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Kinetic and pharmacological properties of [^{3}H]-histamine transport into cultured type 1 astrocytes from neonatal rats

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Abstract. *Objective and design:* Astrocytes actively participate in the inactivation of neurotransmitters. In this work we elucidated the contribution of astrocytes in clearance of histamine, a process which has not yet been fully clarified.
Methods: The characteristics of [^{3}H]-histamine uptake were determined in cultured neonatal rat type 1 astrocytes and histamine-N-methyl-transferase expression was determined using RT-PCR.
Results: These cells transport [^{3}H]-histamine in a time- and concentration-dependent manner. The histamine clearance by astrocytes was described by a mathematical model including two processes: electrodiffusion and active transport. A further analysis of kinetic parameters of a carrier-operated transport revealed a single transport system with Michaelis constant (K_m) of 3.5 ± 0.8 μM and a maximal uptake rate (V_{max}) of 7.9 ± 0.3 pmol/mg protein/min. From drugs tested amitriptyline, desipramine, mepyramine and cimetidine significantly decreased [^{3}H]-histamine uptake. Taken-up histamine could be metabolically degraded in cultured astrocytes, since they express mRNA for enzyme histamine-N-methyltransferase.
Conclusions: Astrocytes participate in the clearance of extracellular histamine by electrodiffusion and active transport by a yet not identified carrier. Taken up histamine can be converted to tele-methylhistamine within astrocytes thus indicating the involvement of astrocytes not only in clearance but also in the inactivation of histamine.

Key words: Histamine – Histamine uptake – Histamine electrodiffusion – Astrocyte – Rat

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Introduction

The role of histamine as a neurotransmitter in the central nervous system was first recognized in the 1950s as a result of prominent sedation caused by the first generation of histamine H1 receptor antagonists. Since 1984, we definitely know that brain histamine is localized mainly in neurons of the posterior hypothalamus whose projections innervate most brain regions [1, 2]. Further studies have demonstrated the existence of four distinct histamine receptor subtypes H1, H2, H3, and H4, all members of the G protein coupled receptor family. The receptor subtypes H1, H2 and H3, like histamine itself, are found throughout the brain [3], whereas H4 expression is very low in the rodent central nervous system (CNS) [4, 5].

Synthesis of histamine in histaminergic neurons occurs through the action of histidine decarboxylase on histidine. Later on, synthesised histamine is taken up into synaptic vesicles by the vesicular monoamine transporter 2 (VMAT2) [6] and released into the synaptic cleft upon depolarization stimuli. The released histamine is metabolised by the enzyme histamine N-methyltransferase (HNMT) producing tele-methylhistamine [7, 8], that is in turn degraded by monoamino oxidase B (MAO B) [8, 9] and aldehyde dehydrogenase which are all intracellular enzymes. In order to be enzymatically degraded or recycled, histamine must be transported either into the presynaptic neuron or into surrounding glial cells. There is very little evidence to suggest neuronal or non-neuronal uptake as part of the mechanism of clearance for synaptic histamine.

Reports from Huszti's laboratory described histamine uptake by cultured glial cells prepared from embryonic chicks [10] and neonatal rats [11–13], which was Na^+-dependent [14] and bidirectional [10, 12]. In order to demonstrate histamine uptake into cultured glial cells, astrocytes from neonatal (P0–P6) rats required maintenance in culture for at least 19 days [11, 14]. Glial cells were also found to be involved in the continuous removal of neuronal histamine from the synaptic cleft in *in vivo* conditions [15].

Stuart and co-workers [16, 17] described a Na^+-dependent histamine uptake into arthropod photoreceptors, which use histamine as their neurotransmitter, as well as into surrounding glia. Interestingly, preferential uptake of histamine into the arthropod photoreceptor terminals occurred under constant light exposure, when the cells were depolarized, while in the dark histamine was taken up by glial cells. Busch and co-workers [18] described the expression of message and protein for human polyspecific organic cation transporter (hOCT2) in neurons of the human cerebral cortex and in various subcortical nuclei. Gründemann and co-workers [19] described the existence of an extraneuronal monoamine transporter (EMT) also referred as OCT3, which is thought to comprise the uptake-2 system involved in the clearance of different catecholamine neurotransmitters. The message for OCT3/EMT is located in human astrocytes, [20] human myocardium, vascular, and glandular tissue [21]. This transport protein can transport histamine along with other monoamines [21]. The K_ms for histamine uptake by different OCTs are all in the 10–900 μM range [22] and the authors suggested that the transporter may function on monoamines in the interstitial cleft that have escaped their high affinity uptake systems. Recently, Yanai's group [23] reported a high and low affinity, Na^+-, Cl^-, and HCO_3^- -dependent [^{3}H]histamine transport into rat brain synaptosomes and messages for organic cationic transporters OCT1, OCT2 and OCT3 were expressed in the cultured neonatal Sprague-Dawley rat cortical astrocytes [24].

In the previous work [25] we found that [^{3}H]histamine is taken up by cultured type 1 astrocytes from neonatal and adult rats, and that the transport of histamine is Na^+- but not Cl^--dependent. To obtain Na^+-dependence and ouabain sensitivity of [^{3}H]-histamine uptake into neonatal rat cultured astrocytes, the cells had to be cultured for three weeks.

In the present study we examined the kinetic properties of [^{3}H]-histamine uptake into cultured neonatal rat type 1 astrocytes. In order to better understand the processes involved in the clearance of [^{3}H]-histamine we constructed a mathematical model of the histamine uptake into astrocytes. In particular, we were interested in the nature of uptake at different temperatures, since we expected a suppression of the active transport across the membrane at low temperature. In the model, two compartments representing the intracellular and extracellular space were separated by the membrane across which [^{3}H]-histamine is transported. By comparing the predicted uptake with the experimentally determined one and including relevant contributions in the model, it was possible to indicate which processes are important in the transmembrane transport of [^{3}H]-histamine at different temperatures. Later on, we checked the influence of some antidepressant drugs and histamine receptor antagonists on the uptake of [^{3}H]-histamine, and the expression of histamine degrading enzyme HNMT in cultured astrocytes.

Materials and methods

Materials

All tissue culture reagents, except fetal bovine serum (Cambrex, Belgium) were obtained from GIBCO, Great Britain. [^{3}H]-histamine (669.7 GBq/mmol) was purchased form Perkin Elmer, USA. The RNA isolation kit was bought from Promega, USA, GeneAmp® Thermostable rTth Reverse Transcriptase RNA PCR Kit and primers from Applied Biosystems, USA. Test compounds were obtained from Sigma USA (mepyramine maleate, clomipramine-HCl, nortriptyline-HCl, citalopram hydrobromide, fluoxetine-HCl, fluvoxamine maleate, and famotidine), Sandoz Switzerland (desipramine hydrochloride, amitriptyline HCl), Pliva Croatia (maprotyline).

Astrocyte Culture

Rats were used in accordance with the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals and Permission for Use of Laboratory Animals 323-02-232/2005/2 issued by Veterinary Administration of Republic of Slovenia. Astrocyte cultures were prepared from the cortex of neonatal Wistar rats (3 days old, both sexes) and cultured as previously described [26]. Cells were grown in high-glucose Dulbecco's modified Eagle's medium (DMEM), containing 10 % fetal bovine serum (FBS), 1 mM pyruvate, 2 mM glutamine, and 25 μg/ml gentamycin in 95 % air-5 % CO_2. Confluent cultures were shaken at 225 rpm overnight, and the medium was changed the next morning; this was repeated a total of three times. After the third overnight shaking, the cells were trypsinized and cultured for 24 h in 10 μM cytosine arabinoside. After reaching confluence again the cells were subcultured into 12-well clusters and grown for additional 3 weeks.

Uptake of [^{3}H]-histamine into cultured astrocytes

Monolayer cultures in 12-well clusters were preincubated for 30 min in uptake buffer (25 mM HEPES, 125 mM NaCl, 4.8 mM KCl, 1.2 mM KH_2PO_4, 1.2 mM $MgSO_4$, 1.4 mM $CaCl_2$, and 5.6 mM glucose, pH 7.4) at 37 °C (total uptake) and at 4 °C (non-specific uptake). Test compounds (0.06 mL) were added and incubated for 20 minutes at 37 °C, only. Histamine transport was initiated by the addition of [^{3}H]-histamine at a final concentration of 0.125 μM, except for the dose-response curves when the concentration range from 1 nM to 100 μM was used. After 20 min, unless the time course was examined, the reaction was stopped by placing the dishes into an ice-water bath. The buffer was quickly removed and the dishes were washed twice with ice-cold uptake buffer. The cells were lysed in 0.6 mL of 0.5 M NaOH. 0.5 mL of each sample was transferred to a scintillation vial and the radioactivity was measured. Protein content was determined in the remaining aliquots (0.1 ml) using Bio-Rad method.

Reverse transcriptase-polymerase chain reaction (RT-PCR)

The total RNA isolation from tissue samples was performed with RNAgents® Total RNA Isolation System or from astrocyte cultures with SV Total RNA Isolation System according to the manufacturer's protocol. The quantity and purity of isolated total RNA was estimated by measuring the absorbance at 260 and 280 nm with the Perkin-Elmer MBA 2000 spectrophotometer.

Equal amounts (250 ng per reaction) of total RNA were used for RT-PCR which was performed with GeneAmp® Thermostable rTth Reverse Transcriptase RNA PCR Kit. Reverse transcription was carried out for 15 min at 70 °C, followed by PCR: 1 min incubation at 95 °C, 35 cycles of 95 °C for 10 sec and 60 ° C for 15 sec, and 7 min at 60 °C. Cyclophilin gene was used as a positive control and samples without a reverse transcription step as a negative control. Products were verified by electrophoresis on 2 % agarose gel containing ethidium bromide. The primers used were as follows: HNMT (sense) 5'-TGCTTGGCATAAGGAGACA-3', product 153 bp, HNMT (antisense) 5'-GCTAAGAGACCGTGGAAGAAT-3' and cyclophylin (sense) 5'-TGGACCAAACACAAATGGTT-'3, product 161 bp, cyclophilin (antisense) 5'- TGACTTCTTGCTGGTCTT-3'.

Data analysis

The uptake experiments were routinely carried out in triplicates and each experiment was repeated twice. The results are expressed as means ± SEM. The kinetic parameters (K_m and V_{max}) and IC_{50} values were calculated by a non-linear regression method using software Prism4 version 4.00 (GraphPad Software Inc., San Diego, USA). Comparison of data among groups was carried out using either ANOVA and Bonferroni's post test or Student`s paired t-test, if only two groups of data were compared. Differences were considered significant at $p<0.05$. Correlation was tested by calculating Pearson's coefficient.

Results

Time course

Astrocytes were prepared from cortices of neonatal rats (postnatal day 3) as described [26]. At that point, the cultures contained 93–100 % type 1 astrocytes, determined by immunochemical staining for glial fibrillary acidic protein [25].

Because the method of preparation, culture conditions and kind of test animal used can affect the molecular properties of cultured astrocytes, we first examined the time course and temperature-dependence of [^{3}H]-histamine (125 nM) transport into type 1 cultured astrocytes. Figure 1 shows the uptake of [^{3}H]-histamine in fmol/mg of protein in time dependence at two different temperatures. It can be seen that uptake of [^{3}H]-histamine occurred also at 4 °C. At both temperatures used [^{3}H]-histamine uptake initially increased and then gradually reached a plateau. However, the height of the plateau and the rate of reaching it were temperature-dependent processes. The mean uptake of 125 nM [^{3}H]-histamine at 20 minutes at 37 °C was 670 ± 85 fmol/mg of protein. The amount of taken up [^{3}H]-histamine measured at 4 °C was significantly lower than that measured at 37 °C from 5 minutes of incubation period on ($p<0.0002$) (Figure 1), but not completely inhibited. However, it can be seen from Figure 1 for both temperatures that the uptake was saturated after 20 minutes of incubation.

Based on these experiments and previously published data [25] further experiments were performed using an uptake incubation period of 20 minutes.

Description of [^{3}H]-histamine transport by astrocytes

It is considered that histamine can in general enter cells by electrodiffusion through the membrane and by active transport. At higher temperature T_1 = 310 °K (37 °C) both processes of transport across the membrane take place. Within this assumption, we derived the time dependence (t) of the ratio between the number of histamine molecules inside the cells and the number of histamine molecules outside the cells (N_{in}/N_{out}) (Appendix, equations (A5) and (A6)),

$$\frac{N_{in}}{N_{out}} = \frac{V_{in}}{V_{out}}\frac{1}{\alpha_1}\left(1+\frac{b}{a_1}\frac{\theta}{1+\theta}\right)\left(1-\exp\left(-a_1 t\right)\right). \quad (1)$$

At lower temperature T_2 = 277 °K (4 °C) we assumed that the active transport does not occur, so that (N_{in}/N_{out}) increases with time according to (Appendix, equations (A16) and (A17)),

$$\frac{N_{in}}{N_{out}} = \frac{V_{in}}{V_{out}}\frac{1}{\alpha_2}\left(1-\exp\left(-a_2 t\right)\right). \quad (2)$$

Here, V_{in} and V_{out} are the volume of the inner and of the outer solution, respectively, while b, a_1, a_2, α_1, α_2 and θ are model constants and parameters (please see the detailed description of the derivation and the quantities in Appendix). The quantities V_{in}/V_{out}, b, a_1 and a_2 were determined by fitting the experimental data.

Figure 2 shows the ratio between the numbers of [^{3}H]-histamine molecules within astrocytes and in the outer solu-

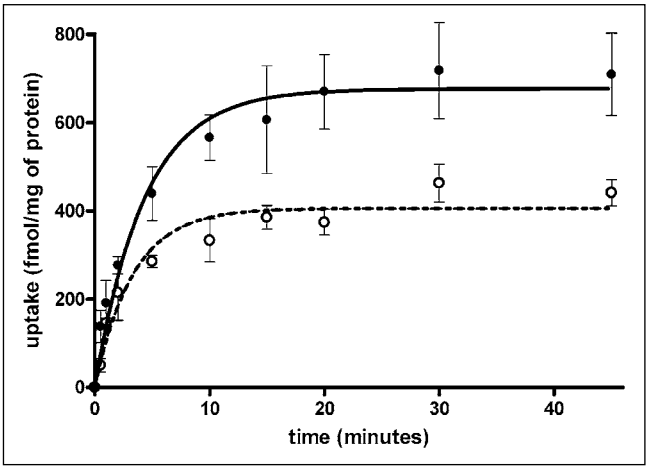

Fig. 1. Time course of [^{3}H]-histamine uptake into cultured neonatal rat astrocytes. Monolayer cell cultures were incubated at 37 °C (•) and 4 °C (○) in uptake buffer for the indicated times. Each point represents the mean and SEM of six determinations. The uptake of [^{3}H]-histamine at 4 °C was significantly lower ($p<0.0002$) than the uptake measured at 37 °C.

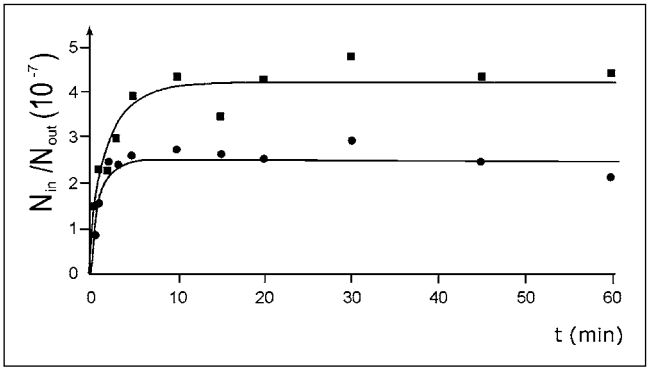

Fig. 2. The time course of the ratio between the numbers of [^{3}H]-histamine molecules within the neonatal rat cultured astrocytes (N_{in}) and in the outer solution (N_{out}) for two temperatures. Monolayer cell cultures were incubated at 37 °C (■) and 4 °C (•) in uptake buffer for the indicated times. Each point represents the mean of six determinations. The lines are the result of the best fit mathematical model for the two corresponding temperatures (Eqs.(1) and (2), respectively).

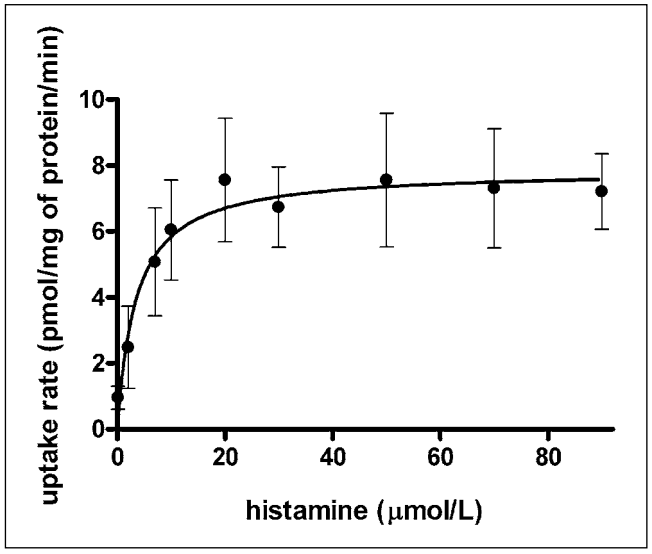

Fig. 3. Concentration-dependent specific [^{3}H]-histamine uptake rate into neonatal rat astrocytes. The cultured astrocytes were incubated for 20 min at concentrations from 0.125 to 90 μmol/L at 37 °C and 4 °C. Specific uptake of [^{3}H]-histamine was calculated as the difference between the total ([^{3}H]-histamine uptake at 37 °C) and non-specific (uptake of [^{3}H]-histamine at 4 °C). Specific [^{3}H]-histamine uptake was saturable with K_m of 3.5 ± 0.8 μM and V_{max} of 7.9 ± 0.3 pmol/mg protein/min determined by the best fit to $V = V_{max}\ x\ X\ /\ (K_m + X)$, where V is the rate of uptake, V_{max} – maximal rate of uptake, X – concentration of [^{3}H]-histamine and K_m – Michaelis constant. Each point represents the mean ± SEM of six determinations.

tion (N_{in}/N_{out}) in time dependence (t) for both temperatures (277 °K and 310 °K). The dependences shown in Figures 1 and 2 differ as the intracellular content of [^{3}H]-histamine is expressed in fmol per mg of protein (measured at given times) in Figure 1. It can be seen from Figure 2 that the proposed model agrees well with the experimental data. We obtained the optimal parameters a_1=0.51/min, a_2=1.01/min, V_{out}/V_{in} = 155192 and b = 27018/min. At lower temperature the transport was faster than at higher temperature, which was attributed to a more effective net movement of histamine through the membrane at T = 277 °K (larger effective diffusion coefficient – D_{eff} (Eq. (2)). The height of the plateau was determined by the effect of the active transport. Since there was assumingly no active transport present at 277 °K, the plateau (which was linearly dependent on parameter b (Eq.A10)) yielded a significantly higher saturation at 310 °K than at 277 °K.

Kinetics of specific [^{3}H]-histamine uptake

The kinetic characteristics of [^{3}H]-histamine transport into cultured rat astrocytes were determined (Figure 3). Astrocyte cultures were incubated for 20 minutes with [^{3}H]-histamine in a concentration range from 0.125 to 90 μM at 37 °C and 4 °C. In order to measure specific histamine uptake, the concentrations of [^{3}H]-histamine within astrocytes at 4 °C were subtracted from the concentrations determined at 37 °C. Kinetic analysis of specific [^{3}H]-histamine uptake into cultured neonatal type 1 astrocytes, as calculated by non-linear regression analysis, yielded Michaelis constant (K_m) of 3.5 ± 0.8 μM and a maximal rate (V_{max}) of 7.9 ± 0.3 pmol/mg protein/min for a single transport system.

Effects of different antidepressant drugs and some histamine receptor antagonists on [^{3}H]-histamine uptake

We investigated the effects of various antidepressant drugs and some histamine receptor ligands on [^{3}H]-histamine transport into cultured neonatal rat cultured astrocytes. IC_{50} values for the inhibition of [^{3}H]-histamine uptake were calculated from the inhibition curves presented in Figure 4.

From available antidepressant drugs we tested two non-selective reuptake inhibitors (amitriptyline and clomipramine), three selective serotonin reuptake inhibitors (citalopram, fluoxetine and fluvoxamine) and three selective noradrenaline reuptake inhibitors (desipramine, maprotyline and nortriptyline). [^{3}H]-histamine uptake into neonatal rat astrocytes was significantly inhibited only by amitriptyline (Figure 4A) and desipramine (Figure 4C). 10 μM amitriptyline and desipramine inhibited more than 50 % of the total [^{3}H]-histamine transport into cultured astrocytes. Some inhibition was achieved by fluoxetine, citalopram (Figure 4B) and nortriptyline (Figure 4C) in the μM concentration range, whereas clomipramine (Figure 4A), fluvoxamine (Figure 4B) and maprotyline (Figure 4C) did not at all affect [^{3}H]-histamine uptake into neonatal rat astrocytes.

We also observed the effect of different histamine receptor ligands (mepyramine, cimetidine and famotidine) on the uptake of [^{3}H]-histamine into neonatal rat cultured astrocytes. [^{3}H]-histamine transport was significantly (up to 50 % of the total uptake) inhibited by 10 μM mepyramine (inverse agonists of histamine H1 receptor subtype) and cimetidine (H2 receptor subtype antagonists), however, H2 receptor antagonist famotidine only slightly altered [^{3}H]-histamine transport into cultured astrocytes (Figure 4D).

Inhibitory potencies (IC_{50} values) were in the rank order: cimetidine (0.25 μM) > amitriptyline (0.32 μM) > desipramine (1.74 μM) > mepyramine (3.3 μM) and they did not correlate with the pK_a values of the compounds (r = -0.56).

HNMT expression

Further on, we were interested in what happened with histamine inside the astrocytes. Since histamine from CNS is metabolized almost exclusively by HNMT, we investigated the expression of HNMT in cerebral cortex tissue and astrocyte culture. As it is seen from Figure 5, RT-PCR results demonstrated mRNA for HNMT in brain cortical tissue and in astrocyte cultures, which is in agreement with results obtained by Garbarg and co-workers [27].

Discussion

The concentration of histamine in foetal rat brain is high, reaching a peak at 17 days of gestation. Histamine then sharply declines but subsequently increases again and reach-

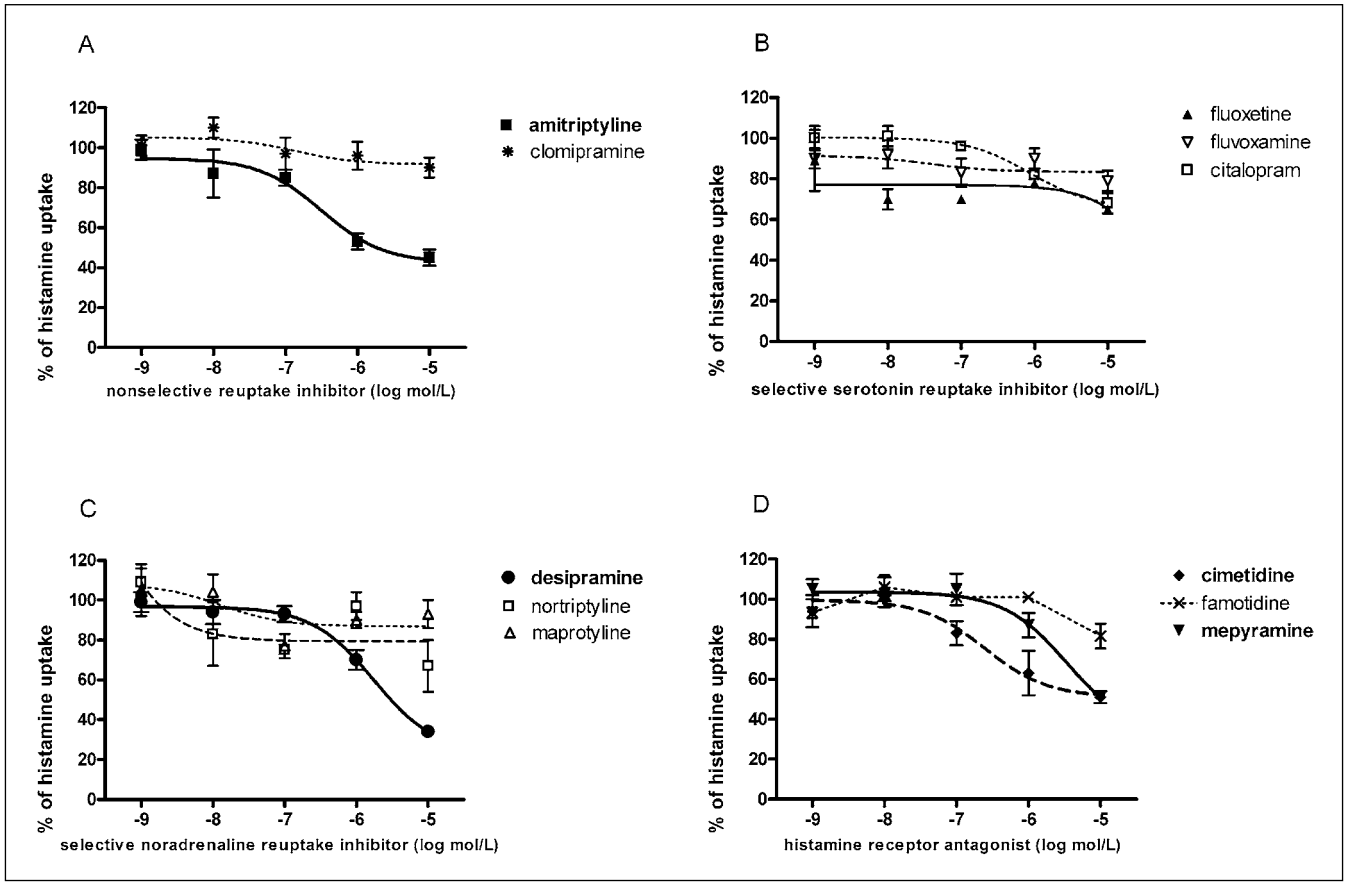

Fig. 4. Effects of various compounds on [^{3}H]-histamine uptake into neonatal rat cortical astrocytes. Astrocytes were preincubated with various compounds: tricyclic non-selective antidepressants **(A)**, selective serotonin reuptake inhibitors **(B)**, selective inhibitors of noradrenaline uptake **(C)** and histamine receptor antagonists and inverse agonist **(D)** for 20 min. The uptake of 125 nmol/L [^{3}H]-histamine was determined after 20 min at 37 °C. Results are given as a percentage of the total uptake measurement 670 ±85 fmol/mg proteins. Each point represents the mean and SEM of six determinations.

es the second peak 5–10 days after birth, and then declines again to the adult levels [28]. The high content both embrionically and early postnatally correlates with the period of rapid cell proliferation and growth of the rat CNS. Interestingly, at these time periods, rat brain histamine had been localized mostly in the nuclear fraction, further supporting its role in growth and maturation [29]. Analysis of subcelullar localization of histamine in the adult rat brain demonstrated its presence in the synaptosomal fractions [29]. These data suggest that histamine functions as a neurotransmitter only in the adult rat brain.

Regardless of the fact that at postnatal day 3 histamine is mainly a modulator of cell growth and not a neurotransmitter, histamine is like other neurotransmitters taken up by astrocytes prepared from 3 days old rats with a half time that is similar (Figure 1) to its physiological elimination time in the blood (0.5–2 min) [30]. We suggest that [^{3}H]-histamine is taken up into neonatal rat astrocytes by both, passive and active transport. In our model, we have assumed for simplicity that at higher temperature (37 °C) both processes contribute considerably to the clearance of histamine, while at low temperature (4 °C) the active transport is negligible. As there is an agreement between the experimental and theoretical data, we assume that transport occurring at 4 °C indeed represents mainly passive electrodiffusion that depends on the transmembrane concentration gradient of [^{3}H]-histamine, whereas the process observed at 37 °C is a result of passive electrodiffusion and a substantial contribution of additional carrier-mediated active transport (Figure 2).

Surprisingly, it was found that [^{3}H]-histamine accumulates within astrocytes at a slightly lower rate at 37 °C than at 4 °C. (Figure 1 and 2). [^{3}H]-histamine transport at 37 °C might be affected by simultaneous binding of [^{3}H]-histamine to histamine receptor binding sites present on astrocyte cell membranes, since it is known that cultured astrocytes possess histamine H1 and H2 receptor subtypes [31, 32]. The amount of histamine electrodiffusion occurring at 4 °C observed in this study was comparable to the amount of histamine transport, observed previously [25], which was ouabain-insensitive and Na^+-independent.

The K_m value of [^{3}H]-histamine uptake into cultured rat type 1 astrocytes was 3.5 μM (Figure 3). The measured K_m value was significantly higher than K_m obtained by Huszti et al. [11] - 0.19 μM in astrocytes and 0.30 μM in rat cerebral endothelial cells [33], but similar to the K_m value of histamine uptake occurring in the P2 fraction of rat synaptosomes

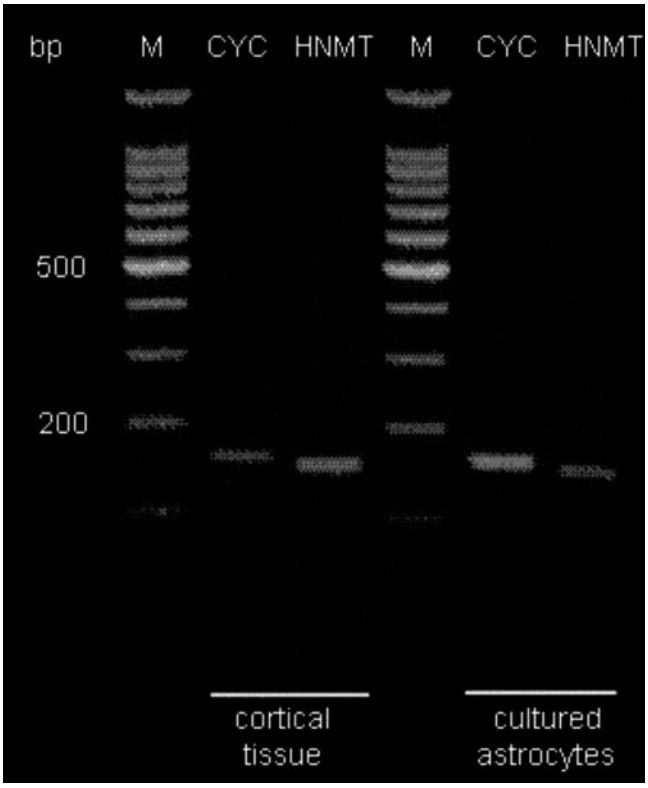

Fig. 5. The expression of histamine N-methyl-transferase in cerebral cortex of neonatal rat tissue and astrocyte cultures from the same brain regions. The figure represents ethidium bromide-stained agarose gel. RNA was prepared as described in Materials and methods. The samples shown were positive for HNMT (153 bp) and housekeeping gene cyclophilin (CYC) (161 bp).

–1.2 μM [23]. Cultured rat astrocytes express also OCT1, OCT2 and OCT3 [18], of which OCT2 and OCT 3 can also transport histamine, but the K_m values for histamine transport by OCTs are significantly different (180–940 μM) [24] than the affinity for histamine uptake obtained in our study, so the observed carrier seems to represent a new entity.

Additional evidence that histamine is not transported by one or more OCTs into cultured neonatal astrocytes is the result of inhibition studies using several antidepressant drugs and histamine receptor antagonists. The basic mechanism of action of antidepressant drugs is the inhibition of monoamine neurotransmitter(s) reuptake. Citalopram, fluoxetine and fluvoxamine are all selective serotonin reuptake inhibitors (SSRIs). Desipramine, maprotyline and nortriptyline preferably inhibit noradrenaline uptake, whereas amitriptyline and clomipramine *in vivo* act as non-selective antidepressant drugs. Since tricyclic antidepressants bind to a different site on the serotonin transporter than SSRI, as well as to other drug targets, we expected that they would more likely interfere with [^{3}H]-histamine transport into cultured astrocytes than SSRIs. Some tricyclic antidepressant drugs e.g. trimipramine, doxepin and dothiepin were found to inhibit histamine transport into rat synaptosomes [23]. Out of all tricyclic antidepressant drugs used in our experiments, only amitriptyline and desipramine significantly decreased [^{3}H]-histamine uptake. The IC_{50} values of amitriptyline and desipramine inhibiting [^{3}H]-histamine uptake into cultured type 1 astrocytes were within human therapeutic serum levels. Since the experiments were performed *in vitro*, no metabolites of antidepressant drugs influenced [^{3}H]-histamine transport into cultured astrocytes. The latter fact might explain the result when observing the effect of clomipramine on [^{3}H]-histamine uptake into cultured astrocytes. The parent compound clomipramine inhibits only serotonin re-uptake in presynaptic neurones, whereas its metabolite N-desmethylclomipramine inhibits the reuptake of noradrenaline and contributes to less selective antidepressant mechanism of action [34]. Maprotyline, desipramine and nortriptyline are all tricyclic antidepressants and selective inhibitors of noradenaline uptake, but only desipramine significantly and nortriptyline slightly decreased [^{3}H]-histamine transport into cultured neonatal rat astrocytes (Figure 4C). Therefore, the inhibition of histamine uptake into cultured astrocytes by amitriptyline, nortriptyline and desipramine might be either a consequence of direct binding of these compounds to a not yet identified histamine carrier or a consequence of indirect action. Amitriptyline, nortriptyline and desipramine interact and inhibit enzyme Na^+ K^+- ATP-ase [35]. The activity of this enzyme is essential for maintainace of the electrochemical gradient that drives histamine uptake into cultured astrocytes [11, 25].

With exception of cimetidine and famotidine, which are weakly basic (pK_a values 7.09 and 7.10 respectively), antidepressant drugs and mepyramine have pK_a values from 8.1 to10.4. Many of these drugs interact with OCTs. Cimetidine, famotidine and desipramine are known inhibitors of OCT1 and citalopram of all human OCTs [22]. Some antidepressant drugs (amitriptyline, nortriptyline and desipramine) in addition to inhibiting serotonin and/or noradrenaline transporter also decrease GABA uptake by GAT1 and GAT3 [36] and, as we observed, also affect histamine transport into cultured astrocytes. Histamine H1 receptor inverse agonist mepyramine and cimetidine (H2 receptor antagonist) affected [^{3}H]-histamine transport into rat type 1 astrocytes at concentrations exceeding their K_D values, proving that the uptake and not receptor binding of [^{3}H]-histamine was inhibited.

Rat astrocytes might represent the main clearance site for brain histamine since the rate of [^{3}H]-histamine transport into astrocytes [11, 12] is significantly greater (Figure 3), than the rate of [^{3}H]-histamine uptake into rat synaptosomes [23]. The rate of rat synaptosomal histamine uptake is in the range of fmol/mg of protein/min [23]. These findings also support the idea proposed back in 1987 [37], that astrocytes represent the main inactivation site of histamine in the rat central nervous system when it was documented that histamine had been more readily transported into the astrocyte enriched fraction than into the synaptosomal fraction while the opposite was observed when uptake of histidine was studied. This finding was recently published for *Drosophila melanogaster* where histamine is the main neurotransmitter involved in photoreceptor signalling [38]. After sensory neuron stimulation histamine is released into the synaptic cleft and the majority of the released histamine is then cleared into perisynaptic astrocytes, where it is converted into its metabolite carcinine by the enzyme ebony. Further on, carcinine is released from astrocytes and taken up by the inebriated neurotransmitter

transporter into photoreceptor cells, where it is converted back to neurotransmitter histamine. Drosophila retinal astrocytes actually participate in recycling of neurotransmitter histamine.

As we found that type 1 astrocytes express mRNA for HNMT, the cytosolic enzyme responsible for histamine degradation in CNS (Figure 5), histamine can be converted into its metabolite tele-methylhistamine within astrocytes, but further studies are needed to confirm that astrocytes participate in the recycling of neurotransmitter histamine in rodent CNS.

This report provides additional neurochemical evidence for the existence of a high affinity histamine transport system in neonatal rat astrocytes, but a gene for this high affinity histamine transporter has yet to be identified. In addition, histamine is not just cleared into astrocytes but can also be metabolized within, thus indicating a possible involvement of astrocytes in the recycling of neurotransmitter histamine. Overall, these results suggest that one of the important functions of astrocytes is to regulate excess neurotransmitter levels, including those of histamine, in the extracellular space.

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Appendix

Mathematical model of [^{3}H]-histamine transport by astrocytes

In the model, two compartments representing the intracellular and extracellular space were separated by a membrane which transports [^{3}H]-histamine. Two processes were considered: electrodiffusion (which we describe by the Goldman equation) and active transport, so that the number of molecules of histamine inside the cells N_{in} increased as,

$$\frac{\mathrm{d}\dfrac{N_{in}}{N_{out}}}{\mathrm{d}t} = A_1\left(1-\frac{N_{in}}{N_{out}}\right) + B\left(\frac{\theta}{1+\theta} - \frac{\theta\dfrac{N_{in}}{N_{out}}\dfrac{V_{out}}{V_{in}}}{1+\theta\dfrac{N_{in}}{N_{out}}\dfrac{V_{out}}{V_{in}}}\right), \quad \text{(A1)}$$

Where

$$A_1 = \frac{D_{eff,1}S}{dV_{out}(1-\alpha_1)}\frac{ve_0\Delta\psi}{kT_1} \quad \text{(A2)}$$

$$B = \frac{SD_{complex}c_{carrier}}{dN_{out}}. \quad \text{(A3)}$$

The first term in Eq. (A1) describes the electrodiffusion [39, 40], while the second term describes the active transport. Here, t is time, $D_{eff,1}$ is the effective diffusion coefficient of histamine in the membrane at $T_1 = 310\,^\circ$K, S is the membrane area, V_{out} is the volume of the outer and, V_{in} is the volume of the inner solution, d is the thickness of the membrane, V is the valence of the histamine molecule, e_0 is the elementary charge, $\Delta\psi$ is the transmembrane potential, k is the Boltzmann constant, N_{out} is the number of histamine molecules in the outer solution, $D_{complex}$ is the diffusion coefficient of the complex composed of the carrier (transporter) molecule and histamine, $c_{carrier}$ is the concentration of the carrier (transporter) molecules in the membrane, $\theta = Kc_{out}$, $K = 0.025$ l/μmol is the adhesion constant for binding of histamine to the carrier molecules, $c_{out} = 1.25$ nmol/l is the concentration of histamine in the outer solution and

$$\alpha_1 = \exp\left(-\frac{e_0\Delta\psi}{kT_1}\right). \quad \text{(A4)}$$

For clarity, equation (A1) is rewritten in the form

$$\frac{\mathrm{d}y}{\mathrm{d}t} = \alpha_1(1-y) + b\left(\frac{\theta}{1+\theta} - \frac{\dfrac{\theta y}{\alpha_1}}{1+\dfrac{\theta y}{\alpha_1}}\right), \quad \text{(A5)}$$

where

$$y = \frac{N_{in}}{N_{out}}\frac{V_{out}}{V_{in}}\alpha_1, \quad \text{(A6)}$$

$$a_1 = A_1\alpha_1\frac{V_{out}}{V_{in}}, \quad \text{(A7)}$$

and

$$b = B\alpha_1\frac{V_{out}}{V_{in}}. \quad \text{(A8)}$$

It is taken in the denominator of the second term of Eq. (A5) that $\theta\, y/\alpha_1 \ll 1$ and that $b\,\theta/\alpha_1 \ll a_1$, so that the solution of Eq. (A5) is

$$y = \left(1+\frac{b}{a_1}\frac{\theta}{1+\theta}\right)\left(1-\exp\left(-a_1 t\right)\right). \quad \text{(A9)}$$

It follows from Eq. (A9) that at higher temperature T_1 the uptake saturates to

$$y(t\to\infty) = \left(1+\frac{b}{a_1}\frac{\theta}{1+\theta}\right). \quad \text{(A10)}$$

At lower temperature $T_2 = 277\,^\circ$K we assume that the active transport is absent, therefore the number of histamine molecules in cells changes according to equation

$$\frac{\mathrm{d}\dfrac{N_{in}}{N_{out}}}{\mathrm{d}t} = A_2\left(1-\frac{N_{in}}{N_{out}}\right) \quad \text{(A11)}$$

where

$$A_2 = \frac{D_{\text{eff},2} S}{dV_{\text{out}}(1-\alpha_2)} \frac{ve_0\Delta\psi}{kT_2} \quad \text{(A12)}$$

$D_{\text{eff},2}$ is the effective diffusion coefficient of histamine in the membrane at $T_2 = 277\,^{\circ}\text{K}$ and

$$\alpha_2 = \exp\left(-\frac{e_0\Delta\psi}{kT_2}\right). \quad \text{(A13)}$$

Eq. (A11) is rewritten in the form

$$\frac{dy'}{dt} = a_2(1-y'), \quad \text{(A14)}$$

where

$$a_2 = A_2\alpha_2\frac{V_{\text{out}}}{V_{\text{in}}}, \quad \text{(A15)}$$

while the solution of Eq. (A14) is the exponential function,

$$y' = 1-\exp(-a_2 t). \quad \text{(A16)}$$

with

$$y' = \frac{N_{\text{in}}}{N_{\text{out}}}\frac{V_{\text{out}}}{V_{\text{in}}}\alpha_2\,. \quad \text{(A17)}$$

The parameters a_1, a_2, b and $V_{\text{out}}/V_{\text{in}}$ are adjusted according to the least squares method to yield the best agreement of the prediction by the model and the experimental data. The parameters a_1 and a_2 describe the rate of histamine molecule movement across the membrane at 277 °K and 310 °K, respectively.

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