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Short communication

# Tryptophan ingestion by gestant mothers alters prolactin and luteinizing hormone release in the adult male offspring

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**Abstract**

The effect of tryptophan administration to pregnant rats on the development of serotonergic systems and serotonin-related hormones in the offspring was studied. The male offspring of rats treated with tryptophan (200 mg/kg/day) during the second half of gestation showed a 4- to 7-fold increase in serum prolactin 40 and 70 days after birth and a 2-fold increase in serum luteinizing hormone 70 days after birth. The forebrain of adult offspring of tryptophan-treated rats showed an increase in serotonin and 5-hydroxyindoleacetic acid levels. Present data suggest that tryptophan regulates serotonergic differentiation during early development. © 1997 Elsevier Science B.V.

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In addition to their action as neurotransmitters during adulthood, monoamines could act as a differentiation signal during early neurogenesis [1,19,22]. We have previously reported that oral tyrosine administration to pregnant rats induces in the fetus a short-lasting increase in tyrosine and catecholamine brain levels [5] and in the adult offspring a persistent modification of dopamine and 3,4-dihydroxyphenylacetic acid (DOPAC) levels in several brain loci [22], dopamine agonists response [22] and different behavioral patterns [1,21]. Despite tryptophan administration to pregnant rats induces in the fetal brain a similar short-lasting modification of serotonergic neurotransmission [2], its action on the ontogenic development of serotonergic systems has received little attention.

In animals and humans, the serotonergic cell differentiation occurs prenatally and their dendritic growth and terminal synaptogenesis take place during postnatal life [2,8,24,27]. The present study was designed to assess the effect of oral tryptophan administration to pregnant rats on the postnatal development of serotonergic systems in the offspring. The regulation of the neuroendocrine system is one of the main roles of serotonergic cells during adulthood. Amongst other hormones, serotonergic neurons regulate prolactin release [4,6,11,13,14,25], and the activity of the hypothalamic-pituitary-gonadal system [23,26]. In order to determine whether prenatal tryptophan action on serotonin systems has persistent functional consequences during postnatal life, the effect of prenatal administration of tryptophan on prolactin and luteinizing hormone (LH) was also examined.

Experiments were carried out on male Sprague–Dawley rats (Letica, Barcelona). Animals were housed at 22°C, two per cage, under normal laboratory conditions on a standard light–dark schedule (12:12 h with 3.00–15.00 h light on) and with free access to food and water.

L-Tryptophan (200 mg/kg; Sigma, St. Louis, MO) was given daily by stomach intubation to young pregnant rats (150–200 g body weight before gestation). The amino acid or its saline vehicle was administered during the light cycle from day 15 to day 21 of gestation. Timing of pregnancies was determined by daily checking vaginal washings for sperm; the day on which sperm was found was regarded as day 0. Only the rats that were born on day 21 of gestation were included in the study. Sixty rats were divided into six groups of 10 rats each. In order to prevent a litter effect each of the animals included in a group descended from a different mother. The rats were sacri-

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ficed by decapitation at the onset of the dark period of days 40 or 70 after birth. Blood was collected from the trunk and allowed to clot at room temperature. Serum was separated by centrifugation ($2000 \times g$), divided into aliquots, and stored at $-40$°C until assay. Immediately after extraction, the brain was laid on its dorsal surface and the mesencephalon and forebrain dissected from the forebrain structures by the mesencephalic flexure and according to a previously described procedure [2]. The brain pieces were rapidly removed and weighed in conical 5 ml test tubes. Two ml of 0.1 M perchloric acid containing $4 \times 10^{-5}$ M sodium metabisulphite was pipetted into the tubes to avoid the metabolization or oxidation of monoamines. Thus, 2 min after rat sacrifice the brain monoamines were protected and the solution became stabilized. The mixture was then sonicated at 100 W for about 12 s while on ice, and the homogenate was centrifuged for 15 min at $15\,000 \times g$. The supernatant and the pellet were kept in separate tubes. All the biochemical studies were made in the following 2 months after brain dissection and to avoid deterioration of the biological sample, it remained cooled ($-70$°C) until the biochemical determinations were begun. Using this procedure the concentration of the studied compounds is not significantly modified prior to the biochemical determinations [3].

Serum LH and prolactin radioimmunoassays were performed using the materials provided by the NIAMDD and the results were expressed in terms of the respective RP-3 preparation according to previously validated procedures [18].

Brain serotonin (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA) levels were measured by liquid chromatography with electrochemical detection according to previously validated procedures [2,3,22]. An aliquot of the supernatant was injected into the chromatographic column ($300 \times 3.9$ mm stainless steel column packed with Nova-Pack c18, 4 $\mu$m particle size (Waters, Milford, MA, USA). The mobile phase consisted of 0.1 M $NaH_2PO_4 \cdot H_2O$, 0.5 mM EDTA, 1 g/l sodium heptanesulphonate (PIC B7), 6% acetonitrile. The final solution (pH = 4.35) was filtered (0.45 $\mu$m Millipore filter) before use. Standards of serotonin and 5-HIAA (Sigma, St. Louis, MO) were dissolved in perchloric acid (PCA) containing $4 \times 10^{-5}$ M sodium metabisulphite and kept as stock solutions at $-20$°C. They were diluted with ice-cold PCA/$Na_2O_5S_2$ shortly before chromatographic injection. All separations were performed isocratically at a flow-rate of 1.0 ml/min at room temperature. The electrochemical detector used was a Waters Model 460. The detector potential was 0.82 V. Quantifications were performed from standard curves of peak height.

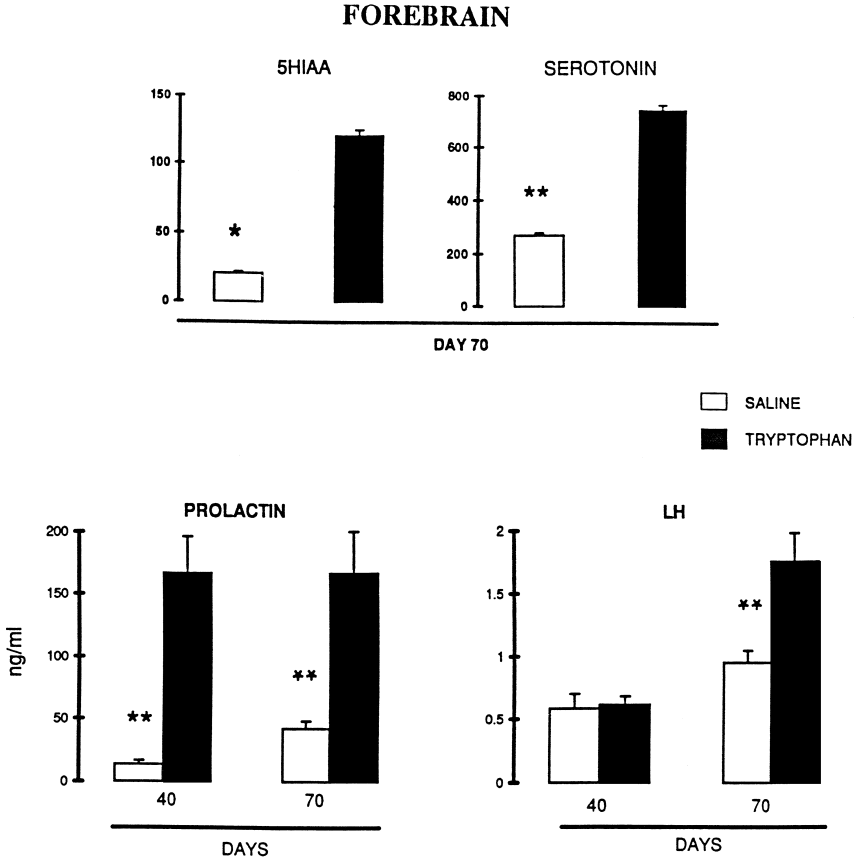


Fig. 1. Top: 5-HIAA and serotonin levels in the forebrain and mesencephalon of 70-day-old rats. Bottom: serum prolactin and LH levels of 40- and 70-day-old rats. The bars represent mean values ± S.E.M. $^{*}P < 0.05$; $^{**}P < 0.001$.

The statistical analyses were performed with the Complete Statistical System (CSS) of StatSoft and using a *t*-test. Differences were judged significant when associated with a probability of 5% or less.

As Fig. 1 shows, oral administration of tryptophan to gestant mothers induced, in the male offspring, a marked increase in serum prolactin (days 40 and 70), serum LH (day 70) and forebrain levels of both serotonin and 5-HIAA. No statistical differences were found for serotonin and 5-HIAA, in the forebrain (5-HT $t_{18} = 1.0$, 5-HIAA $t_{18} = 0.87$) and mesencephalon (5-HT $t_{18} = 0.11$, 5-HIAA $t_{18} = -0.15$) of 40-day-old groups and in the mesencephalon ($t_{18} = -1.41$, n.s., serotonin for tryptophan vs. vehicle groups; $t_{18} = -1.08$, n.s., 5-HIAA for tryptophan vs. vehicle groups) of 70-day-old groups.

We have previously reported that tryptophan administered to pregnant rats crosses the placental barrier and induces a dose-related increase in tryptophan concentration in the placenta, body and brain of the fetus [2]. Because tryptophan hydroxylase activity is not saturated by its substrate, this increase in tryptophan concentration produces a parallel increase in 5-HT and 5-HIAA levels in the fetal brain [2]. The effect begins 1 h after tryptophan intake and persists for 24 h [2]. The offspring of tryptophan-treated mothers showed 70 days after birth a synthesis (5-HT levels) and metabolization (5-HIAA levels) rise for 5-HT. These data indicate that, in addition to the transitory modification of 5-HT metabolism in the fetal brain, maternal intake of tryptophan during gestation facilitates serotonergic neurotransmission in offspring during adulthood. We found no effects in prepuber rats. Serotonergic neurons differentiate during early ontogenesis and their fibers reach the hypothalamus in the last days of prenatal life [2,8,24,27]. However, the final termination density and the formation of precise termination patterns are not reached until adulthood [10]. Mechanisms involved in the regulation of this delayed synaptogenesis [9] may be at the basis of the prepuber-postpuber differences observed here for the effect of mother intake of tryptophan on serotonergic system development of the offspring.

During adulthood, serotonin is involved in the neural regulation of prolactin release [4,11–13,15,23,25]. The peripheral administration of 5-hydroxytryptophan [12], the intravenous administration of 5-HT [20] or the administration of serotonergic agonists [12] facilitates prolactin release to plasma. The drug blockade of serotonergic neurotransmission decreases the serum prolactin level [7]. Thus, 5-HT facilitates prolactin release from the anterior pituitary gland. Fig. 1 shows a serum prolactin level increase in the offspring of tryptophan mothers. These data suggest that the prenatal administration of tryptophan induces not only a long-lasting activation of serotonergic neurotransmission but also a persistent promotion of the facilitatory activity of 5-HT on prolactin release.

Serotonergic transmission is also involved in the neural regulation of the hypothalamic-pituitary-gonadal system [18,20,23,26]. The intraventricular injection of high concentrations of 5-HT evokes LH release in male rats [11]. The intraventricular injection of the serotonergic neurotoxin 5,7-dihydroxytryptamine decreases LH release [26]. Thus, and despite serotonergic inervation of preoptic area [18] and hypothalamus [23] probably having a different involvement in the regulation of LH release, these previous data show that an overall facilitation of brain serotonergic neurotransmission during adulthood promotes LH release [14]. Fig. 1 shows that, corresponding to the 5-HT increase, the prenatal administration of tryptophan induces a serum LH concentration increase that can be detected 70 days after birth. Taken together, present data show that prenatal administration of tryptophan induces a long-lasting facilitation of central serotonergic neurotransmission, disrupting the serotonergic regulation of pituitary hormone release.

In conclusion, the present data suggest that excessive ingestion of amino acids during gestation could have important consequences on the brain development of the offspring. In humans, it has been reported that transient tyrosinemia during neonatal life can induce permanent intellectual impairment 8–10 years later [16,17]. Further studies are warranted to evaluate the functional consequences of the neonatal increase in serum tryptophan on human development. According to the hypothesis previously proposed for tyrosine and the cathecolaminergic systems [1,5,19,21,22], present data support the idea that serotonin may have a neurotrophic role prior to the evolutionary onset of neurotransmission.

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