ted line), obtained by the addition of the individual values for TsTX and ouabain 10^{-7} and 10^{-6}M were identical to the experimental values ($p > 0.3$). Thus, by the association of TsTX and ouabain an additive effect could be observed. Increasing the ouabain concentration to 10^{-5} and 10^{-4}M eliminated the additive effect. Ouabain up to the level of 10^{-6}M caused practically no interference with the oxygen consumption and energy processing in slices of rabbit brain tissue[5]. At higher concentrations, 10^{-5} and 10^{-4}M, ouabain had an inhibitory effect of about 40%. The reduction in oxygen consumption and energy for brain metabolism could be responsible for a reduction in the levels of ACh synthesis. Since it seems that at the nerve endings the processes of ACh synthesis and ACh release are linked[6], it is reasonable to assume that a reduced rate for the synthesis of ACh will affect the rate of ACh release. Thus, a reduction in the synthesis of ACh obtained either by a drug such as hemicholinium[7,8] or by absence of glucose[3], inhibited the release of ACh elicited by TsTX.

The present findings, and further results in which TsTX did not inhibit Na^+, K^+, ATPase[4], and the comparison of the effects of tetrodotoxin and EGTA on the release of ACh evoked by TsTX and by ouabain is contrary to the suggestion of Vizi[9] that inhibition of Na^+, K^+, ATPase is the only mechanism needed to explain the release of ACh in the central nervous system. Finally, the fact that the effects of TsTX in increasing the release of ACh[3,4] and the influx of sodium[4] were both inhibited by tetrodotoxin would confirm Birks' suggestion that sodium is essential for ACh synthesis and ACh release[10].

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Preliminary evidence that a dopamine receptor antagonist blocks the prolactin-inhibitory effects of melatonin in anosmic male rats[1]

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Summary. Previous studies have shown that daily afternoon injections of melatonin in anosmic male rats result in depressed accessory sex organ weights and serum prolactin levels. The present data indicate that the prolactin-inhibitory effect of melatonin may be mediated via the dopaminergic system.

Recently, we demonstrated that daily afternoon injections of a putative pineal hormone, melatonin (Mel), into anosmic male rats resulted, after several weeks, in a reduction in serum prolactin (Prl) levels[2]. Additionally, these animals had markedly hypotrophic seminal vesicles and ventral prostate glands. Inasmuch as Mel has no direct effect on pituitary Prl secretion either in vivo[3] or in vitro[4], we hypothesized that the Prl-inhibitory effects of Mel might be mediated via an increase in the secretion of Prl-inhibitory factor(s) (PIF). Since hypothalamic dopamine (DA) may represent a physiological PIF[5], we wanted to test the possibility that Mel's inhibitory action might be mediated via an interaction with the dopaminergic system.

Materials and methods. At 26 days of age, male Sprague-Dawley rats (Simonsen Labs, Gilroy, Calif.) weighing 60–70 g were rendered anosmic by bilateral olfactory bulbectomy, under ether anesthesia, according to a previously described method[6]. Following bulbectomy 4–5 animals were housed per metal cage in a temperature- and light-controlled room (25–26 °C; 14 h L:10 h D; lights on 06.00–20.00 h). The animals were provided with food and tap water ad libitum.

2 days following bulbectomy the animals began receiving s.c. implants of beeswax pellets containing 1.2 mg of the dopamine receptor blocker, pimozide (Pim) (McN-JR-6238; generously supplied by McNeil Labs, Inc.) under ether anesthesia. The method for preparing the Pim pellets was similar to that described for Mel beeswax pellets[7]. During the first 3 weeks of the study, rats received 2 implants per week; thereafter, animals received only 1 implant per week until the termination of the experiment. Control animals received beeswax pellets devoid of Pim. All implantations were performed between 09.00 and 12.00 h. Additionally, rats began receiving daily injections of either Mel (Sigma Chem. Co., Lot No. 77C-0319) or 0.9% saline diluent, 2 days following bulbectomy. The dose of Mel was 50 µg per injection, administered s.c. on the dorsum of the back in 0.1 ml of saline between 17.00 and 18.00 h. Fresh Mel solutions were prepared daily just prior to injection.

Animals were treated with Mel and/or Pim for 5 weeks, after which each was weighed and decapitated. Truncal blood was collected and the serum assayed for Prl by radioimmunoassay. Seminal vesicle and ventral prostate glands were weighed on a torsion balance. The data were statistically analyzed by Student's t-test.

Results. As seen in the figure Mel injections in animals receiving beeswax pellets without Pim resulted in a significant ($p < 0.01$) decrease in serum Prl levels compared to control animals. However, this effect was obviated in rats receiving Pim implants as evidenced by significantly ($p < 0.05$) elevated Prl levels in Mel-Pim-treated animals compared to Mel-treated rats. Seminal vesicle and ventral prostate weights were significantly reduced in the Mel-treated animals (table). This effect was not significantly affected by the Pim implants. Furthermore, the ventral

Mean (± SEM) body, seminal vesicle and ventral prostate weights in 62-day-old anosmic male rats receiving daily afternoon injections of melatonin (50 µg) and weekly s.c. implants of beeswax containing 1.2 mg of pimozide for 5 weeks. The animals were olfactory bulbectomized at 26 days of age and began receiving the injections and implants at 28 days of age. Implants were administered twice weekly for the first 3 weeks and once weekly thereafter. Controls received beeswax pellets devoid of pimozide

Treatment	N	Body weight (g)	Organ weights (mg) Seminal vesicles	Ventral prostates
Saline + beeswax	5	249 ± 15	318.3 ± 14.1 (130.1 ± 10.9)	212.9 ± 24.3 (86.8 ± 11.1)
Melatonin + beeswax	5	214 ± 6	130.3 ± 55.9[b] (58.6 ± 23.9)[c]	88.3 ± 19.4[a] (37.7 ± 9.4)[a]
Melatonin + pimozide	5	224 ± 14	206.2 ± 54.5 (88.0 ± 21.9)	103.3 ± 24.0[d] (44.3 ± 10.4)[d]
Saline + pimozide	4	254 ± 14	330.7 ± 25.6 (132.2 ± 14.6)	187.1 ± 11.2 (74.8 ± 7.3)

N = number of rats per group. Numbers in parentheses represent the mean relative organ weights (mg/100 g b.wt). [a] vs saline control, $p < 0.01$; [b] vs saline control, $p < 0.02$; [c] vs saline control, $p < 0.05$; [d] vs saline-pimozide control, $p < 0.05$.

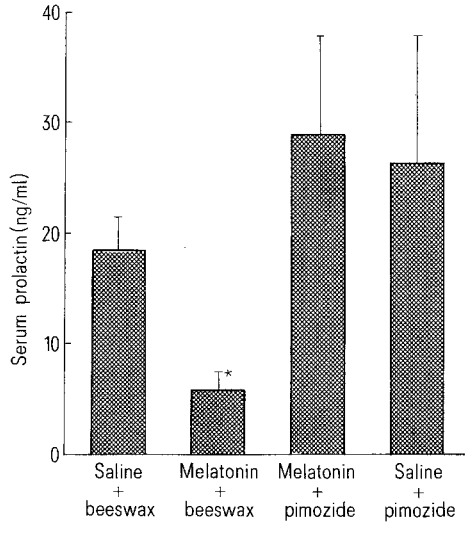
prostate weights in the Mel-Pim-treated animals were lower ($p < 0.05$) than those of rats treated with Pim implants alone.

Discussion. The present investigation corroborates our earlier finding that Mel inhibits Prl secretion in the anosmic male rat. Furthermore, the Prl-inhibitory effect of Mel is completely prevented by s.c. implants of beeswax pellets containing Pim. Pim is a specific DA receptor antagonist and has been shown to block the Prl-inhibitory action of DA directly at the level of the anterior pituitary[8]. In vivo, the acute effect of Pim is manifested as an elevation in blood levels of Prl[9]. On this basis it seems reasonable to suggest that inhibitory influence of Mel on Prl secretion in the anosmic rat may be mediated via DA and a dopaminergic receptor.

Since daily injections of Mel do not inhibit Prl secretion in normal male rats, perhaps anosmia makes the dopaminergic system more sensitive to the effects of Mel. It is possible that Mel stimulates the secretion of DA from the medial basal hypothalamus resulting in diminished Prl levels. Pim, by interacting with DA receptors on the lactotrophs, inhibits the action of DA, and thus Mel, on Prl secretion. Alternatively, Mel may stimulate dopaminergic neurons in the hypothalamus to release DA which, in turn, interacts postsynaptically with DA receptors on presumptive peptidergic PIF[10] neurosecretory cells. If Mel were to work via this mechanism, then it is conceivable that Pim could block the postsynaptic effect of DA on the PIF neurosecretory cell. This mechanism seems unlikely, however, since the medial basal hypothalamus has been shown to be devoid of dopamine receptors[11]. Although Mel does not exert a direct effect on pituitary Prl secretion in normal rats it is possible that in olfactory-deprived animals the anterior pituitary is sensitized to direct inhibitory effects of Mel. This hypothesis could be easily tested by exposing pituitaries from anosmic rats to Mel in organ culture and measuring the release of Prl into the medium. This could be done in the presence or absence of Pim to determine if the DA receptor is involved.

Although Pim is a highly specific DA receptor antagonist[12] the possibility exists that Pim also blocks a non-dopaminergic Prl-inhibitory system (perhaps peptidergic) which is responsive to Mel. In such a scheme while the effect of Pim on Mel-induced Prl inhibition would be the same, its mechanism of action would be different.

Since the accessory sex organs consist of Prl-responsive tissues, the Mel-induced depression in serum Prl may partially account for the reduction in accessory organ weight. However, it is unlikely that a decrease in the secretion of Prl alone was responsible since Pim did not reverse the effects of Mel on accessory organ weight as it did in the case of Prl. Other effects such as a Mel-induced depression of luteinizing hormone (LH) and/or testosterone output would have to be postulated.



Mean serum prolactin levels (ng/ml) in 62-day-old anosmic male rats treated with daily afternoon injections of melatonin (50 µg) and weekly s.c. implants of beeswax containing 1.2 mg of pimozide for 5 weeks. For experimental conditions see text. Vertical lines from top of bars signify SEM. * $p < 0.01$ vs saline + beeswax; $p < 0.05$ vs melatonin + pimozide.

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