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The Effects of Short Photoperiod, Pinealectomy, and Melatonin Treatment on Oxytocin Synthesis and Release in the Male Syrian Hamster

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The pineal gland has been shown to affect plasma oxytocin (OT) levels, but the mechanism of this action is not apparent. In the present study, the ability of the photoperiod to affect plasma OT levels, neurointermediate lobe (NIL) OT content, and hypothalamic OT mRNA levels was studied in male Syrian hamsters. In addition, the ability of pinealectomy to prevent and melatonin (MEL) to mimic the short photoperiod-induced changes were also determined. Exposure to short days (SD) led to the expected decrease in testes weight and plasma PRL levels, but plasma OT levels were unchanged. However, NIL OT content was increased in the SD-exposed animals. Hypothalamic OT mRNA levels were not significantly altered by SD exposure. Pinealectomy blocked the effects of SD on testes weight, whereas afternoon MEL injections mimicked the effects of SD. In long day (LD)-exposed hamsters, pinealectomy induced a decrease in NIL OT content without altering hypothalamic OT mRNA levels. In SD-exposed animals, NIL OT content was not affected by pinealectomy. Melatonin injections had no significant effect on NIL OT content or hypothalamic OT mRNA levels. The data from the present study suggest that exposure of male Syrian hamsters to short photoperiods influences some aspects of OT synthesis and/or transport to produce its increased accumulation in the NIL, but does not affect OT release. These changes are apparently not the result of SD-induced changes in MEL secretion, but conceivably could be related to the previously documented effects of SD on hypothalamic catecholamine turnover.

Key Words: Short photoperiod; melatonin; pinealectomy; oxytocin.

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Introduction

Release of neurohypophyseal hormones exhibits diurnal changes, which are presumably related to the effects of environmental light. Diurnal rhythmic variations of oxytocin (OT) levels in the cerebrospinal fluid (CSF) were found in primates with peak and nadir levels during light and dark hours, respectively (Reppert et al., 1984). Diurnal variations in the concentration of OT in the hypothalamus, neurohypophysis, and plasma were also described in the rat (Windle et al., 1992) and were found to be dependent on the stage of the estrous cycle (Windle and Forsling, 1993). The effects of light–dark cycles on neurohypophyseal function may be mediated in part by the pineal gland. In the rat, pinealectomy decreases OT content in the hypothalamus and neurohypophysis (Juszczak and Guzek, 1983, 1988), and increases plasma levels of OT (Demaine et al., 1990). The pineal hormone melatonin (MEL) can modify OT release from the rat hypothalamo-neurohypophyseal system in vivo (Juszczak et al., 1986; Juszczak and Guzek, 1988) and in vitro (Juszczak et al., 1992; Yasin et al., 1993).

The present study was undertaken to determine whether long-term exposure to altered photoperiod will affect OT gene expression, and OT levels in the neurohypophysis and in the peripheral plasma. We were also interested in determining whether effects of chronic changes in photoperiod on OT levels are mediated by the pineal and MEL. For these studies, we selected adult male Syrian hamsters. Syrian hamsters are seasonal breeders with a reproductive system that is very sensitive to changes in the length of the daily exposure to light. Under conditions of short photoperiod (SD; <12.5 h light/d), male and female reproductive organs undergo atrophy. These effects of photoperiod are pineal-dependent, i.e., can be prevented by pinealectomy (Px), and are mediated by shifts in the diurnal pattern of MEL release (Reiter, 1985). In the male hamster, SD-induced testicular atrophy is accompanied by reduction in peripheral levels of luteinizing hormone (LH), follicle-stimulating hormone (FSH), prolactin (PRL), and testosterone (Bartke, 1985 and

Table 1
Effects of Exposure to LD or a SD for 1, 4, or 10 wk (SD1, SD4, SD10) on Body, Testes, and Pituitary Weights and Plasma PRL Levels in Male Syrian Hamsters (mean ± SEM)

Photoperiod	Body wt, g	Testes wt, g	Anterior pituitary, mg	Plasma PRL, ng/mL
LD	144 ± 1.5^{a}	3.63 ± 0.056^{a}	2.90 ± 0.121^{a}	185 ± 69.4^{a}
SD 1	143 ± 2.5^{a}	3.73 ± 0.075^{a}	2.67 ± 0.085^{a}	66 ± 11.5^{ab}
SD 4	147 ± 2.1^{a}	3.74 ± 0.081^{a}	2.37 ± 0.070^{b}	25 ± 4.4^{b}
SD 10	163 ± 3.2^{b}	2.44 ± 0.256^{b}	2.42 ± 0.064^{b}	16 ± 2.8^{c}

$^{a, b, c}$ Within each column, the values without the same letter in the superscript are significantly different ($P < 0.05$).

references therein). Suppression of gonadal activity by SD is accompanied by alterations in dopamine (DA) and norepinephrine (NE) metabolism in the hypothalamus (Steger and Bartke, 1991) and the neurointermediate lobe of the pituitary (Steger et al., 1995). Moreover, MEL can reduce DA content in the neurointermediate lobe of the pituitary (NIL) in the male Syrian hamster (Alexiuk and Vriend, 1993). These findings suggest potential mechanisms for photoperiod-related changes in OT release, because in other species, OT release is known to be controlled by noradrenergic (Randle et al., 1986; Parker and Crowley, 1993) and dopaminergic (Bridges et al., 1976; Crowley et al., 1991; Crowley and Armstrong, 1992) mechanisms. Therefore, it was of interest to determine whether photoperiod, Px, and MEL influence the acute effect of pharmacological blockade of catecholamine synthesis on OT release.

Findings reported in this article indicate that exposure of male hamsters to SD increases OT content in NIL without altering hypothalamic OT mRNA or plasma OT levels. We believe that this effect may be related, directly or indirectly, to the previously described SD-induced alterations in DA transmission.

Results

Experiment 1

Body weight was slightly, but significantly elevated after 10 wk of SD conditions. Exposure of animals to SD was followed by the expected significant decrease of testis weight. Anterior pituitary weight was reduced after 4 and after 10 wk in SD (Table 1).

In saline-injected hamsters, OT content in NIL was significantly increased after 10 wk of SD exposure (Fig. 1A). The levels of OT mRNA in the hypothalamus were not significantly altered at this time or after shorter periods of exposure to SD conditions (Fig. 1B). A single dose of alpha methyl paratyrosine (αMPT) administered 1 h before sacrificing the animals had no significant effect on NIL OT content in any of the groups.

There were no differences in plasma OT levels among all groups of saline-injected animals (Fig. 2), whereas plasma PRL levels were diminished significantly after 4 and 10 wk of SD-exposure (Table 1). Injection of αMPT resulted in a marked increase in plasma OT levels in all groups examined (Fig. 2). Plasma OT levels measured after injection of aMPT, were lower in animals exposed to SD for 1 wk than in the remaining groups (Fig. 2; $P<0.05$).

Experiment 2

Body weight was not altered by Px or MEL injections, as compared to the corresponding control animals (Table 2). However, in long photoperiod (LD)-exposed animals, body weight in pinealectomized hamsters was significantly lower than in those injected with MEL. Anterior pituitary weight was diminished in SD-exposed as well as in MEL-treated animals when compared to vehicle-treated controls. Px reversed the effect of SD on anterior pituitary weight (Table 2). Both exposure to SD and MEL treatment reduced testis and seminal vesicle weights when compared with LD-exposed vehicle-treated hamsters. Px prevented the decrease of testis and seminal vesicle weights in response to SD (Table 2).

In LD-exposed animals, pinealectomy significantly reduced OT content in NIL, whereas MEL injections had no effect (Fig. 3). In LD-exposed animals that were given a single dose of αMPT before autopsy, the apparent suppressive effect of Px on NIL OT content was numerically smaller and not statistically significant. In SD-exposed hamsters, Px failed to alter OT levels in NIL (Fig. 3). There were no significant differences between saline and α-MPT-injected animals within any of the groups.

The levels of OT mRNA did not differ between any of the groups. The apparent reduction of OT mRNA levels in animals injected with MEL as compared to vehicle-treated controls was not statistically significant (Fig. 4).

Plasma OT levels in animals not receiving αMPT were not affected by either Px or MEL (Fig. 5). However, plasma PRL levels in LD-exposed hamsters were significantly reduced by MEL.

Plasma levels of both OT and PRL (Fig. 5) were greatly elevated after administration of αMPT in all groups examined. In LD-exposed hamsters, the levels of plasma PRL measured following αMPT were significantly lower in MEL-treated or pinealectomized animals than those in ethanol vehicle-injected controls. In contrast, in SD-exposed

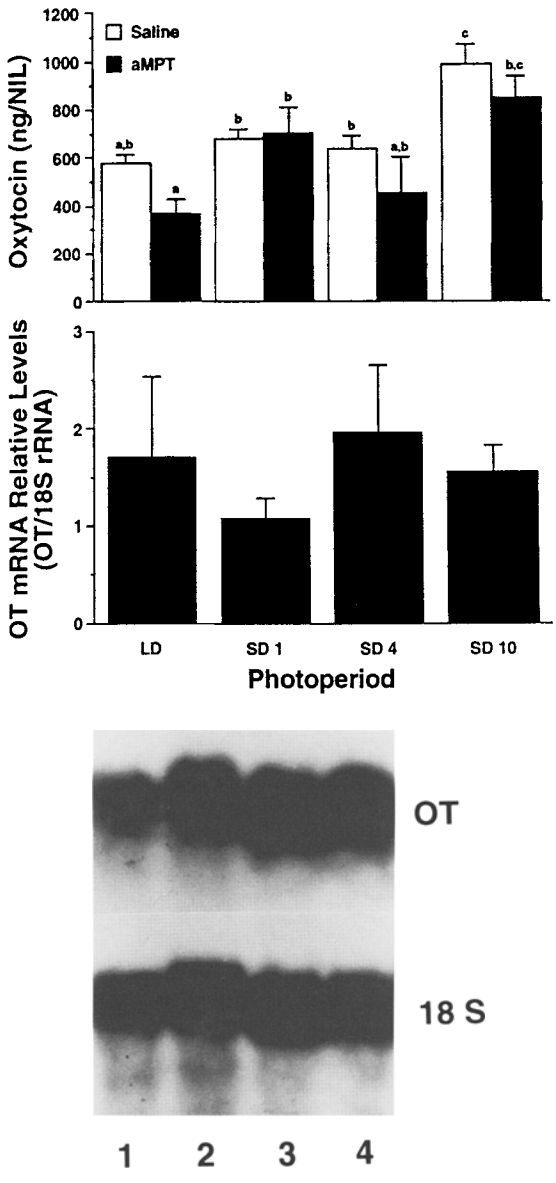

Fig. 1. Effects of exposure to SD for 1, 4, or 10 wk (SD1, SD4, SD10) and acute treatment with tyrosine hydroxylase inhibitor, αMPT, on the content of OT in the NIL (top) and OT mRNA content in the hypothalamus in relative densitometer units (middle). Bottom: Results of Northern blot analysis of OT gene expression in a representative experiment; lane 1—LD, lane 2—SD4, lane 3—SD10, lane 4—SD1. After hybridization, the blots were normalized against the levels of 18 S rRNA to correct for loading accuracy. Relative levels (OT/18 S) of expression of OT mRNA are shown in the middle panel. Values expressed as mean ± SEM (OT, n = 8–12 hamsters/group, OT mRNA; n = 4/group). Different letters denote statistical significance ($P < 0.05$).

hamsters, plasma OT and plasma PRL responses to αMPT were significantly increased by Px (Fig. 5).

Testosterone levels in the testis changed in parallel with the changes in testis weight, i.e., were lower in SD-exposed than in LD-exposed animals, significantly reduced following treatment of LD animals with MEL, and significantly higher in pinealectomized SD hamsters than in the corresponding controls (Fig. 6).

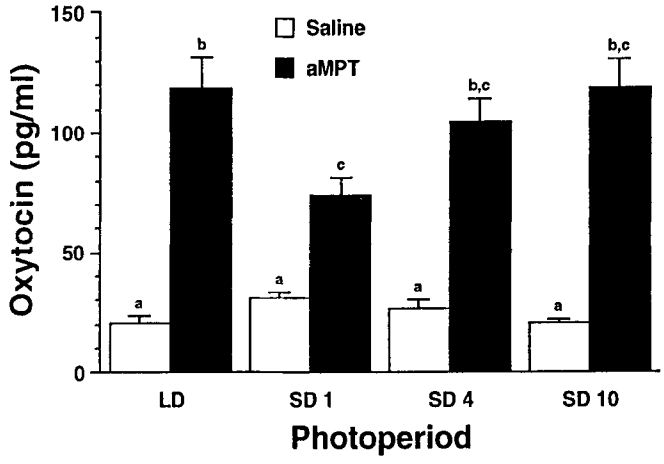

Fig. 2. Effects of SD exposure for 1, 4, or 10 wk and acute treatment with tyrosine hydroxylase inhibitor, αMPT, on plasma OT. Values expressed as mean ± SEM (n = 8–12 hamsters/group). Different letters denote statistical significance ($P < 0.05$).

Discussion

The Effect of SD on OT Synthesis and Release

Oxytocin content in the hypothalamo-neurohypophyseal system represents a net result of several processes, i.e., OT synthesis by magnocellular neurons situated in the supraoptic (SON) and paraventricular (PVN) nuclei of the hypothalamus, infundibular transport of the hormone toward the neural lobe of the pituitary, and finally the secretion of OT into the blood. The present results show, for the first time, that exposure to SD can increase OT content in NIL in the Syrian hamster. This effect was evident after 10 wk of SD exposure, but not after 1 or 4 wk in SD. However, there was no corresponding significant change in hypothalamic OT mRNA levels. Considerable variation between the results of individual experiments (please *see* SEM values in Fig. 1) may have precluded detection of photoperiod-induced changes in OT mRNA levels. Plasma OT levels did not reflect the changes in OT content in NIL, and were not altered after 1, 4, or 10 wk of SD exposure. Several studies demonstrated that stimuli that are known to increase OT release from the neurohypophysis are also capable of inducing a rapid elevation of OT mRNA level in the hypothalamus (Arnauld et al., 1993; Crowley et al., 1993; Crowley and Amico, 1993). The present results indicate that exposure of male hamsters to SD may alter some aspects of OT synthesis in the hypothalamus and/or transport toward the neurohypophysis without significantly increasing OT release into the blood or altering steady-state levels of OT mRNA in the hypothalamus.

We suspect that the previously documented effects of SD on hypothalamic dopaminergic and noradrenergic transmission in this species (Steger et al. 1983, 1984, 1995; Benson, 1987) could mediate the SD-induced changes in OT content. The PVN and, to a lesser extent, the SON as well as posterior pituitary are innervated by dopaminergic

Table 2
Effects of Px and MEL Treatment on Body and Organ Weights (Mean ± SEM)

Treatment	Body wt, g	Testes wt, g	Seminal vesicle wt, mg	Anterior pituitary wt, mg
Con SD	157 ± 3.7^{ab}	1.84 ± 0.283^{a}	555 ± 36^{a}	2.15 ± 0.11^{a}
Px SD	156 ± 2.9^{ab}	3.15 ± 0.232^{b}	691 ± 62^{ab}	2.42 ± 0.08^{b}
Et LD	154 ± 2.6^{ab}	3.58 ± 0.138^{b}	807 ± 56^{b}	2.64 ± 0.15^{b}
Px LD	149 ± 3.9^{a}	3.43 ± 0.091^{b}	915 ± 70^{c}	2.49 ± 0.06^{b}
MEL LD	163 ± 3.6^{b}	1.73 ± 0.206^{a}	548 ± 34^{a}	2.26 ± 0.05^{a}

SD = short photoperiod; LD = long photoperiod, Et = ethanol vehicle.
[a, b, c]Within each column, the values without the same letter in the superscript are significantly different ($P < 0.05$).

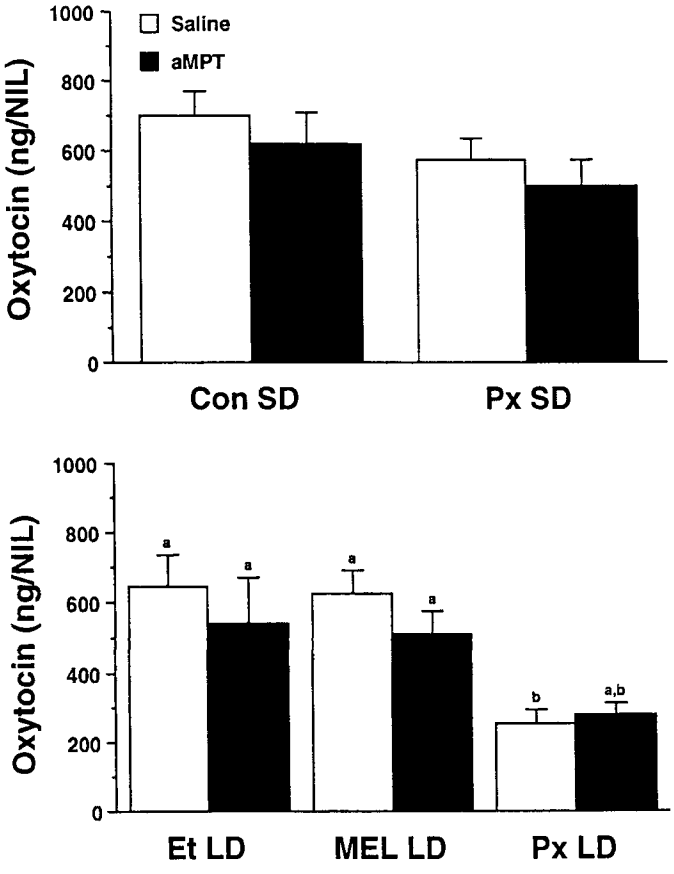

Fig. 3. Effects of Px in hamsters exposed to SD (top), and effects of Px and MEL or ethanol vehicle (Et) in hamsters exposed to LD on OT content in the NIL. Some of the animals received an injection of tyrosine hydroxylase inhibitor, αMPT. Values expressed as mean ± SEM (n = 6–8 hamsters/group). Different letters denote statistical significance ($P < 0.05$).

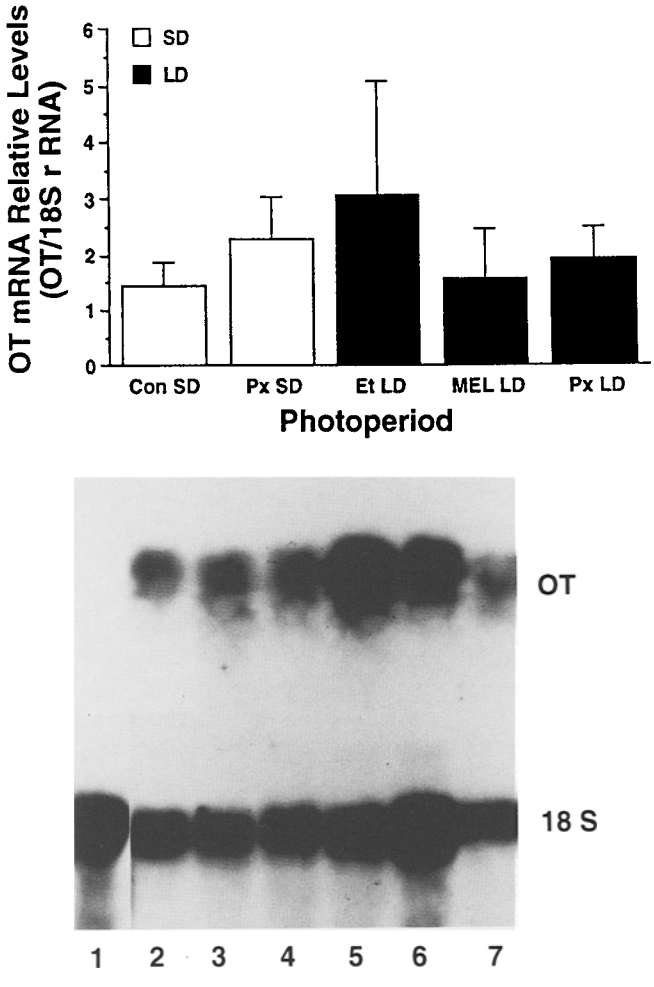

Fig. 4. Effects of Px in hamsters exposed to SD, and effects of Px and MEL or Et in hamsters exposed to LD on hypothalamic OT mRNA content in relative densitometer units (n = 3–4/group) (top) and results of Northern blot analysis of OT gene expression in a representative experiment (bottom). Some of the animals received an injection of tyrosine hydroxylase inhibitor, αMPT. Lane 1—negative control (liver), lane 2—Px SD, lane 3—MEL LD, lane 4—SD, lane 5—Et LD, lane 6—Px LD, lane 7—positive control (hypothalamus). After hybridization, the blots were normalized against the levels of 18 S rRNA to correct for loading accuracy. Relative levels of expression of OT mRNA are shown in the top panel.

and noradrenergic neurons (Palkovits, 1981; Saavedra, 1985; Crowley and Armstrong, 1992 and references therein). Dopaminergic innervation of NIL originates mostly from the arcuate nucleus (tuberohypophyseal DA system), whereas noradrenergic innervation originates partly from the brain and partly from the superior cervical ganglia (Saavedra, 1985). Inhibitory influence of DA on the in vitro release of OT from the isolated hypothalamo-neurohypophyseal system was reported by Seybold et al.

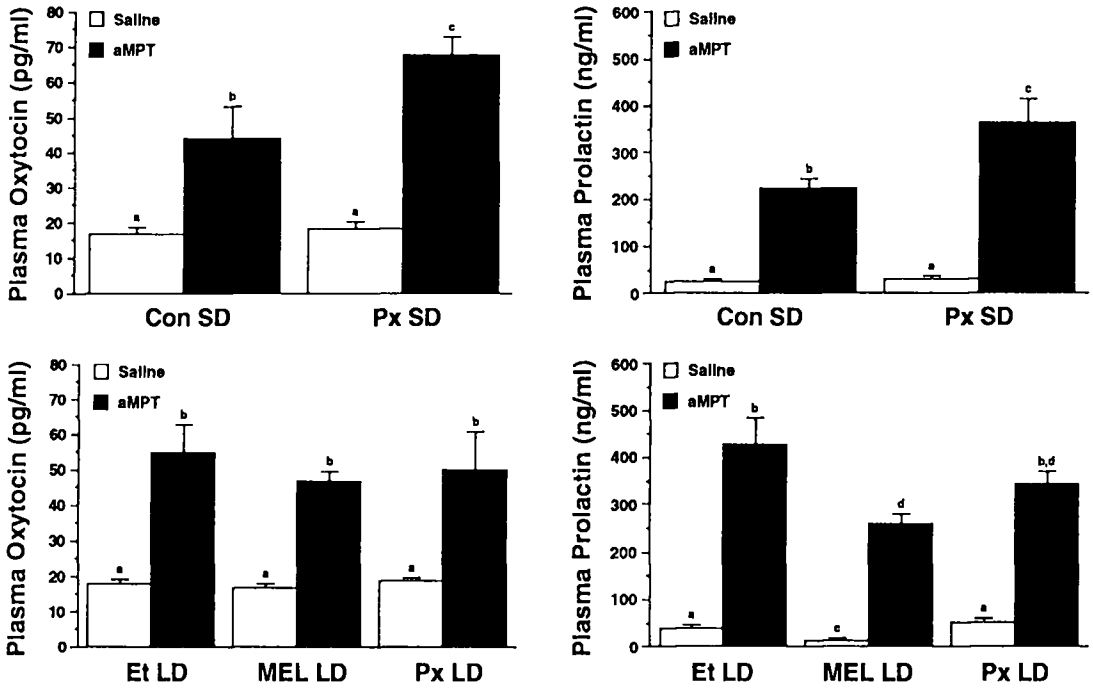

Fig. 5. Effects of Px in hamsters exposed to SD (top), and effects of Px and MEL or Et in hamsters exposed to LD on plasma OT (left) and PRL (right) levels in tyrosine hydroxylase inhibitor (αMPT)-injected and control hamsters. Values expressed as mean ± SEM (n = 6–8 hamsters/group). Different letters denote statistical significance ($P < 0.05$).

(1978). However, more recent in vitro studies demonstrate that the inhibitory effect of DA on OT release, exerted via the D-2 DA receptors, is not exerted at the level of the neural lobe (Crowley et al., 1991). An excitatory role for DA in the control of OT release, especially during lactation, has also been postulated. This effect is mediated by D-1 DA receptors and occurs, at least in part, at the level of the neural lobe (Crowley et al., 1991). In this context, it is important that both D-1- and D-2-binding sites were shown to be present in the neurohypophysis (Crowley and Armstrong, 1992 and references therein). Using tissues from animals used in the present study, we have recently demonstrated that DA turnover in the hypothalamus was significantly decreased within 1 wk of exposure to SD and remained diminished up to 10 wk, whereas DA turnover in NIL was sharply increased after 1 and 4 wk at SD conditions, and subsequently declined toward the values measured in LD-exposed animals (Steger et al., 1995). The significant increase in plasma OT level after αMPT injection may also suggest inhibitory influence of DA on OT release. However, in SD-exposed hamsters, DA metabolism in NIL was increased and DA turnover in the hypothalamus started to decline well in advance of the elevation in OT content in NIL. Perhaps DA was exerting inhibitory action on both synthesis and release of OT. Moreover, the possible peripheral effects under conditions of diminished catecholamine synthesis and their influence on the function of oxytocinergic neurons (e.g., by modified afferentation of baroreceptor origin) cannot be excluded.

Significant changes in plasma PRL levels were first seen at 4 wk after transfer from LD to SD, whereas DA turnover in NIL was markedly increased after 1 and 4 wk of SD exposure (details and discussion in Steger et al., 1995). Increased OT accumulation in NIL appeared later than diminution in PRL release and elevation in NIL DA metabolism. Such a course of events suggests that the effects of SD on OT levels may have been secondary to the changes in PRL release, consistent with the findings obtained in other models (Parker et al., 1991).

The Effect of Px and MEL Treatment on OT Synthesis and Release

Px resulted in a diminution of NIL OT content in LD-exposed animals. These results are in agreement with data obtained in rats where Px decreased OT content in the hypothalamus and neurohypophysis within 8 wk after surgery (Juszczak and Guzek, 1983, 1988). It is of interest that, in the present study, Px reduced NIL OT content without altering steady-state levels of OT mRNA in the hypothalamus. Further studies will be required to distinguish between several mechanisms that could account for this effect. The mechanisms by which SD may influence oxytocinergic neurons in intact or in pinealectomized animals are still unknown. Nevertheless, the neural connections among the eyes, PVN, and pineal gland are now well established. It is therefore possible that neural input originating in the retina and reaching PVN affects the synthesis and release of neurohypophyseal hormones.

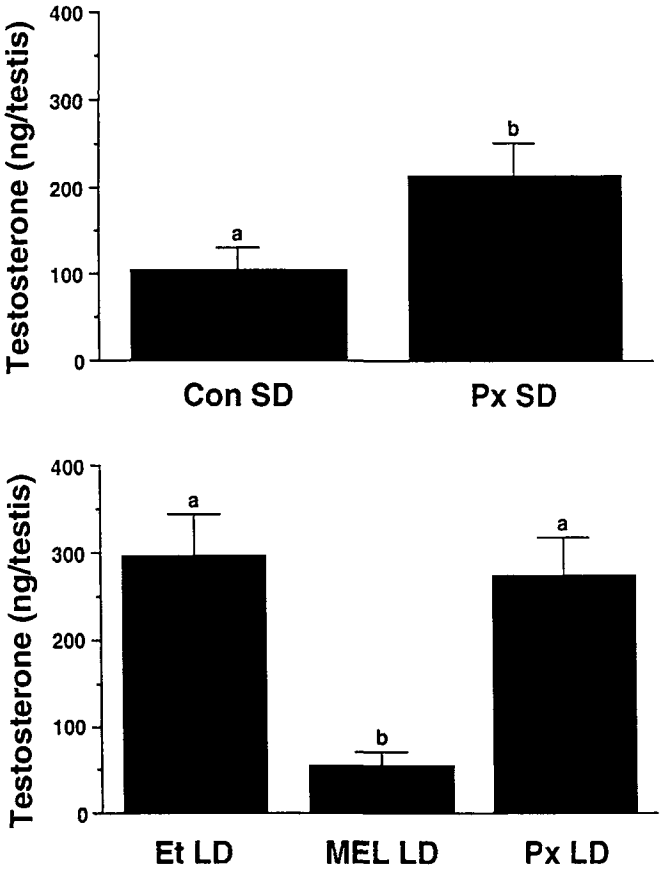

Fig. 6. Effects of Px in hamsters exposed to SD (top), and effects of Px and MEL or Et in hamsters exposed to LD on testosterone levels in the testis. Values expressed as mean ± SEM (n = 6–8 hamsters/group). Different letters denote statistical significance ($P < 0.05$).

In the present study, long-term (60 days) treatment with MEL injected at the end of the light phase was able to mimic the SD-induced testicular, seminal vesicle, and anterior pituitary weight changes, as well as diminution of testicular testosterone level, in agreement with previously published observations (Steger et al., 1984; Reiter, 1985; Steger and Gay-Primel, 1990; Steger and Bartke, 1991). However, this treatment failed to modify OT content in NIL or plasma OT level. The levels of OT mRNA in the hypothalamus appeared to be diminished after MEL treatment, but this decrease was not statistically significant.

Several previous studies addressed the effects of MEL on OT release. Experiments in vitro showed that MEL inhibited both basal and K^+-stimulated OT release from the isolated rat hypothalamus. Maximum inhibition of OT release was obtained at $4.3 \times 10^{-7}M$ MEL, whereas higher concentrations tended to increase basal OT release (Yasin et al., 1993). On the other hand, our previous studies (Juszczak et al., 1992) demonstrated that whereas higher concentrations of MEL (10^{-3} and $10^{-6}M$) stimulated OT release from rat NIL both under basal and K^+-stimulated conditions, MEL at the concentration of $10^{-7}M$ was ineffective. In the Syrian hamster NIL, MEL inhibited OT release at concentrations of 10^{-7}, 10^{-9}, and $10^{-11}M$ (Juszczak et al. 1995). Dissimilar effects of MEL on OT levels in NIL in our previous in vivo studies (Juszczak et al., 1986; Juszczak and Guzek, 1988) and in the present experiments may be owing to differences in the dose of MEL or to species differences. Juszczak et al. (1986) and Juszczak and Guzek (1988) used rats and high doses of MEL (100 μg MEL/100 g body wt), whereas in the present experiment, we used 25 μg MEL/animal in the Syrian hamster. This dose of MEL was previously reported to cause gonadal atrophy in the Syrian hamster (Tamarkin et al., 1976; Reiter, 1985) and was effective in this regard in the present study.

General Discussion and Conclusions

The possible mechanism of SD-induced and pineal-related changes in the function of oxytocinergic neurons could also involve alterations in hypothalamic catecholamine metabolism. In previous studies, MEL reduced DA release from the hypothalamus (Zisapel et al., 1982, 1983) and reduced DA content in NIL (Alexiuk and Vriend, 1993), whereas SD consistently reduced DA turnover in the ME, but not in the mediobasal hypothalamus (MBH) (Steger and Bartke, 1991; Steger et al., unpublished observations). In the present study, the increase in plasma OT levels in response to blocking catecholamine synthesis with αMPT was reduced by exposure to SD for 1 wk and increased by Px of SD-exposed hamsters. This observation would seem consistent with the inhibitory effect of MEL on OT release, which was found in previous in vitro studies (Yasin et al., 1993; Juszczak et al. 1995).

As expected from previous observations (Orstead and Blask 1987; Steger and Gay-Primel 1990), treatment with MEL decreased plasma PRL levels similarly to the effect of SD exposure and reduced the increase in plasma PRL after αMPT administration, whereas Px reversed the effects of SD exposure on plasma PRL level.

The present results demonstrate that exposure of male Syrian hamsters to SD for 10 wk increases the accumulation of OT in the NIL, but does not affect the release of OT into the circulation. The apparent effects of SD on some aspects of OT synthesis and/or transport may be related to changes in catecholamine turnover in the hypothalamus and, in particular, to the inhibitory DA mechanisms. In our experimental conditions, the effects of SD on the oxytocinergic system of the male Syrian hamster were apparently not mediated by MEL, but may have been related to the alterations in PRL release.

Material and Methods

Animals

Adult male Syrian (golden) hamsters, purchased from the National Cancer Institute, Frederick Cancer Research and Development Center (Frederick, MD) were housed in quarters with controlled temperature (23 ± 1°C) and light–

dark cycles (16 h light:8 h darkness; lights on from 0700–2300 h) before the experiments. They had free access to standard pelleted food (Lab Diet, Purina Mills, Inc., St. Louis, MO) and tap water throughout the experiments.

Experimental Design

In the first experiment, hamsters were divided into four groups, using 19 or 20 animals/group: animals kept under LD (16 h light and 8 h darkness; 16L:8D) throughout the whole experiment (group 1; LD), animals transferred to SD (6L:18D) for 1 wk (group 2; SD-1), 4 wk (group 3; SD-4), or 10 wk (group 4; SD-10). All animals were killed by decapitation on the same day (between 0900 and 1200 h) 1 h following an ip injection with saline (11–12 animals from each group) or with the tyrosine hydroxylase inhibitor, αMPT (250 mg/kg; 8 animals from each group). Results concerning regulation of PRL release in these animals have recently been reported (Steger et al., 1995).

In the second experiment, animals were divided into five groups, using from 13–16 animals/group: intact males exposed to SD for 10 wk (group 5; Con SD), pinealectomized males exposed to LD (group 6; Px LD) or SD (group 7; Px SD) for 10 wk, and intact animals exposed to LD and injected sc once daily for 60 d, with 100 μL of vehicle (1% ethanol in 0.9% sodium chloride, group 8; Et LD) or with MEL (*N*-acetyl-5-methoxytryptamine, Sigma Chemical Co., St. Louis, MO, lot 73H0986), 25 μg/animal in 100 μL of vehicle (group 9; MEL LD). All injections were given 1–1.5 h before lights were off, i.e., between 2130 and 2200 h. The durations of MEL treatment and exposure to SD in the second experiment were selected based on the results of the first experiment. The animals in groups 6 and 7 were pinealectomized according to procedures described by Kuszak and Rodin (1977) with minor modifications (Juszczak and Guzek, 1983, 1988). Animals were allowed to recover for 5–7 d before transferring half of them to SD (Px SD group).

Ten wk after pinealectomy and LD or SD exposure, or on the next day after the last MEL or vehicle injection, half of the animals in each group received αMPT, whereas the remaining animals received saline. One hour later, between 0900 and 1200 h, the animals were decapitated.

Experimental Procedures

In both experiments, blood from the trunk was collected into glass tubes (containing 6% EDTA, Bacitracin—0.2 mg/mL and Aprotinin—25 μg/mL), centrifuged at 1800*g*, 4C, for 20 min, and plasma stored at –20°C until used for measurements of OT and PRL levels.

After decapitation, the brain of each hamster was removed, immediately frozen on dry ice, and stored at –70°C. The pituitary was removed and the NIL was separated from the anterior lobe. The neurointermediate lobe was then homogenized by sonication in 100 μL of 0.25% acetic acid in double-distilled water. A 50-μL aliquot of tissue suspension was stored at –70°C for subsequent catecholamine determination, and the remaining 50 μL aliquot was transferred into a polypropylene tube and heated for 5 min in a boiling water bath (to inactivate proteolytic enzymes present in the tissue) and then centrifuged at 1800*g*, at 4°C, for 20 min. The supernatant was removed, brought up to a constant volume with the same solution of acetic acid, frozen, and stored at –70°C until assayed for OT. Effects of SD exposure on catecholamine levels and turnover in these animals have already been reported (Steger et al., 1995).

Extraction of Total RNA

RNA from the hypothalamic tissue was obtained according to the method described by Chomczynski and Sacchi (1987). Tissue samples (i.e., two halves of the hypothalamic fragments pooled from two animals from the same group) were homogenized in 500 μL of solution D (4*M* guanidinum thiocynate, 25 m*M* sodium citrate, 0.5% *N*-lauryl-sarcosine, and 0.1*M* β-mercaptoethanol). The homogenate was extracted with 100 μL 2*M* sodium acetate (pH 4.1), 500 μL 0.1 M Tris-saturated phenol, and 200 μL chloroform:isoamylalcohol (49:1). The solution was mixed for 10 s, chilled on ice for 15 min, and centrifuged for 20 min at 12,000*g*, at 4°C. The aqueous phase was removed, RNA precipitated after addition of 500 μL ice-cold isopropanol, and incubated for 1 h at –20°C, followed by centrifugation at 12,000*g*, at 4°C, for 20 min. The RNA pellet was redissolved in 150 μL solution D and reprecipitated at –20°C overnight. After washing with 250 μL of 80% ethanol and centrifugation, the final RNA pellet was redissolved in 25 μL DEPC-treated water. The concentration of total RNA was quantified by measuring the absorbance at 260 nm in a UV spectrophotometer.

Northern Blot Analysis

Ten micrograms of total RNA from each sample were fractionated on a 1.5% agarose gel containing 17.2% formaldehyde in MOPS buffer. Relative amounts of 28S and 18S ribosomal RNAs were assessed under UV light and ribonucleic acids were then transferred to a nylon membrane overnight. The membrane was baked at 80°C for 2 h and then placed into a plastic bag for prehybridization. Prehybridization was performed for at least 2 h at 63°C in 10 mL buffer containing 50% formamide, 5X SSC, 0.02% SDS, 0.1% *N*-lauryl-sarcosine, and 2% blocking solution (Boehringer Mannheim, Indianapolis, IN). Immediately after prehybridization, the membrane was hybridized overnight at 63°C in the same buffer with the addition of digoxigenin-labeled cRNA OT-specific probe and then washed twice with 2X SSC–0.1% SDS for 5 min at room temperature, once in 0.1X SSC–0.1% SDS for 15 min at 63°C, and once in the same washing buffer for 15 min, but at 68°C. For detection of digoxigenin-labeled nucleic acid, the standard protocol (with Lumigen PPD) was used according to the manufacturer (Boehringer Mannheim). Blots were finally exposed to Kodak X-Omat film for 1 or 2 h to obtain optimal signal intensity and prevent overexpo-

sure. For rehybridization, the probe was removed from the membrane by washing at 75°C in probe-stripping solution (50 m*M* Tris-HCl, pH 8.0, 1% SDS, 60% formamide) for 1 h. Membranes were rehybridized with a digoxigenin-labeled bovine 18S ribosomal RNA according to the same procedure as that for the cRNA OT-specific probe. The 18S bovine ribosomal RNA probe is a 250-bp *Eco*RI fragment subcloned into pGEM4. This was generously provided by Michael D. Uhler, Mental Health Research Institute, University of Michigan, Ann Arbor, MI. Bands showing specific hybridization were assessed by densitometry. The autoradiographic bands (signal intensties) were quantified with a Quick Scan Jr (Helena Laboratories, Beaumont, TX) densitometer, and the data were normalized against the signal intensities of 18S bovine rRNA used as an internal control to correct for RNA loading accuracy. The results were expressed in terms of arbitrary units. Changes in signal intensity for OT blots were compared to the signal intensity of 18S rRNA.

Preparation of cRNA Probe

Oxytocin cDNA probe was kindly provided by Thomas G. Sherman, Department of Behavioral Neuroscience, University of Pittsburgh, Pittsburgh, PA. The antisense OT cRNA probe was prepared by linearizing the pGEM4-OT3c plasmid (a full-length OT cDNA subclone inverted into the *Eco*RI site of pGEM4; Sherman et al., 1988) with *Hin*dIII, followed by synthesis of a digoxigenin-labeled 579 cRNA using SP6 RNA polymerase.

Preparation of Plasma Samples for OT Radioimmunoassay (RIA)

Plasma samples diluted with 4% acetic acid (v/v) were filtered through C-18 Sep-Pak cartridges (Waters, Millipore Corp., Milford, MA), and prewashed with 5 mL of methanol and 10 mL of deionized water. After the samples were passed through the cartridges (1 mL), they were washed with 10 mL of 4% acetic acid and eluted with 3 mL of 70% acetonitrile in 4% acetic acid. The eluates were lyophilized, stored at −20°C, and reconstituted with assay buffer for RIA. Recovery of OT from plasma following extraction exceeded 90%, and results were reported without correction for recovery.

RIA Procedures

OT levels were determined in duplicate, using a double-antibody RIA. On the first day, buffer (0.5% bovine serum albumin in phosphate-buffered saline; BSA-PBS) was added to unknown samples and standards. Standard curve constructed with synthetic OT (Cambridge Research Biochemicals, Wilmington, DE; batch no: 08568) ranged from 0.5–500 pg OT/tube. Antiserum (kindly provided by Janet Amico, Department of Medicine, University of Pittsburgh School of Medicine, Pittsburgh, PA, and prepared under NIH Grant AM 16166 by Alan G. Robinson) was diluted 1:18,000 in 1% normal rabbit serum diluted with in 0.1*M* EDTA-PBS, pH 7.4, with 0.2 mL being dispensed in each tube. On the following day, 10,000 cpm of ^{125}I-OT (New England Nuclear, Wilmington, DE), dissolved in 0.5% BSA-PBS, were added to each tube. The tubes were further incubated for 3 additional days. The separation of bound and free OT was achieved by adding a goat antirabbit γ-globulin (diluted in PBS buffer) followed by the addition of 1 mL of 10% polyethylene glycol to hasten the precipitation of the complex (Desbuquois and Aurbach, 1971). All incubations were carried out at 4°C. After centrifugation, the supernatant was aspirated and the radioactivity contained in the precipitate was measured in a γ-counter. All samples from animals tested within one experimental paradigm were measured in the same RIA to avoid interassay variability; the intraassay coefficient of variation was 4.2%. Assay sensitivity determined at 95% of binding was approx 0.5 pg OT/tube with 50% of binding corresponding to 17–20 pg OT/tube. Crossreactivity of the antibody was 0.2% with arginine vasopressin, and <0.1% with lysine vasopressin, arginine vasotocin, Pro-Leu-Gly-NH_2, Neurophysin, thyrotropin-releasing hormone (TRH), adrenocorticotropin (ACTH), and PRL (Amico et al., 1985).

The concentration of PRL in the plasma samples was measured using a homologous hamster PRL assay with reagents supplied by F. Talamantes according to a previously published procedure (Steger et al., 1983).

Testicular testosterone (T) concentration was determined using the RIA system described previously (Russell et al., 1993). Briefly, testicular tissue was homogenized in double-distilled water, extracted with anhydrous diethyl ether (Aldrich Chemical Company, Milwaukee, WI), and the extracts were dried under nitrogen. After drying, a 500-μL aliquot of 0.05*M* PBS, pH 7.4, containing 0.2% gelatin was added to all tubes. The antitestosterone serum (ICN Biomedicals, Inc., Costa Mesa, CA) was diluted to 1:340,000 in buffer (PBS and 1 m*M* EDTA, pH 7.4). This dilution was found to bind ^{125}I-T (ICN Biomedicals, Inc., Costa Mesa, CA) at approx 40%. First antibody (100 μL) and 100 μL of tracer were added to the assay tubes, and incubated at 4°C for 24 h. Free and bound T were separated using an antirabbit γ-globulin. Assay sensitivity expressed as 85% of the total binding was 5 pg/tube.

Statistical Evaluation of the Results

Significant differences between the means were identified using *t*-tests for comparisons of two means and analysis of variance (ANOVA) followed by Fisher PLSD or Dunnett's tests for comparisons of three or more means. $P < 0.05$ was used as the minimal level of significance. In experiment 2, LD- but not SD-exposed animals were given daily vehicle injections. Therefore, the effects of experimental treatments were analyzed, and reported separately for the LD groups and the SD groups.

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References

Alexiuk, N. A. M. and Vriend, J. P. (1993) Brain Res. Bull. **32,** 433–436.

Amico, J. A., Ervin, M. G., Leake, R. D., Fisher, D. A., Finn, F. M., and Robinson, A. G. (1985). *J. Clin. Endocrinol. Metab.* **60,** 5–12.

Arnauld, E., Wattiaux, J. P., Arsaut, J., Rostene, W., and Vincent, J. D. (1993). *Neurosci. Lett.* **149,** 177–181.

Bartke, A. (1985). In: *The hamster: reproduction and behavior.* Siegel, H. I. (ed.). Plenum, New York, pp. 73–98.

Benson, B. (1987). *Exp. Brain Res.* **65,** 371–376.

Bridges, T. E., Hillhouse, E. W., and Jones, M. T. (1976). *J. Physiol.* **260,** 647–666.

Chomczynski, P. and Sacchi, N. (1987). *Anal. Biochem.* **162,** 156–159.

Crowley, R. S. and Amico, J. A. (1993). *Endocrinology* **133,** 2711–2718.

Crowley, W. R. and Armstrong, W. E. (1992). *Endocrine Rev.* **13,** 33–65.

Crowley, W. R., Parker, S. L., Armstrong, W. E., Wang, W., and Grosvenor, C. E. (1991). *Neuroendocrinology* **53,** 493–502.

Crowley, R. S., Insel, T. R., O'Keefe, J. A., and Amico, J. A. (1993). *Endocrinology* **133,** 2704–2710.

Demaine, B. C., Forsling, M. L., Kelestimur, H., and Stoughton, R. P. (1990). *J. Physiol.* **423,** 12P.

Desbuquois, B. and Aurbach, G. D. (1971). *J. Clin. Endocrinol.* **33,** 732–738.

Juszczak, M. and Guzek, J. W. (1983). *Acta Physiol. Polonica* **34,** 41–46.

Juszczak, M. and Guzek, J. W. (1988). *J. Pineal Res.* **5,** 545–552.

Juszczak, M., Guzek, J. W., and Lewy, A. (1986). *J. Pineal Res.* **3,** 199–211.

Juszczak, M., Stempniak, B., and Guzek, J. W. (1992). *J. Pineal Res.* **12,** 1–6.

Juszczak, M., Debeljuk, L., Bartke, A., and Stempniak, B. (1995). *Neuroreport* **6,** 2453–2456.

Kuszak, J. and Rodin, M. (1977). *Experientia (Basel)* **33,** 283–284.

Orstead, K. M. and Blask, D. E. (1987). *Neuroendocrinology* **45,** 182–190.

Palkovits, M. (1981). *Neuroendocrinology* **33,** 123–128.

Parker, S. L. and Crowley, W. R. (1993). *Endocrinology* **132,** 658–666.

Parker, S. L., Armstrong, W. E., Sladek, C. D., Grosvenor, C. E., and Crowley, W. R. (1991). *Neuroendocrinology* **53,** 503–510.

Randle, J. C. R., Mazurek, M., Kneifel, D., Dufresne, J., and Renaud, L. P. (1986) *Neurosci. Lett.* **65,** 219–223.

Reiter, R. J. (1985). In: *The hamster: reproduction and behavior.* Siegel, H. I. (ed.). Plenum, New York, pp. 99–118.

Reppert, S. M., Perlow, M. J., Artman, H. G., Ungerleider, L. G., Fisher, D. A., and Klein, D. C. (1984). *Brain Res.* **307,** 384–387.

Russell, L. D., Corbin, T. J., Borg, K. E., De Franca, L. R., Grasso, and P., Bartke, A. (1993). *Endocrinology* **133,** 2062–2070.

Saavedra, J. M. (1985). *Neuroendocrinology* **40,** 281–284.

Seybold, V., Miller, J. W., and Lewis, P. R. (1978). *J. Pharmacol. Exp. Ther.* **207,** 605–610.

Sherman, T. G., Day, R., Civelli, O., Douglas, J., Herbert, E., Akil, H., and Watson, S. J. (1988). *J. Neurosci.* **8,** 3785–3796.

Steger, R. W. and Bartke, A. (1991). *Biol. Reprod.* **44,** 76–82.

Steger, R. W. and Gay-Primel, E. (1990). *Biol. Reprod.* **42,** 217–221.

Steger, R. W., Bartke, A., Goldman, B. D., Soares, M. J., and Talamantes, F. (1983). *Biol. Reprod.* **29,** 872–878.

Steger, R. W., Reiter, R. J., and Siler-Khodr, T. M. (1984). *Neuroendocrinology* **38,** 158–163.

Steger, R. W., Juszczak, M., Fadden, C., and Bartke, A. (1995). *Endocrinology* **136,** 3000–3006.

Tamarkin, L., Westrom, W. K., Hamill, A. I., and Goldman, B. D. (1976). *Endocrinology* **99,** 1534–1541.

Windle, R. J. and Forsling, M. L. (1993). *J. Endocrinol.* **136,** 305–311.

Windle, R. J., Forsling, M. L., and Guzek, J. W. (1992). *J. Endocrinol.* **133,** 283–290.

Yasin, S. A., Costa, A., Besser, G. M., Hucks, D., Grossman, A., and Forsling, M. L. (1993). *Endocrinology*, **132,** 1329–1336.

Zisapel, N., Egozi, Y., and Laudon, M. (1982). *Brain Res.* **246,** 161–163.

Zisapel, N., Egozi, Y., and Laudon, M. (1983). *Neuroendocrinology* **37,** 41–47.