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The presence of histamine and *tele*-methylhistamine in the pineal gland of chick

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Abstract. The levels of histamine (HA) and *tele*-methylhistamine (*t*-MeHA) in chick pineal gland were measured by RIA at two time points, i.e. at the end of the light (L) phase and in the middle of the dark (D) phase of 12 h: 12 h L:D cycle. The chick pineal gland showed high HA levels (12.19 and 13.09 ng/pineal at L and D, respectively). The *t*-MeHA content of chick pineal gland was about 20-times lower than HA level (650.2 and 536.4 pg/pineal at L and D, respectively). An aminoacid precursor of HA, L-histidine (1 g/kg, ip), given to chicks during L or D significantly increased both HA and *t*-MeHA content of the pineal gland. The L-histidine-evoked elevations in HA level were 2-4-times higher than changes in *t*-MeHA content. Enzymatic study showed the presence in chick pineal of a moderate activity of L-histidine decarboxylase (HDC), and well expressed activity of HA-methyltransferase (HMT), HA synthesizing and inactivating enzyme, respectively, which suggests that both HA and *t*-MeHA may be produced within the gland. It is suggested that the metabolic dynamics of the pineal HA may be higher during lighthours than darkhours of the daily light:dark illumination cycle.

Short communication

Key words: histamine, *tele*-methylhistamine, pineal gland, chick

Histamine (HA) is an established neurotransmitter/neuromodulator in the central nervous system (CNS) of mammals (Schwartz et al. 1991) and some non-mammalian species (Nowak 1993, 1994). One of the widely reported actions of HA is stimulation of cAMP production, the effect observed, among other tissues, in brains of guinea pig, rabbit and chick (Nahorski et al. 1977, Palacios et al. 1978, Al--Gadi and Hill 1985, Nowak and Sęk 1991, Zawilska and Nowak 1995). Recently, we have studied in parallel the effects of HA on cAMP synthesis in cerebral cortex, retina and pineal gland of chick, and found the latter tissue to be highly sensitive to the amine (Nowak 1993, Nowak and Sęk 1994, Nowak et al. 1995, Nowak and Zawilska 1995). This unexpected observation seemed interesting for at least two reasons: (1) the HA-evoked cAMP response in the pineal gland was much stronger than that in other tested tissues (including mammalian ones), and (2) the regulatory mechanisms underlying the activity of the cAMP generating system of the chick pineal are still poorely recognized, although it is widely accepted that the nucleotide plays a crucial role in the induction process of serotonin *N*-acetyltransferase, the rate-limiting enzyme in melatonin biosynthesis (Takahashi et al. 1989). Taking the above into consideration, any substance capable of modulating cAMP accumulation/level in the vertebrate pineal gland may be regarded as a potential regulator of the pineal activity, including melatonin formation.

This work is a continuation of our study of a possible role of HA in the pineal physiology. In light of a powerful effect of HA on cAMP accumulation in avian pineal a question has arisen as to the origin and localization of the amine. Therefore, we were interested whether the chick pineal gland (1) contains HA, and its main - at least in mammals - metabolite *tele*-methylhistamine (*t*-MeHA), and (2) has the potential to synthesize HA locally. In addition we have asked whether levels of HA and *t*-MeHA (if present) fluctuate according to environmental lighting conditions. The latter question refers to the rhythmic throughout the day production of the pineal hormone melatonin, with high and low values occurring during the dark and light phase of a daily light:dark illumination cycle, respectively (Takahashi et al. 1989).

Experiments were performed on 2-3-weeks old male white leghorn chicks (*Gallus domesticus*) weighing 100-125 g. The animals were obtained on the day of hatching, and were kept in warmed brooders with *ad libitum* standard food and water. Chicks were maintained under a photoperiodic schedule of 12 h light: 12 h dark (L:D; lights on between 23:00 and 11:00) for a minimum of ten days before use. Lighting was provided by fluorescent strip lights yielding 150 lux at the bottom of the brooder. The experiments were carried out in strict accordance with the Polish governmental regulations concerning experiments on animals and rules followed at the Department of Biogenic Amines.

In one set of experiments the animals received intraperitoneal injections (i.p.) of L-histidine (Serva, Heidelberg, Germany) or vehicle (0.9% NaCl) one h prior to lights offset (L) or during the fifth h of the dark phase (D) of the L:D illumination cycle. Chicks were sacrificed by decapitation 1 h later; pineal glands were quickly isolated and frozen on dry ice. Tissues were stored at -70°C untill assayed (maximally for 3 days). All injections and tissue dissections during the dark phase were performed under dim red light.

HA and *t*-MeHA contents of the pineal gland were measured in separate materials, each material being pooled from 4 glands per group in one experiment (3 experiments were in total) by commercially available sensitive and specific [^{125}I]RIA kits: HA (Immunotech Int., S.A.; Marseille, France); *t*-MeHA (Pharmacia Diagnostics AB; Uppsala, Sweden). The detection limit of HA and *t*-MeHA was 0.1 nM of the analyzed substance in the sample.

The activities of the chick pineal L-histidine decarboxylase (HDC) and histamine *N*-methyltransferase (HMT) were measured in two separate tissue homogenates, with the aid of established radioisotopic assays, using as substrates [^{3}H]-L-histidine (50.3 Ci/mmol; DuPont-NEN) and [^{14}C]-S-adenosyl-L-methionine (58 mCi/mmol; DuPont-NEN), respectively, as described in detail previously

(Nowak and Kuliński 1986). The tissue homogenate was prepared from 12-15 pineals of animals killed at the end of the light phase.

Data are expressed as mean ± standard error of mean (SEM), and were analyzed for statistical significance by analysis of variance with Newman-Keul's test (using a computer programme The Primer of Biostatistics; McGraw-Hill Comp., N.Y.). The number given in parenthesis refers to the number of assays. The obtained results, i.e. amine levels or enzyme activities, were recalculated per one pineal gland.

Preliminary enzymatic studies showed the presence in the chick pineal gland of both HDC and HMT activity. The obtained results (in dpm/pineal/60 min) were: for HDC - 268.6 ± 24.9(9), with blank value of 51.3 ± 22.7(6), and for HMT - 1152.1 ± 83.5(10), with blank value of 276.2 ± 40.6(4). This is the first demonstration of HDC activity in this avian tissue, whereas our "HMT" data confirm earlier findings by Mezei (1975).

In agreement with the results by Mezei and Mezei (1978), the chick pineal gland showed relatively high HA levels (L = 12.19 ± 0.40, D = 13.09 ± 0.61 ng/pineal; n = 8/group). The amine content in glands isolated at the end of the L phase of the L-D illumination cycle was not significantly different from HA level found in tissues taken in the middle of the D phase. Systemic administration of L-histidine (1 g/kg, 1 h), the amino acid presursor of HA, markedly increased HA content of the pineal gland, both under L (by 93%) and D (by 48%) conditions (Fig. 1).

The pineal level of *t*-MeHA was comparatively low and accounted for about 5% of HA content (L - 650.2 ± 27.1*, D - 536.4 ± 20.3 pg/pineal; n = 6/group, *P< 0.05 vs. D). *t*-MeHA level found at the end of the L phase was significantly higher (by 21%; P<0.05) than that measured in the middle of the D phase. L-Histidine (1 g/kg), given to chicks during the L or D phase, produced similar increases

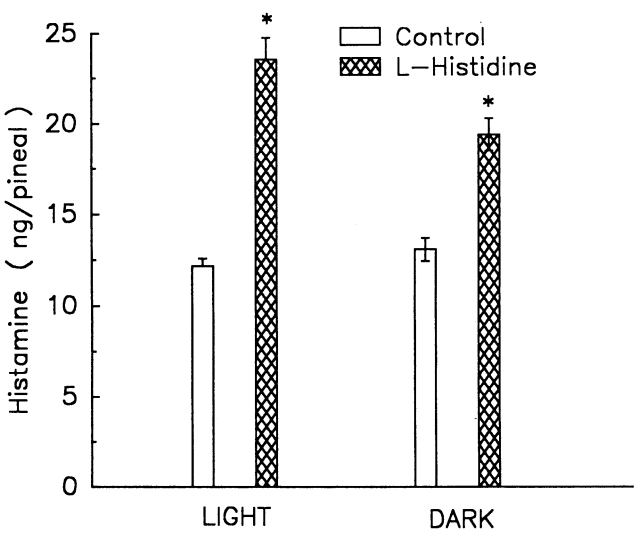

Fig. 1. Histamine content in the pineal gland of control and L-histidine treated chicks. The animals received i.p. injections of L-histidine (1 g/kg) or vehicle one h prior to lights offset (Light) or during the fifth h of the dark phase (Dark) of the L:D illumination cycle. Chicks were decapitated one h later; pineal glands were isolated, pooled, and then used for determination of histamine content. Data are expressed in ng/pineal. Each column represents mean ± SEM of 8-10 determinations collected from 3 independent experiments. *P <0.05 vs. Control.

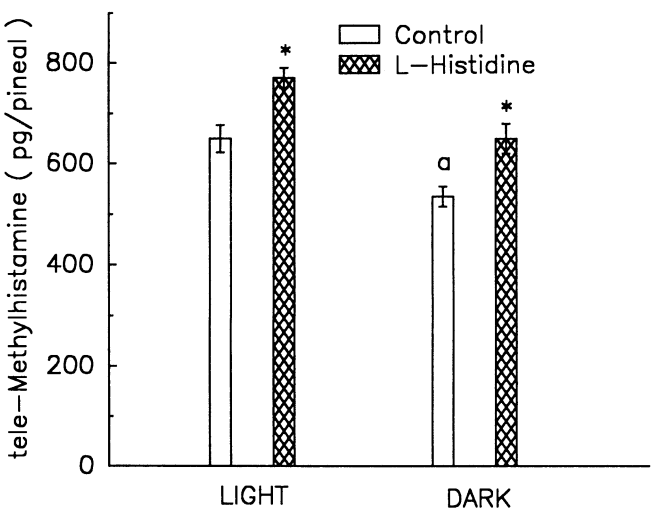

Fig. 2. *tele*-Methylhistamine content in the pineal gland of control and L-histidine treated chicks. The animals received i.p. injections of L-histidine (1 g/kg) or vehicle one h prior to light offset (Light) or during the fifth h of the dark phase (Dark) of the L:D illumination cycle. Chicks were decapitated one h later; pineal glands were isolated, pooled, and then used for determination of *tele*-methylhistamine content. Data are expressed in pg/pineal. Each column represents mean ± SEM of 5-6 determinations. *P <0.05 vs. Control, [a]P <0.05 vs. Light.

(by 19-21%) in *t*-MeHA content of the pineal gland (Fig. 2).

Thus, peripheral administration of L-histidine to chicks led to marked increases in the pineal levels of HA, and, to a lesser extent, *t*-MeHA, indicating that the aminoacid rapidly undergoes decarboxylation to HA, with its subsequent ring-methylation to *t*-MeHA. This resembles the situation occurring in mammalian brain (Schwartz et al. 1991, Nowak 1993). Since the chick pineal contains both HDC and HMT activity it seems very likely that in untreated and L-histidine-treated animals such a two-step metabolic process: L-histidine → HA → *t*-MeHA, catalized by the two mentioned enzymes, takes place physiologically in the avian pineal gland.

Similar to mammalian CNS (Schwartz et al. 1991), *t*-MeHA did not stimulate cAMP production in the chick pineal gland (Nowak and Sęk 1994) and cerebral cortex (Zawilska and Nowak 1995), the finding being consistent with an idea that biologically inactive *t*-MeHA is a physiological metabolite of HA not only in mammals, but also in avian species. However, at present we cannot ultimately conclude that imidazole ring-methylation is the only pathway of HA inactivation in the chick pineal. In our earlier (Nowak 1993) and recent experiments (Sęk and Nowak, unpublished data) we were unable to detect in the chick CNS any measurable activity of diamine oxidase (DAO), another HA-catabolizing enzyme operating in some peripheral tissues in mammals (Shaff and Beaven 1976). Yet, it has been demonstrated that HA may be degraded, to some extent - depending on the species, by *N*α-acetylation (mammals, arthropods), or γ-glutamylation (rat brain, snail nervous system)(see Nowak 1993), and the problem of the existence in the chick pineal of an alternative route for HA inactivation remains an open question. On the other hand, comparatively (vs. HA) low levels of *t*-MeHA might result from the fact that this substance is a physiological substrate for monoamine oxidase type B (MAO-B; Waldmeier et al. 1977), an enzyme occurring ubiquitously in high levels in animal tissues (Yu 1986).

The avian pineal gland is directly photosensitive, and its main biological activity - the rhythmic throughout the day cAMP-dependent production of melatonin - is highly intensified at night in darkness (Takahashi et al. 1989, Zatz 1989). If HA is somehow involved in the regulation of the hormone formation (*via* its action on cAMP system), one could anticipate a daily rhythm of the amine metabolic activity in the pineal gland. The obtained findings (Figs. 1 and 2) seem to suggest the existence of such a dependence on environmental lighting. Although the control levels of HA in "light" and "darkness" did not differ, those of *t*-MeHA (121% vs. dark value), as well as the amine levels in L-histidine-injected chicks (193% vs. 148% of control value) did, being significantly ($P<0.05$) higher in light-exposed than in dark-adapted animals. These data may suggest that the production of HA in the chick pineal is more intensive during light/day-hours than dark/night-hours. Obviously, more study is needed to solve this issue since, at present, it is unknown whether such differences in HA formation are dependent merely on lighting conditions, or on the time of day, or both.

In conclusion, based on the present results, and our earlier findings (Nowak and Sęk 1994, Nowak et al. 1995), it can be stated that the chick pineal gland contains the whole functional HA-ergic system, consisting of the amine synthesis, inactivation, and receptors, whose stimulation leads to both an increased production of the pineal cAMP and activation of cationic channels in acutely isolated and cultured pineal cells (Nowak and Sęk 1994, Nowak et al. 1995). Although the type of the pineal cells capable of synthesizing and storing HA, as well cells responsive to HA, remain to be established, the data give an additional support to the idea that HA may be one of physiological regulators of the pineal activity in at lease some vertebrates.

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