DNA. Thus, due to the increment detected in the concentration of these polycations at the time when testicular atrophy was maximal and the circulating levels of LH and FSH (two known triggers of steroidogenesis and spermatogenesis) were still low, we could speculate that PA might act as trophic factors inducing the subsequent regrowth of testicular tissue during the recrudescence period. In this context, Shubhada et al (1989) described ODC activity in different testicular cell types of rat giving clear evidence that PA may play a role in mediating the effects of hormones and growth factors. A highly selective mode of action of PA, during mitotic and meiotic cell

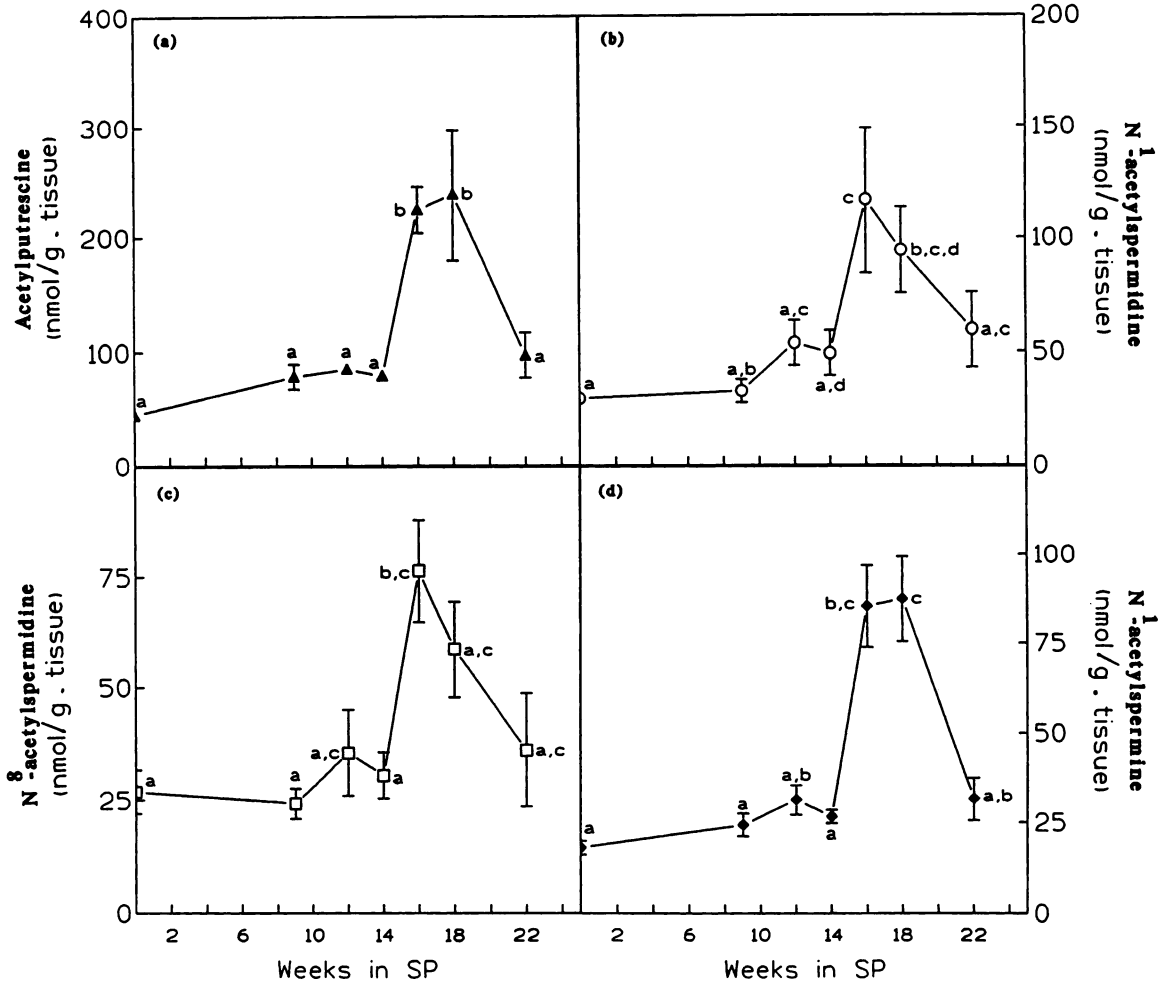

FIG. 5. Concentration of APu **(a)**, N¹ASd **(b)**, N⁸ASd **(c)**, and N¹ASp **(d)** in SV from adult male golden hamsters exposed to LP or to SP during 9, 12, 14, 16, 18, or 22 weeks. The data represent the mean ± SEM (n = 8–10 animals per group). Where SEM lines are not present, the SEM was encompassed by the area of the symbol. Values without the same letter are significantly different at least at $P < 0.05$.

cycles in spermatogenesis, has been described in mice (Hakovirta et al, 1993). Nevertheless, we cannot exclude a second explanation for the increase seen in PA concentrations in the regression phase. The latter might be a consequence of a differential PA production by different testicular cell types and of the morphological changes occurring in testis during this period. If gonadal regression involved a selective loss of those cells that produce low levels of PA and, in hence, an enrichment in the testicular cells containing high levels of these polycations, these

Table 3. *Effect of long photoperiod (LP) and short photoperiod (SP) on content (nmol per SV) of acetylputrescine (APu), N¹-acetylspermidine (N¹-ASd), N⁸-acetylspermidine (N⁸-ASd), and N¹-acetylspermine (N¹-ASp) in seminal vesicle (SV) from adult male golden hamsters*

	APu (nmol per SV)	N¹ASd (nmol per SV)	N⁸ASd (nmol per SV)	N¹ASp (nmol per SV)
LP	9.23 ± 0.60[a]	5.88 ± 0.51[a]	5.47 ± 0.93[a]	3.79 ± 0.37[a]
9 weeks SP	6.32 ± 0.28[b]	2.60 ± 0.12[b]	2.00 ± 0.23[b]	1.95 ± 0.05[b]
12 weeks SP	5.37 ± 0.31[b]	2.88 ± 0.47[b]	2.24 ± 0.60[b]	1.97 ± 0.27[b]
14 weeks SP	6.13 ± 0.19[b]	3.34 ± 0.49[b]	2.38 ± 0.48[b]	2.10 ± 0.20[b]
16 weeks SP	4.55 ± 0.08[b]	2.83 ± 0.25[b]	1.67 ± 0.26[b]	1.66 ± 0.16[b]
18 weeks SP	4.63 ± 0.12[b]	1.73 ± 0.15[b]	1.12 ± 0.11[b]	1.83 ± 0.38[b]
22 weeks SP	5.80 ± 0.39[b]	3.45 ± 0.46[b]	2.01 ± 0.43[b]	1.91 ± 0.22[b]

The data represent the mean ± SEM (n = 8–10 animals per group). Values without the same letter are significantly different at least at $P < 0.05$.

changes in the cellular composition could result in an apparent increase of the PA concentration in the regressed organ. In order to clarify this controversial point, experiments that measure PA levels in isolated Sertoli cells, Leydig cells, and germ cells from hamsters exposed to LP and SP should be designed.

In SV, the pattern of PA was similar to the testis. The PA content and ODC activity (expressed per gland) changed concomitantly with SV weight. Nevertheless, Pu, Sd, and Sp concentration (as nmol/g of tissue) presented a sharp increase by 16 weeks in SP. If PA concentrations are expressed per mg of protein or per mg of DNA, Pu and Sp also increase during the involution period, while Sd remains unchanged (data not shown). Sd is a regulatory key of the interconversion pathway of the PA acting as substrate of Sp synthase and/or as precursor during putrescine formation via acetyltransferases and oxidases (Bolkenius and Seiler, 1987).

ODC activity (in terms of mg of protein, g of tissue, or mg of DNA) exhibited a profound inhibition in the regressed SV. Thus, the increase observed in PA concentration during the involution period is not due to *de novo* synthesis of these polycations. In previous reports, data obtained in our laboratory showed that, in rat and mouse SV, ODC activity is regulated mainly by PRL (Gonzalez et al, 1991, 1994). From the present results, which show that ODC activity and circulating PRL levels are decreased during regression, it seems likely, that in hamsters, PRL would also regulate ODC activity in SV. Intracellular PA concentration is modulated through a balance between uptake and biosynthesis on the one hand and retroconversion and efflux on the other (Shappell et al, 1993). When biosynthesis has been inhibited or inactivated, growth can be sustained by exogenous PA (Kramer et al, 1989; Pilz et al, 1990; Nicoll et al, 1994). Therefore, transport of PA in and out of the cells is critical for maintaining the intracellular PA pool (Seiler and Dezeure, 1990). We could speculate that, regardless of the *de novo* synthesis, other sources are mobilized to reestablish the PA levels, and consequently, an active ODC enzyme may not be a prerequisite for the restitution of SV integrity. One plausible explanation is that the increased PA concentration in SV is likely due to their intake from systemic circulation. These PA might be of testicular origin.

In spite of this, an apparent increment of the PA concentration in regressed SV resulting from changes in the cellular composition of the gland during the involution phase and a preferential loss of those cell types that contain low PA levels cannot be excluded. The relative contribution of these factors to an accurate explanation of increased PA concentration in regressed hamster SV must await additional investigation.

The PA acetylation is important in the maintenance of normal PA levels and function (Casero and Pegg, 1993). Moreover, there is clear evidence that certain effects of PA are expressed through their derivatives (Canellakis et al, 1989). In the present work, the total content of *N*-acetyl derivatives in testis and SV changed with organ weight. However, no variations were detected in the testicular NAPA concentration. On the other hand, in SV, the increment of PA concentration during the regression period was accompanied by a sharp elevation of APu, N^1ASd, and N^1ASp concentrations. These and other previously reported observations (Seiler and Dezeure, 1990; Casero and Pegg, 1993; Shappell et al, 1993) suggest that these compounds are probably acting as homeostatic regulators of the intracellular PA pool in order to adapt to physiological needs.

Although the mechanism through which PA and their *N*-acetyl derivatives affect cell function has not yet been established, it is accepted that, unless PA are present, cells do not grow or survive (Kramer et al, 1989).

In conclusion, the present study demonstrates the existence of PA and their *N*-acetyl derivatives in the testis and SV of adult golden hamsters maintained in LP and SP. Furthermore, the photoperiodic related changes detected in PA levels might act as a putative trigger for the regrowth and regeneration of the reproductive organs during the reactivation phase or as a secondary signal mediating the action of trophic factors that cause spontaneous recrudescence.

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