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Changes in mediobasal hypothalamic dopamine and GABA release: A possible mechanism underlying taurine-induced prolactin secretion

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Summary. Taurine (Tau), a putative inhibitory amino acid neurotransmitter, has been shown to stimulate prolactin (PRL) release. Using ovariectomized, estrogen-replaced adult rats we investigated initially the effect of this amino acid, injected by different routes, on PRL secretion in vivo. Tau (100–500 mg/kg) had no effect on PRL release when given i.p.; 15 min after i.c.v. injection of Tau (3 μmoles), a significant increase in serum PRL levels was observed (78 $\pm$ 9 ng/ml over basal levels, $p < 0.01$ vs. controls). In vitro (cultured anterior pituitary cells) PRL release was not affected by a 5 h incubation with Tau (10^{-3}–10^{-8} M). Basal dopamine (DA) or gamma-aminobutyric acid (GABA) output from superfused mediobasal hypothalamic fragments (MBH) was not affected by Tau (10^{-3} M or 10^{-5} M). However, during stimulation with KCl (50 mM), Tau (10^{-3} M) significantly lowered DA release, and increased GABA output. It is concluded that Tau acts at a central level to increase PRL secretion, most probably by modulating the hypothalamic release of neurotransmitters controlling lactotroph function.

Keywords: Amino acids – Taurine – Prolactin – Dopamine – GABA – HPLC – Hypothalamus

Introduction

In the brain of different vertebrate species, taurine (Tau) acts as a putative inhibitory amino acid neurotransmitter (or, at least, neuromodulator), exerting a hyperpolarizing effect probably caused by changes in Cl^- conductance (for review, see Huxtable, 1989). In a similar way to gamma-aminobutyric acid (GABA), Tau has been shown to affect hypothalamic/pituitary hormone secretion, e.g.: 1) somatostatin release by median eminence tissue is increased

by Tau (Aguila and McCann, 1985); 2) Tau inhibits stimulated LH and LHRH release (Price et al., 1978; Arias et al., 1994); 3) a stimulatory effect of this amino acid on prolactin secretion has been described (Scheibel et al., 1980; Ikuyama et al., 1988; Panula-Lehto et al., 1989).

Except for its probable hypothalamic site of action, little is known about the mechanisms involved in Tau-induced PRL release. To further characterize the role of Tau on PRL secretion we performed following in vivo and in vitro investigations: a) in adult, ovariectomized, estrogen-treated rats, the PRL release response to Tau, administered intraperitoneally (i.p.) and centrally, in the lateral ventricle (i.c.v.), was evaluated; b) the effect of different Tau concentrations on PRL secretion was tested in pituitary cell cultures, and c) the influence of Tau upon basal and KCl-stimulated dopamine and GABA release was investigated using superfused mediobasal hypothalamic fragments.

Material and methods

Adult female Wistar rats (220–250 g, ovariectomized 2 weeks prior to the experiments) were used. Animals were kept in group cages (3/cage) under controlled conditions (temperature: 22–24°C, lights on 6–20 h), and had free access to tap water and food. All animals received estradiol benzoate (100 μg/kg) 30 h prior to the experiments.

In vivo experiments

During the i.p. experiments, animals (n = 8–10 group), fitted with indwelling atrial cannulae, received 100–250 or 500 mg/kg Tau (dissolved in water) or saline. Blood samples were taken before, and regularly thereafter. For the i.c.v. challenge, a guide cannula (30 g), fitted with a steel mandrill, was implanted stereotaxically under ketamine-diazepam anesthesia into the right lateral ventricle (A: 0 mm, L: 1.5 mm, V: 3 mm; according to Pellegrino et al., 1967), and anchored to the skull using screws and dental cement. Three days thereafter, animals were fitted with indwelling atrial cannulae. On the next day, between 8 and 11 h, the mandrill was replaced by the infusion needle (33 g) attached to a 10 μl syringe by a silicone tubing. Ninety minutes thereafter infusion (3 μmoles Tau in 6 μl distilled water) was performed by a syringe pump in 10 min (n = 5 rats). Control animals (n = 6) received saline infusions. Blood sampling was performed as indicated in Fig. 1. Serum, obtained after clotting at room temperature and centrifugation (10 min, 3,000 rpm) was frozen at −20°C until determination.

In vitro experiments

a) Pituitary cell cultures. Minimum Essential Medium for Suspension (SMEM), Dulbecco's Minimal Eagle's Medium (DMEM), Hepes, Bovine Serum Albumin (BSA), horse serum, Fetal Calf Serum (FCS) and antibiotics were purchased from Gibco (Karlsruhe, Germany). Trypsin, DNAse, LHRH, taurine and Earle's balanced salt solution (EBSS) from Sigma (Deisenhofen, Germany), and Percoll from Pharmacia (Freiburg, Germany). After decapitation (9.00 h), the anterior lobe of the pituitary was isolated and minced. Enzymatic dispersion (trypsin 0.1%, DNAse 0.005% in 10 ml SMEM, 45 min, 37°C) followed; cells were harvested, washed and centrifuged twice, and resuspended in 5 ml serum medium (DMEM containing 10% horse serum, 2% FCS, 100 UI/ml penicillin and 100 μg/ml streptomycin), then layered on 40 ml of 60% Percoll and centrifuged (600 g, 15 min) to remove erythrocytes; the cell pellet was diluted (2.5×10^5 cells/ml) and seeded in 96 well microtiter plates (250 μl/well). Cell viability (trypan

blue exclusion test) was always >90%. Cells were incubated at 37°C in a 10% CO_2, 10% air atmosphere for 72 h, and formed during this period a confluent layer at the bottom of the well. On the experimental day medium was aspirated and adherent cells were washed three times with BSA medium (DMEM supplemented with 0.1% BSA, 20 mM Hepes and antibiotics). Wells were then filled with 270 μl BSA medium and 30 μl of the test solution (BSA for controls or BSA containing 10^{-2} to 10^{-7} M Tau), and the cells incubated for 5 h. Thereafter, medium was carefully aspirated, centrifuged, and the supernatant stored at −20°C until assay.

b) Superfusion of hypothalamic fragments: The mediobasal hypothalami (MBH; limits: optic chiasm, hypothalamic grooves, mammilary bodies; cut depth: ca. 1.5 mm; n = 6–8/group) were dissected on ice after decapitation and placed into superfusion chambers (medium: EBSS, pH 7.4, gassed with O_2 95% – CO_2 5%; flow rate 50 μl/min). After a 90 min wash-out phase 10 min fractions were collected on ice, acidified with 0.1 N citrate buffer (pH 3.1), and stored frozen until determination. The first four fractions were used for estimating basal neurotransmitter (NT) release; thereafter Tau (10^{-3} M or 10^{-5} M) was added to the medium; sampling continued then for 60 min. Control experiments (without Tau supplementation) were performed in parallel. During the last 20 min NT release was stimulated in all groups by KCl (50 mM, fractions 9–10).

Analytics and statistics

PRL concentrations in serum samples and in cell culture supernatants were determined as described previously by Niswender et al. (1968) using a double antibody radioimmunoassay procedure (standards and antibodies were generously supplied by the NIADDK). Results are expressed in terms of the RP2 standard; detection limit was 1 ng/ml, and the intraassay coefficient of variation 5.4%. Dopamine (DA) levels were measured using reverse phase HPLC and electrochemical detection, according to Jarry et al. (1986). GABA concentrations were assayed using an enzymatic-fluorimetric method (Mansky et al., 1982).

Results are presented as mean values ± SEM. In pituitary cell culture experiments, PRL concentrations from control incubations (BSA medium alone) were averaged and the mean set as 100%. All data were then expressed in relation to this 100% value. In hypothalamic superfusion studies, data from fraction periods 3–4 (basal), 6–8 (Tau) and 9–10 (KCl 50 mM + Tau) were averaged. Differences between control and Tau-treated groups were tested for significance using a one-way analysis of variance and Tukey's multiple comparison test (Tukey, 1949) or Wilcoxon's rank sum test, as appropiated. Differences were considered significant when $p < 0.05$.

Results

PRL secretion (in vivo and in vitro studies)

Serum PRL levels were not affected by the i.p. administration of 100,200 (data not shown) or 500 mg/kg Tau (Fig. 1). On the contrary, i.c.v. Tau elicited a prompt and significant increase in PRL secretion: 15 min after infusion, PRL levels increased by 78 ± 9 ng/ml ($p < 0.01$ vs. control animals; Fig. 1). PRL release by cultured pituitary cells (Fig. 2) was not affected by different Tau concentrations (10^{-3} to 10^{-8} M).

Hypothalamic neurotransmitter release

The influence of Tau on basal and KCl-stimulated DA and GABA release by superfused MBH fragments is depicted in Fig. 3. The addition of Tau to the

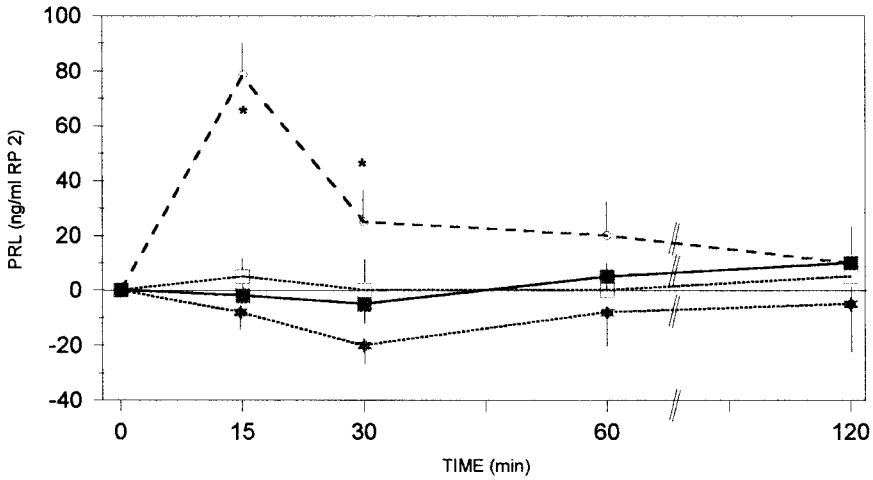

Fig. 1. Prolactin release (increase over basal values) elicited by taurine administration to ovariectomized, estrogen-replaced adult rats. Filled squares: i.p. controls (n = 9), empty squares: i.p. taurine (0.5 g/kg, n = 8), filled stars: i.c.v. controls (n = 6), empty circles: i.c.v. taurine (3μmoles, n = 5). *p < 0.05 vs. corresponding control

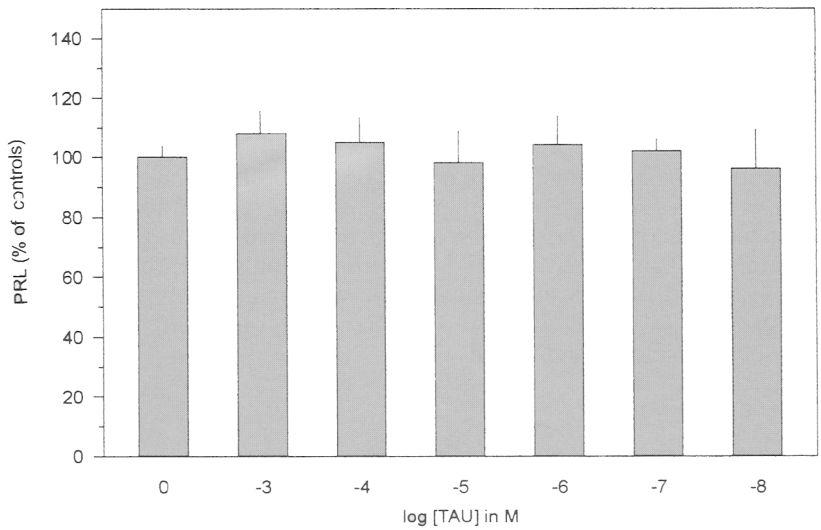

Fig. 2. Effect of different taurine (*TAU*) concentrations on prolactin (*PRL*) secretion by cultured pituitary cells obtained from adult ovariectomized, estrogen-primed rats (125,000 cells/well; n = 20 experiments/TAU concentration)

perifusion medium did not affect basal DA or GABA release; however, a significant (p < 0.05 vs. controls) decrease in KCl-stimulated DA release was observed during perifusion with 1 mM Tau (Fig. 3A). Conversely, Tau (1 mM) clearly increased stimulated GABA output in these preparations (Fig. 3B).

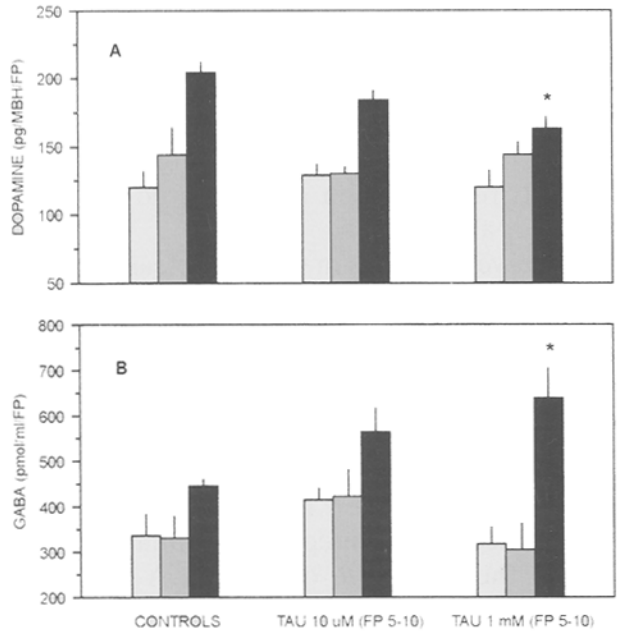

Fig. 3. Effect of taurine (*TAU*) on basal and KCl-stimulated **A** Dopamine and **B** GABA release by perifused mediobasal hypothalamic fragments obtained from adult ovariectomized, estrogen-primed rats. Empty bars: Fraction periods (FP) 4–6 (basal release); dotted bars: FP 7–8 (*TAU*); full bars: FP 9–10 (TAU + KCl 50 mM). Control experiments (without TAU supplementation) were run in parallel. *$p < 0.05$ vs. controls

Discussion

According to our results, Tau does not modify PRL release from monolayer pituitary cell cultures. Neither does intraperitoneally administered Tau affect in vivo PRL secretion, probably due to the low transport rate of Tau into the brain, as described previously (Oja et al., 1976). We were not able to demonstrate an increase in cortical or hypothalamic levels of Tau after i.p. injection of 100–500 mg/kg Tau to juvenile rats aged 30 days (unpublished observations). Furthermore, intravenous or intrapituitary administration of Tau had no effect on PRL levels, as reported by Scheibel et al (1980).

As demonstrated before (Scheibel et al., 1980; Ikuyama et al., 1988; Panula-Lehto et al., 1989), the intracerebroventricular administration of Tau elicits a clear increase in serum PRL concentrations. The microinjection of Tau (125 nmoles) into the arcuate nucleus, but not into other hypothalamic or extrahypothalamic regions also resulted in a significant stimulation of PRL release (Scheibel et al., 1984). It is clear from these experiments that the mediobasal hypothalamus is the most probable site of action of Tau in triggering PRL release.

As to the neurotransmitter systems evaluated, present results show a decrease in KCl-stimulated hypothalamic DA release after adding Tau (1 mM) to the medium bathing mediobasal hypothalamic fragments. It is well known that the removal of the inhibitory dopaminergic control enhances

PRL secretion by lactotroph cells (for review, see Kordon et al., 1994). Intracerebroventricular administration of Tau reduces the activity of central dopaminergic neurons, and increases striatal and hypothalamic DA levels, suggesting a diminished neurotransmitter release (Garcia de Yebenes Prous et al., 1978; Panula-Lehto et al., 1992). It is interesting to note that Tau is without effect on PRL release when administered with the D2 antagonist haloperidol (Scheibel et al., 1980).

The role of the observed changes in GABA release on PRL secretion is far more difficult to interpret. Interactions between the two inhibitory amino acids, namely GABA and Tau, have been the subject of a considerable number of publications. In brief, 1) Tau might exert its inhibitory effects by acting on GABA-A or GABA-B receptors (6,12,15), 2) Tau increases K^+-stimulated GABA release from rat striatal slices (11), and 3) it inhibits GABA reuptake (13).

Contradictory findings have been reported on the effects of GABA on PRL release (4); it is generally accepted that stimulation or inhibition of PRL secretion results from the activation of GABA-A receptors located at the hypothalamus or the pituitary, respectively (see Kordon et al., 1994). However, even acting on the lactotrophes, GABA-A receptor agonists would exert a biphasic effect on prolactin secretion (3). Most probably, increased hypothalamic GABA activity could lead to augmented PRL levels by inhibiting hypothalamic DA release (14,19). However, according to Ondo (17), Tau-induced PRL release would be a specific effect since it is inhibited by TAG (6-aminomethyl-3-methyl-4H, 1,2,4-benzothiadiazine-1, 1-dioxide), a putative Tau antagonist which does not interfere with the action of GABA. Further experiments (e.g. combining the administration of Tau and GABA antagonists, such as bicuculline or baclofen) are needed to clarify this aspect.

We did not evaluate the effect of Tau upon other aminergic or peptidergic systems known to affect PRL release. Tau might potentiate the activity of the opioid peptidergic system, thus augmenting PRL secretion (9). A hypothetical participation of the serotonergic system, as suggested by Scheibel et al. (22) is difficult to support, since Tau does not affect hypothalamic nor SNC concentrations of this monoamine (2,7). The effect of Tau upon the hypothalamic release of peptides affecting PRL release (e.g. ocytocin, vasoactive intestinal peptide or VIP, substance P, etc.) remains to be elucidated.

In conclusion, present results show that 1 mM Tau induces a significant decrease in stimulated dopamine output and an increase in stimulated GABA release by perifused mediobasal hypothalamic fragments. These changes may surely be involved in the PRL-releasing effect induced by central Tau administration.

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