those obtained in the adrenal medulla, where perinatal ethanol causes a definite slowing of catecholaminergic development[3], with a reduction in the number of storage vesicles. It is therefore apparent that the developmental effects of ethanol are different in central vs peripheral neuronal tissues.

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The effects of scorpion venom tityustoxin and ouabain on the release of acetylcholine from incubated slices of rat brain[1]

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Summary. The in vitro release of acetylcholine in slices of rat brain tissue was followed in the presence of tityustoxin and ouabain. At low ouabain concentrations, the release of acetylcholine caused by both ouabain and tityustoxin, was additive. At higher ouabain concentrations the additive effect of tityustoxin and ouabain on the release of ACh was no longer observed.

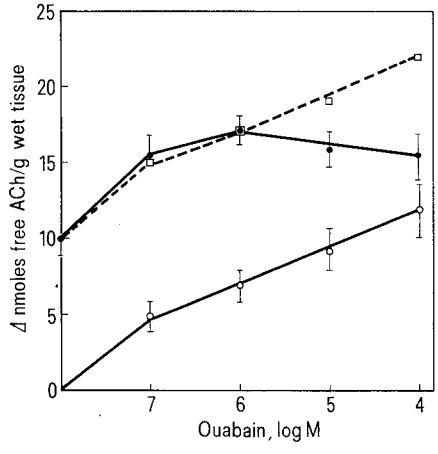
Tityustoxin (TsTX), a toxin purified from the venom of the scorpion *Tityus serrulatus*[2], causes a state of membrane depolarization and an increase in the release of cellular acetylcholine (ACh) in rat brain cortical slices[3]. Previously[4], we have compared the modalities of the release of ACh by TsTX and by ouabain and the effect of these 2 agents on Na^+, K^+, ATPase. Unlike ouabain, TsTX did not inhibit this enzyme activity[4]. Tetrodotoxin failed to reverse the ouabain elicited release of ACh while it successfully antagonized the release of ACh elicited by TsTX[4]. In the presence of Ca^{2+} in the medium, EGTA totally prevented the action of TsTX but not that of ouabain[4]. These results suggest that Na^+, K^+, ATPase was not involved in the release of ACh evoked by TsTX. However, we did not observe[4], as we had expected, an additive effect on the release of ACh by TsTX and ouabain.

The effect of TsTX on ACh release is energy dependent and, at a concentration of 10^{-4} M, ouabain caused almost 40% inhibition in the rates of respiration and ATP hydrolysis in brain cortical slices[5].

Therefore, we decided to compare the release of ACh evoked by TsTX in the presence of different ouabain concentrations that either had or did not have an effect on the rate of respiration and the energy process in brain cortical slices[5].

Material and methods. Tityustoxin (TsTX) was purified by a combination of extraction and chromatographic techniques using Sephadex G-25 and carboxymethylcellulose[2]. Ouabain was obtained from Sigma Chemical Co., Saint Louis, Mo. Albino rats of either sex were decapitated and the brains quickly removed. The preparation of cortical slices, the incubation procedures and the assay were performed as previously described[3].

Results and discussion. The values for the release of ACh were expressed as Δ nmoles of free ACh/g wet tissue (figure). The figures were obtained by subtracting the control value (without TsTX and ouabain) from each of the values obtained in the conditions studied, i.e., in the presence of TsTX or ouabain or combination of the two. Thus, in the absence of ouabain, TsTX 2×10^{-6} M evoked a Δ value of 10.1 ± 0.5 of free ACh/g wet tissue. Ouabain alone, at concentrations of 10^{-7}M and 10^{-6}M, elicited Δ values of 5.0 ± 0.4 and 7.6 ± 0.5 nmoles of free ACh/g wet tissue, respectively. The incubation of TsTX 2×10^{-6}M together with ouabain at the above concentrations, raised the values for the release of ACh to 14.9 ± 0.5 and 17.4 ± 0.6, respectively. The theoretical values (figure, dot-



The effects of tityustoxin (TsTX) and ouabain on the release of acetylcholine from incubated slices of rat brain. ○, Free ACh without TsTX; ●, free ACh with TsTX 2×10^{-6}M; □, theoretical value for TsTX 2×10^{-6} M plus ouabain at the indicated concentrations in abscissa. The mean value ± SEM for 3 experiments are presented. Slices were incubated for 30 min at pH 7.4 in a medium containing (mM): NaCl 136; KCl 2.7; $CaCl_2$ 1.35; NaH_2PO_4 0.36; $NaHCO_3$ 12, glucose 5.5; eserine 0.01. For other details see the text.

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