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OXYTOCIN AND PROLACTIN RELEASE AFTER HYPERTONIC SALINE ADMINISTRATION IN MELATONIN-TREATED MALE SYRIAN HAMSTERS

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The aim of the present investigations was to examine the effects of melatonin (Mel) on oxytocin (OT) release under conditions of osmotic stimulation, brought about by hypertonic saline administration, as well as to determine whether osmotically stimulated OT release in Mel-treated Syrian hamster is associated with alterations in the release of prolactin (PRL) and in norepinephrine (NE) and dopamine (DA) content in the hypothalamus. In both Mel- and vehicle-treated hamsters, injection of hypertonic saline was followed by a significant decrease in OT content in the pituitary neurointermediate lobe (NIL) and elevation of plasma OT and PRL levels. Melatonin injections had no significant affect on NIL OT content in either isotonic- or hypertonic-saline treated animals. Pretreatment with Mel did not alter plasma OT or PRL levels in isotonic saline-injected animals. However, Mel facilitated the release of OT, but prevented the release of PRL after hypertonic saline administration. Melatonin treatment reduced hypothalamic NE content (but not that of DA) in isotonic-saline treated animals. After osmotic stimulation, hypothalamic content of NE and DA was significantly lower in Mel-treated than in vehicle-treated animals. Data from the present study suggest that the osmotically-stimulated release of OT and PRL seems to be related to the activation of noradrenergic rather than dopaminergic transmission. Both dopaminergic and noradrenergic transmission may be, however, involved in mediating the effects of Mel on the osmotically-activated OT and PRL release.

Key words: *melatonin, hypertonic saline, oxytocin, prolactin, dopamine, norepinephrine*

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INTRODUCTION

The existence of functional relationships between the pineal gland and hypothalamo-neurohypophyseal system is now well established. The influence of the pineal gland and melatonin (Mel) on the neurosecretory activity of neurones in the supraoptic (SON) (1) and paraventricular (PVN) (2) nuclei was noted in the rat. However, while the stimulatory action of Mel on oxytocin (OT) release from isolated neurointermediate lobe of the pituitary (NIL) has been described (3), other authors reported that Mel produced a dose-dependent inhibition of OT release from the rat hypothalamus *in vitro* (4). Although *in vitro* data suggest that Mel action depends on its concentration (3, 4), experiments *in vivo* demonstrated that Mel, when administrated under conditions of equilibrated water metabolism, is capable of decreasing OT content in the rat neurohypophysis. This effect was detected after a single Mel injection (5) as well as after treatment continued for up to 8 days (5—7). Moreover, Mel augmented the depletion of OT storage in the neurohypophysis of rats deprived of water for 8 days but not of those dehydrated for 2 or 4 days (5).

The aim of the present study was to examine the effects of Mel on OT release under conditions of osmotic stimulation with hypertonic saline. This hyperosmotic stimulus is known to increase the release of OT (8—10).

Melatonin can inhibit dopamine (DA) release from the retina (11) and from the rat hypothalamus *in vitro* (12), and reduce DA content in the NIL of male Syrian hamsters (13). Both noradrenergic (14, 15) and dopaminergic (16—18) mechanisms are known to control OT release. Melatonin (19), DA (20, 21) and OT (22) can modify PRL release. Moreover, PRL was found to stimulate OT release *in vivo* as well as *in vitro* from isolated NIL (23). It was, therefore, of interest to determine whether osmotically stimulated OT release in Mel-treated Syrian hamster is associated with alterations in the release of PRL and in norepinephrine (NE) and DA content in the hypothalamus.

MATERIAL AND METHODS

Animals

Adult male Syrian (golden) hamsters (body weight on the day of killing, 140±9 g) were purchased from National Cancer Institute, Frederick Cancer Research and Development Center (Frederick, MD 21702). The animals were housed in quarters with controlled temperature (23±1°C) and regulated light-dark cycle (16 h hight : 8 h darkness; light from 0700 to 1100 h) and had free access to standard pelleted food and tap water throughout the experiment.

Experimental design

All hamsters were divided into two groups. Group A: animals injected, once daily for 8 days, with vehicle solution (1% ethanol in 0.9% sodium chloride, 0.1 ml per animal); group B: animals injected on the same schedule with Mel (N-Acetyl-5-methoxytryptamine; Sigma Chemical Co., St. Louis, Mo, lot 73H0986) dissolved in the vehicle (25 μg Mel in 0.1 ml of solution per animal). All injections were made subcutaneously, approximately 1—1.5 hours before lights off. Each group was further divided into two subgroups, as follows: subgroup 1, animals injected intraperitoneally (i.p.) with isotonic saline (0.9% sodium chloride; 1 ml per animal) 1 hour before decapitation; subgroup 2, animals similarly injected i.p. with hypertonic saline (1 ml per animal of 2 N sodium chloride), a treatment known to stimulate OT secretion in the rat (9, 10).

Experimental procedure

On the next day after receiving the last dose of Mel or vehicle, the animals were injected with isotonic or hypertonic saline between 0830 and 0930 h and decapitated 1 hour later. Trunk blood was collected into glass tubes containing 6% EDTA, centrifuged at 2,500 rpm at 4°C for 20 min, and plasma stored at −20°C for measurement of OT and PRL levels. Immediately after decapitation, the pituitary was removed and the NIL separated from the anterior lobe, whereas the brain was immediately frozen on dry ice and stored at −70°C. The NIL was homogenized by sonication in 0.1 ml of 0.25% acetic acid in double distilled water. Tissue suspension was transferred into polypropylene tube, heated for 5 min in a boiling water bath (in order to inactivate the proteolytic enzymes contained in the homogenized tissue), and then centrifuged at 2.500 rpm at 4°C for 20 min. The supernatant was removed and made up to constant volume with the same solution of acetic acid, frozen and stored at −70°C until radioimmunoassay (RIA) for OT.

Neurotransmitters content

Within several days after sacrifice, the brains were partially thawed and hypothalamic blocks were dissected as follows: rostral limit — the frontal plane situated about 1 mm rostral to the anterior margin of the optic chiasma; caudal limit — the frontal plane just before the mammillary bodies; lateral limits — sagittal planes passing, on both sides, through the hypothalamic fissures. The depth of such block of tissue was approximately 1.5 mm. Tissue fragments were subsequently sonicated in 0.32 M perchloric acid containing an internal standard, dihydroxybenzylamine (DHBA). Both standards and unknown samples were neutralized with TRIS buffer, absorbed onto alumina, washed in ice cold water bath for 15 min, and then extracted from the alumina with 0.16 M perchloric acid. The samples were subjected to high-performance liquid chromatography with electrochemical detection (HPLC-EC) on a C-18 reverse phase column eluted with PO_4 buffer containing an ion-pairing reagent (octane sulfonate) and an organic modifier, methanol. Unknown amine concentrations were calculated by comparison to the peak areas of the standards and then corrected for the percent recovery of the internal standard, according to the previously described procedures (24).

Preparation of samples for oxytocin RIA

Plasma samples diluted in 4% (v/v) acetic acid were filtered through C-18 Sep-Pak cartridges (Waters, Millipore Corp., Milford, MA) pre-washed with 5 ml of methanol and 10 ml of deionized water. After sample application (1 ml), the columns were washed with 10 ml 4% acetic acid and

eluted with 3 ml 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.

Oxytocin RIA

Oxytocin levels were determined for each sample in duplicate using a double-antibody RIA. On the first day, buffer (0.5% bovine serum albumin in phosphate-saline; BSA-PBS) was added to unknown samples and standards. A standard curve was set up using synthetic OT purchased from Cambridge Research Biochemicals, Wilmington, DE (batch no: 08568) at doses ranging from 0.5 to 500pg/tube. The antiserum was kindly provided by dr. Janet Amico, Department of Medicine, University of Pittsburgh School of Medicine, Pittsburgh, PA 15261, and prepared under NIH Grant AM 16166 by Dr. Alan G. Robinson. It was diluted 1:18.000 in 1% normal rabbit serum in 0.1 M EDTA-PBS, pH 7.4 and dispensed 200 µl/tube. On the following day, ^{125}J-OT (New England Nuclear, Wilmington, DE, lot number: CU61740) was added to each tube in a volume of 100 µl containing about 10,000 cpm/tube. After 48 hours of incubation a second antibody (100 µl/tube of goat anti-rabbit gamma-globulin diluted in PBS buffer) was used for the separation of bound from free OT. This was followed by addition of 1 ml of 10% polyethylene glycol (25). All incubations were carried out at 4°C. After centrifugation, the supernatant was aspirated, and the radioactivity contained in the precipitate was measured using a gamma counter. All samples from animals tested within one experimental paradigm were measured in the same RIA to avoid interassay variability; the intra-assay coefficient of variation (CV) was 4.7%. Assay sensitivity determined at 95% of binding was 0.5 pg OT/tube with 50% binding occuring between 12—13 pg OT/tube. Cross reactivity of the antibody with the OT was 100%, with arginine vasopressin was 0.2%, and with lysine vasopressin, arginine vasotocin, Pro-Leu-gly-NH_2, Neurophysin, TRH, ACTH and PRL, less than 0.1% (26).

The concentration of PRL in the plasma samples was measured using a homologous hamster PRL assay with reagents supplied by Dr. F. Talamantes according to a previously published procedure (27).

Statistical evaluation of the results

Significance of the differences between means was calculated using analysis of variance (ANOVA) followed by Fisher PLSD and Dunnett's test, using $p < 0.05$ as the minimal level of significance.

RESULTS

In both Mel- and vehicle-treated hamsters, injection of hypertonic saline was followed by a significant decrease in OT content of the NIL. Eight days of treatment with Mel did not affect the pituitary content of OT in either isotonic- or hypertonic-saline-treated animals (*Fig. 1*). Plasma OT levels were elevated after osmotic stimulation in both vehicle- and Mel-treated animals. The

concentration of OT measured after hypertonic saline administration was higher in animals previously injected with Mel than in those pretreated with vehicle (*Fig. 2*).

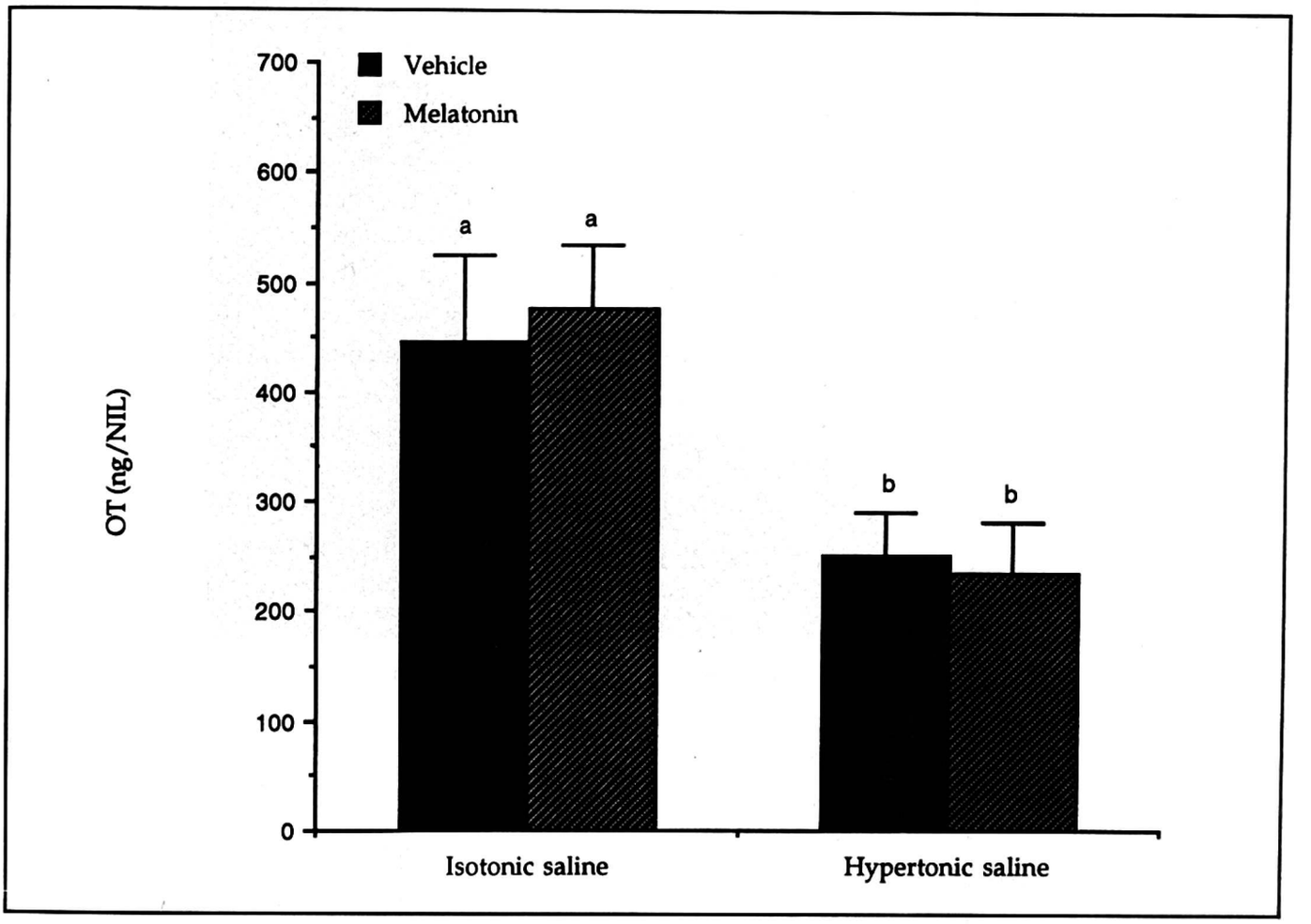

Fig. 1. Effects of hypertonic saline and melatonin on the content of oxytocin (OT) in the neurointermediate lobe of the pituitary in male Syrian hamsters. a, b — values marked with a different letter are significantly different ($P < 0.05$). (Means ± SEM); $n = 8$ per group; details in the text.

Plasma PRL levels in vehicle treated animals were significantly increased after osmotic stimulation. Pretreatment with Mel did not alter plasma PRL level in isotonic saline-injected animals, but prevented osmotically-induced PRL surge (*Fig. 2*).

Melatonin treatment for 8 days reduced hypothalamic NE content (*Fig. 3*); the content of DA also tended to be lower after Mel treatment, but this apparent difference was not statistically significant (*Fig. 3*). After osmotic stimulation, hypothalamic content of NE and DA was significantly lower in Mel-treated than in vehicle-treated animals. Moreover, administration of hypertonic saline to previously vehicle-injected animals resulted in a decrease of the hypothalamic NE content (*Fig. 3*).

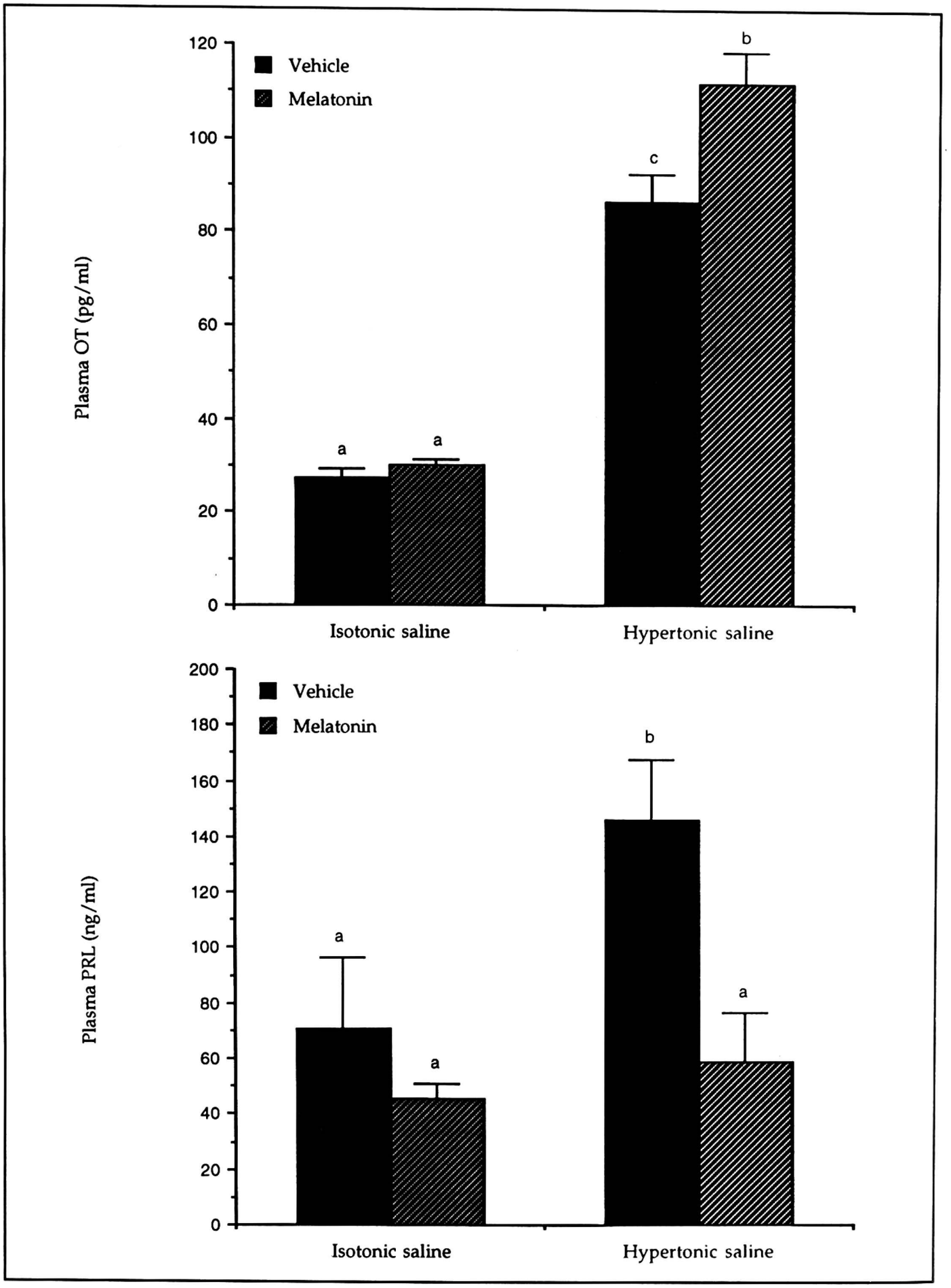

Fig. 2. Effects of hypertonic saline and melatonin on plasma levels of oxytocin (OT; upper panel) and prolactin (PRL; lower panel) in male Syrian hamsters. a, b, c — values marked with a different letter are significantly different ($P < 0.05$). (Means $\pm$ SEM; $n = 8$ per group; details in the text).

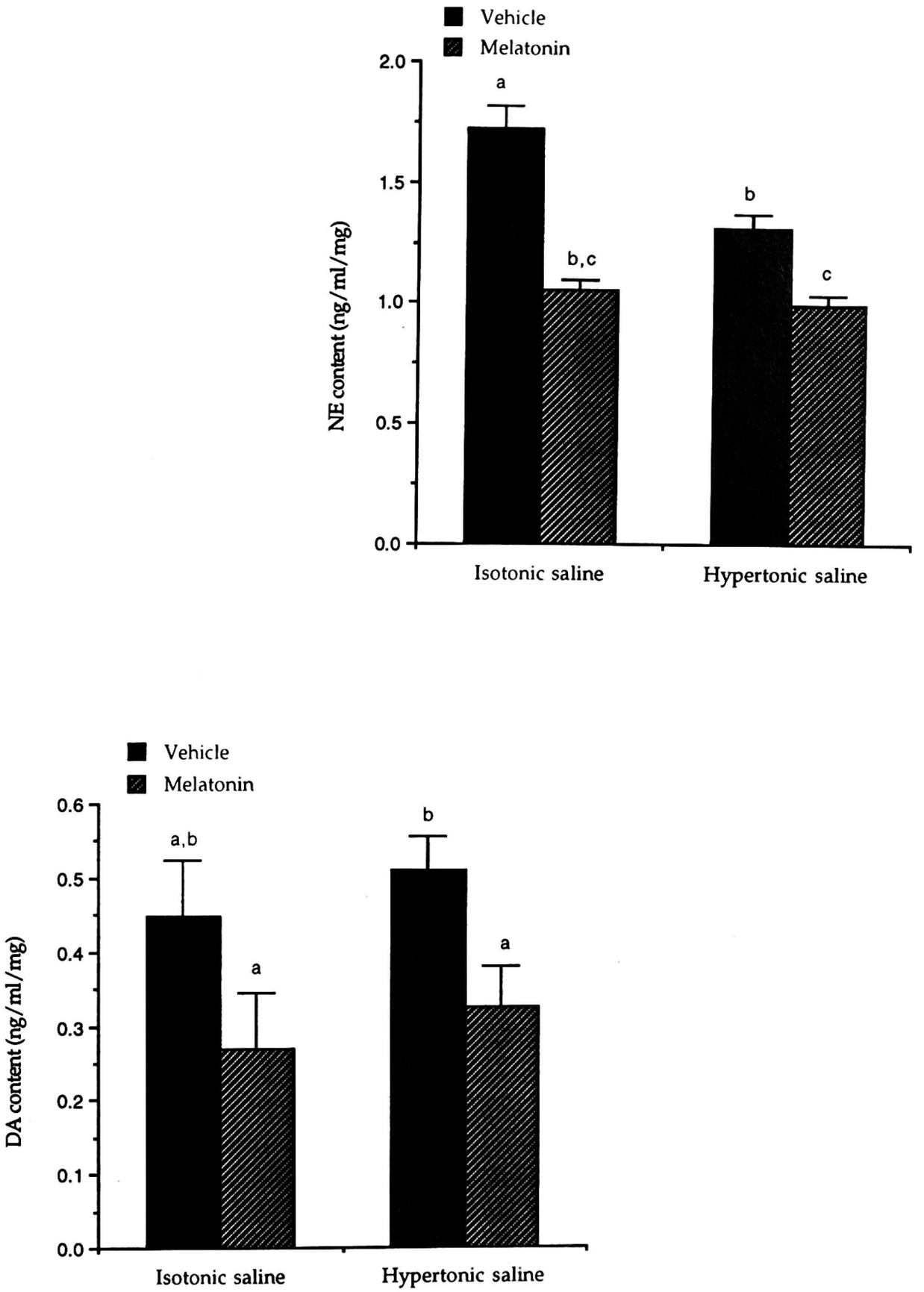

Fig. 3. Effects of hypertonic saline and melatonin on the content of norepinephrine (NE) and dopamine (DA) in the hypothalamus of male Syrian hamsters, a, b, c — values marked with a different letter are significantly different ($P < 0.05$). (Means $\pm$ SEM; $n = 8$ per group; details in the text).

DISCUSSION

The effect of hypertonic saline on OT and PRL release and catecholamines content in the hypothalamus

The stimulatory effect of hypertonic saline on OT release in the Syrian hamster which was observed in the present study was not unexpected. Increase of OT concentration in blood plasma after intravenous (i.v.) or i.p. administration of hypertonic saline in the rat was reported previously (8—10). The increase in OT plasma level after i.p. hypertonic saline administration was found to be comparable with that seen after 48 h dehydration (28). Microdialysis of the hypothalamic supraoptic nuclei (SON) by hypertonic medium significantly increased the intranuclear release of OT and its release from neurohypophysis into the blood (10). Moreover, i.p. administration of hypertonic saline (29) or oral salt loading (30) resulted in a rapid increase of hypothalamic OT mRNA level. Expression of c-fos protein in the magnocellular hypothalamic PVN and SON nuclei, which reflects the activation of oxytocinergic system, was reported as early as 30 min after such treatment (31). In rats drinking hypertonic saline or injected i.p. with hypertonic sodium chloride solution, the content of OT in the NIL was reduced (32, 33). The present findings of reduced OT content in the NIL and increased plasma OT level, i.e., stimulatory action of hypertonic saline on OT release, are in agreement with these observations.

Studies *in vivo* showed that dehydration (known to increase plasma osmolality and OT release; 28) caused significant increase in the accumulation of DOPA in the rat NIL (34). Similarly, increased DA synthesis *in vitro* in the NIL from dehydrated rats was found by Racke et al. (35). In the present study, NE content in the hypothalamus was significantly decreased after hypertonic saline administration whereas DA content was not changed. In as much as changes in catecholamine content may reflect alterations in the activity of the corresponding neurones, these results suggest that the increased OT release from the neurohypophysis into the blood after osmotic stimulation, found in Syrian hamsters in the present study, appears to be related to noradrenergic rather than dopaminergic transmission in the brain.

When studying the effects of chronic hyponatremia on basal and stimulus-induced PRL, OT and vasopressin (AVP) secretion in rats, Dohanics et al. (36) showed that basal plasma PRL, OT and AVP levels were not different between normonatremic and the hyponatremic rats. Chronic hyponatremia did not inhibit PRL response induced by hemorrhage (hemorrhage caused PRL release of equal magnitude in both the normonatremic and the hyponatremic rats) while in contrast, hemorrhage caused large increases in plasma OT and AVP levels in normonatremic but not

in the hyponatremic rats (36). In our present study, we have shown that, in addition to liberation of OT, osmotic stimulation, brought about by hypertonic saline administration, also enhanced PRL release.

The mechanism(s) underlying the modulation of PRL secretion in response to different physiological and pathological stimuli are not clear at present. However, the synthesis and release of PRL by adenohypophysis is known to be controlled by a number of factors of hypothalamic, as well as posterior and anterior pituitary origin. Prolactin secretion from hamster anterior pituitary *in vitro* was significantly depressed by DA (37). Although the best known hypothalamic inhibitory factor for PRL release is DA (20), stimulatory action of DA on PRL release, mediated by D-1 and D-5 DA receptors, has also been reported (21). After hypertonic saline administration in the present study, DA content in the hypothalamus was not altered whereas NE content was significantly decreased. This may suggest that noradrenergic rather than dopaminergic mechanisms are involved in the osmotically induced PRL release in the golden hamster.

The role of the posterior pituitary in the control of PRL release has been extensively studied. Although OT was found to be an important PRL releasing factor (PRF) in the posterior pituitary (22, 38), additional factor(s) present in the NIL were shown to influence PRL release from cultured anterior pituitary cells (39, 40). Since i. v. injection of OT was reported to increase plasma PRL level within 5 minutes (38) it may be hypothesized that, in the present study, increased blood plasma OT level (brought about by osmotic stimulation) could have caused the release of PRL from the anterior pituitary.

The effect of Mel on OT and PRL release and catecholamines content in the hypothalamus

Exogenous Mel is known to cross blood-brain barrier, and the hypothalamus as well as the anterior pituitary (41) seem to be its target areas. Specific melatonin binding sites have been demonstrated in the hypothalamus, especially in the median eminence/pars tuberalis region (42, 43) and Mel was found to alter the metabolism of serotonin, glutamate, noradrenaline (44), dopamine (12) and prostaglandins (45) in the hypothalamus. More recently, Yasin et al. (46) have found that Mel may influence OT release by modifying the metabolism of acetylcholine and/or prostaglandins in the hypothalamus. It is, therefore, possible that Mel modifies the synthesis and/or release of OT and PRL by a direct effect on the hypothalamic neurones or by modified neurotransmission in the brain.

Studies of the effects of Mel on OT release in the rat suggested existence of both stimulatory and inhibitory actions, depending on the dose. Thus, our previous *in vitro* studies (3) demonstrated that relatively high concentrations of

Mel (10^{-3} M and 10^{-6} M) stimulated OT release from rat NIL both under basal conditions and under stimulation by excess potassium, while at a concentration of 10^{-7} M Mel was ineffective. In contrast to these observations, Yasin et al (4) demonstrated that lower doses of Mel inhibited both basal and K^+-stimulated OT release from the isolated rat hypothalamus (maximum inihibition was obtained at 10^{-7} M Mel); higher concentrations increased the basal OT release only. When a similar experiment was performed using Syrian hamsters as NIL donors, Mel at concentrations of 10^{-7} M, 10^{-9} M, and 10^{-11} M inhibited OT release (47).

Previous *in vivo* experiments showed that Mel (100 μg/100 g b. w., injected daily for 8 days, at the end of the light phase of L : D cycle) decreased OT content in the rat hypothalamo-neurohypophyseal system (5, 6). This effect of Mel on OT content in the NIL was not detected in the present experiment. However, Mel enhanced osmotically-stimulated OT release. This indicates that, under certain experimental conditions, Mel may facilitate OT release into the blood. Moreover, it may be suggested that activation of the oxytocinergic system after osmotic stimulation (31) and the transport of OT towards neuropophysis are more pronounced after Mel administration and may partially compensate for the decrease of OT content in the NIL after its release into the blood.

An inhibitory action of Mel on PRL synthesis and release has been postulated. At concentrations 10^{-8} M to 10^{-6} M, Mel reduced both PRL production and secretion *in vitro* from tumor cells isolated from the rat pituitary gland (19). Moreover, Mel has been reported to reduce stimulatory effects of thyrotropin-releasing hormone (TRH), vasoactive intestinal peptide (VIP) and 17β-estradiol on PRL synthesis and release (19). Afternoon injection of Mel severely attenuated PRL secretion from hamster anterior pituitaries and increased the ability of DA to inhibit PRL release *in vitro* (37). However, while subcutaneous Mel injections reduced serum PRL levels (37), oral Mel administration produced an opposite effect (48).

In the present experiment, Mel significantly inhibited PRL release into the blood and reduced DA content in the hypothalamus of hamsters injected with hypertonic saline. This inhibitory effect of Mel on osmotically-induced PRL release may involve dopaminergic transmission in the brain, but studies of DA release or turnover are needed to support this conclusion. In this context, it is of interest that in addition to well-documented inhibitory action of DA on PRL release, stimulatory effects have also been reported (21).

In summary, using Syrian (golden) hamsters, we demonstrated that the osmotically stimulated release of OT and PRL, brought about by hypertonic saline administration, is to be related to the activation of noradrenergic rather than dopaminergic transmission. Moreover, both dopaminergic and noradrenergic transmission may be involved in mediating the effects of Mel on the osmotically-activated OT and PRL release.

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