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L-Histidine is a beneficial adjuvant for antiepileptic drugs against maximal electroshock-induced seizures in mice

R. M. Kamiński [1], **D. Żółkowska** [2,*], **M. Kozicka** [3], **Z. Kleinrok** [3], and **S. J. Czuczwar** [1,4]

[1] Isotope Laboratory, Institute of Agricultural Medicine, Lublin, Poland
[2] Department of Hygiene, Medical University, Lublin, Poland
[3] Department of Pharmacology and Toxicology, Medical University, Lublin, Poland
[4] Department of Pathophysiology, Medical University, Lublin, Poland

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Summary. Endogenous histamine has been reported to be involved in regulation of seizure susceptibility. Enhancement of histamine neurotransmission engendered by L-histidine treatment produces anticonvulsant effects in experimental animals. The present study investigated the influence of L-histidine on the protective effects of carbamazepine and phenytoin against maximal electroshock-induced seizures in mice.

L-Histidine, administered at the doses that did not influence the threshold for electroconvulsions (250–500 mg/kg), enhanced by nearly 30% the protective effects of carbamazepine against maximal electroshock-induced seizures. D-Histidine (1000 mg/kg), an inactive isomer of histidine, was without any effect in this regard. L-Histidine (500 mg/kg) also augmented the protective effects of phenytoin. Importantly, the enhancement of the anticonvulsant effects of these antiepileptic drugs produced by L-histidine co-administration was not associated with augmentation of their unwanted effects on memory and motor performance. A pharmacokinetic interaction was also excluded since the free plasma levels of these antiepileptics remained unchanged in the presence of L-histidine. It may be suggested that L-histidine could serve as a beneficial adjuvant for selected antiepileptic drugs.

Keywords: L-Histidine – Histamine – Antiepileptic drugs – Mice – Epilepsy

Introduction

Histamine in addition to its pivotal role in allergic or inflammatory reactions is also an important neurotransmitter in the central nervous system. It acts through at least three receptor subtypes: H_1, H_2 and H_3 (Bakker et al., 2002). Histamine is synthesized from L-histidine by a specific enzyme named L-histidine decarboxylase (HDC). In the brain, histamine synthesis is restricted primarily to the posterior hypothalamic tuberomammillary nucleus. However, histamine-synthesizing neurons send widespread projections throughout almost all brain regions (Panula et al., 1984; Watanabe et al., 1984).

The accumulating evidence shows significant involvement of histamine neurotransmission in the physiological processes such as sleep-wake cycle, learning and memory, stress or emotion (Brown et al., 2001). Interestingly enough, however, numerous reports show that histamine significantly contributes to the regulation of seizure susceptibility. A study that inspired a cascade of subsequent research was a report by Churchill and Gammon (1949), which showed that anti-histamine drugs are capable of inducing seizures and related symptoms in epileptic patients. Since that time the mechanisms underlying the role of histamine in epileptogenesis and convulsions have been extensively explored.

It is generally agreed that manipulations increasing brain histamine content lead to concomitant reduction of seizure susceptibility. Such observations were obtained by treatment with either L-histidine (Scherkl et al., 1991; Kamei, 2001; Borowicz et al., 2000), which is the substrate for HDC or metoprine (Yokoyama et al., 1992; Kamei, 2001), which inhibits histamine degradation. Conversely, the blockade of H_1 receptor, but not other histamine receptor subtypes, increases vulnerability to seizures in both experimental animals (Scherkl et al., 1991; Swiader et al., 2001) and human subjects (Churchill and Gammon, 1949; Wyngaarden and Seevers, 1951;

* Present address: Integrative Neuroscience Section, Behavioral Neuroscience Branch, NIDA/NIH, 5500 Nathan Shock Drive, Baltimore, MD 21224, U.S.A.

Yokoyama, 2001). Therefore, it was suggested that endogenous histamine may posses anticonvulsant potential; an effect that seems to be mediated exclusively by H_1 receptor. Accordingly, alterations of histamine and L-histidine levels in brain regions responsible for initiation and propagation of seizures were also described (Honda, 1984; Kamei et al., 1998; Toyota et al., 1998; Kamei, 2001). Interestingly, animals fed with diet devoid of L-histidine appeared to be significantly more susceptible to seizures induced by chemoconvulsants (Gietzen et al., 1996). Finally, decreased serum level of L-histidine was found in epileptic patients (Rao et al., 1993).

Recently, it has been shown that an indispensable amino acid, L-histidine, which is a precursor of histamine, possesses anticonvulsant activity in several seizure models (Borowicz et al., 2000). In addition, anti-histamine drugs, particularly H_1 receptor antagonists, reduce anticonvulsant efficacy of several antiepileptic drugs (AEDs) (Swiader et al., 2001). Therefore, we have hypothesized that enhancement of histamine neurotransmission by L-histidine injection may increase the protective effects of antiepileptic drugs. Here, we present data that L-histidine could be a beneficial adjuvant for anticonvulsive therapy.

Material and methods

Subjects

The experiments were carried out on male Swiss mice weighing 25–30 g. The animals were kept under standard laboratory conditions with 12-hour light-dark cycle and free access to food and tap water. The experimental groups, consisting of 8 animals, were chosen by means of a randomized schedule. The control groups were always tested at the same time and day as the respective experimental groups. The temperature in an animal room and during testing was always $21 \pm 1^{\circ}$C. All animal experiments were done according to the Helsinki declaration and conducted in accordance with the guidelines of the European Community Council directive 86/609/EEC. A local ethical committee approved the experimental protocol.

Maximal electroshock test (MES)

Electroconvulsions in mice were induced after stimulation with alternating current produced by a generator (Hugo Sachs type 221; Freiburg, Germany) and delivered via ear-clip electrodes. The stimulus duration was 0.2 s and the current intensity was 25 mA. Full tonic extension of both hind limbs was taken as the endpoint.

Drugs and treatment regimen

L-histidine, D-histidine, carbamazepine (CBZ) and phenytoin (PHT) were purchased from Sigma, St. Louis, MO, USA. Both isomers of histidine were dissolved in sterile saline, while antiepileptic drugs were suspended in a 1% solution of Tween 81 (Loba Chemie, Vienna, Austria). All drugs were injected intraperitoneally (i.p.) in a volume of 0.01 ml/g of body weight. The pretreatment times for PHT, L-histidine (120 min) and CBZ (30 min) were established in pilot experiments showing peak of their anticonvulsant activity. The time of D-histidine pretreatment was equal to that of L-histidine.

At least three groups of animals were injected with varying doses of either CBZ or PHT in order to estimate their ED_{50} values against MES. Subsequently, other groups of animals were pretreated with subthreshold doses of L-histidine (500 mg/kg) and similarly injected with CBZ or PHT for calculation of ED_{50} values. The ED_{50} value of CBZ was also estimated in the presence of biologically inactive isomer, D-histidine (1000 mg/kg); this experiment allowed confirming selectivity of L-histidine effects. We have reported previously that L-histidine at doses equal to or smaller than 500 mg/kg and D-histidine (1000 mg/kg) were devoid of any effects on electroconvulsions (Borowicz et al., 2000).

Chimney test

The effects of AEDs alone or combined with L-histidine on motor performance were evaluated in the chimney test of (Boissier et al., 1960). In this test, mice had to climb backwards up and go out from vertically positioned plastic tube "chimney", which was 25 cm long and 3 cm wide. Animal performance was assumed to be impaired when the task was not completed within 60 sec. The results were shown as a percentage of animals, which failed to perform the task.

Passive-avoidance testing

According to Venault et al. (1986), the step-through passive avoidance task is recognized as a measure of long-term memory. The device for passive avoidance testing consisted of two compartments connected to each other: illuminated box ($10 \times 13 \times 15$ cm) and dark box ($25 \times 20 \times 15$ cm). The dark box was equipped with electric grid floor and entrance to this compartment of pretreated mice was punished by the electric foot-shock (0.6 mA for 2 sec; facilitation of acquisition). On the following day (24 h later), the same animals, without any treatment, were placed into the illuminated box and the time of avoiding entrance to the dark compartment (retention time) was measured up to 180 sec. Retention times of passive avoidance behavior were expressed as medians with 25 and 75 percentiles.

Determination of free plasma levels of antiepileptic drugs

Estimation of the free plasma levels of CBZ and PHT alone or in combination with L-histidine was carried out by immunofluorescence with the use of an Abbott TDx automatic analyzer (Abbott, USA). At least six mice were used per group and the plasma levels were expressed in μg/ml as means $\pm$ S.E.M. Blood samples were collected after decapitation of animals at the time scheduled for experiments. After centrifugation, plasma samples were pipetted into Microcon-30 microconcentrators (Amicon Inc., USA) and again centrifuged for separation of free from protein bound microsolutes. The filtrates of 50 μl were analyzed by the Abbott TDx processor.

Statistics

ED_{50} values and their statistical comparisons were done by computer probit analysis according to the method described by Litchfield and Wilcoxon (1949). The results from chimney test were statistically estimated by Fisher's exact probability test, while results obtained from passive avoidance testing were compared by Kruskal-Wallis test followed by Dunn's test. Plasma levels of AEDs were statistically analyzed by the use of unpaired Student's *t*-test.

Results

Influence of L-histidine upon the protective activity of CBZ and PHT against MES-induced seizures

L-Histidine (500 mg/kg) significantly enhanced the protective activity of CBZ against MES, which was reflected by nearly a 30% reduction of CBZ's ED_{50} value. L-Histidine at 250 mg/kg also potentiated the anticonvulsant efficacy of CBZ by approximately 25%. Only at 125 mg/kg, L-histidine did not affect the activity of CBZ (Fig. 1A). When an inactive isomer of histidine, namely D-histidine (1000 mg/kg), was combined with CBZ, the protective effect of this AED against MES was not affected (data not shown).

L-Histidine (500 mg/kg) also augmented by 25% the protective effects of PHT against MES. However, at a lower dose of 250 mg/kg, L-histidine remained ineffective in this regard (Fig. 1B).

Influence of L-histidine, CBZ and PHT upon the motor performance and memory of mice

L-Histidine (250–500 mg/kg) was devoid of any significant effects upon both motor and memory performance of mice (Table 1). Also, CBZ and PHT at doses equal to their ED_{50} values against MES (12.2 and 10.4 mg/kg respectively), did not change the performance of mice tested for motor and memory deficits. When lower doses of these AEDs were combined with L-histidine (250–500 mg/kg), which afforded the same protection against MES, the motor and memory performance of mice still remained unaffected (Table 1).

Influence of L-histidine upon the free plasma concentration of AEDs

Free plasma level of CBZ following i.p. injection was $0.80 \pm 0.12\ \mu g/ml$ and L-histidine co-administration did

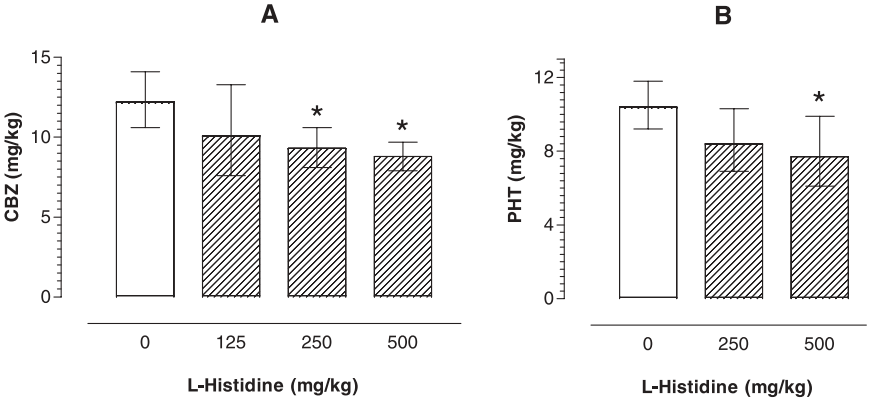

Fig. 1. Effects of L-histidine upon the protective activity of CBZ and PHT against MES in mice. Bar graphs represent ED_{50} values with 95% confidence intervals against MES-induced seizures. (see Methods) $^{*}P \leq 0.05$ vs. vehicle treated animals; according to Litchfield and Wilcoxon (1949) probit analysis

Table 1. Effects of L-histidine, CBZ and PHT upon motor and memory performance of mice

Treatment [1] (mg/kg)	Impaired animals [2] (%)	Median latency [3] (25,75 percentiles)	Number of animals [4] (*N*)
CBZ (12.2)	0	180 (180,180)	10
CBZ (9.3)	0	180 (180,180)	10
CBZ (9.3) + L-histidine (250)	30	180 (180,180)	10
CBZ (8.8)	10	180 (180,180)	10
CBZ (8.8) + L-histidine (500)	20	180 (180,180)	10
PHT (10.4)	20	180 (180,180)	10
PHT (7.7)	20	180 (180,180)	10
PHT (7.7) + L-histidine (500)	30	180 (180,180)	10
L-Histidine (250)	20	180 (180,180)	10
L-Histidine (500)	20	180 (180,180)	10

[1] The doses of antiepileptic drugs were equal to their ED_{50} values obtained in the MES following either single or combined treatment with L-histidine (see Methods)
[2] Motor performance was tested in the chimney test (see Methods)
[3] Memory performance was tested in the passive avoidance paradigm (see Methods)
[4] Separate groups of animals of equal number were used for each of these behavioral tests

Table 2. Influence of L-histidine on the free plasma levels of CBZ and PHT

Treatment (mg/kg)	Free plasma level [1] (μg/ml)	Number of animals (N)
CBZ (8.8)	0.80 ± 0.12	5
CBZ (8.8) + L-histidine (500)	0.78 ± 0.03	5
CBZ (9.3)	0.61 ± 0.03	6
CBZ (9.3) + L-histidine (500)	0.58 ± 0.02	7

[1] Table data are expressed as means ± standard error of the mean (S.E.M.)

not affect this value, which was $0.78 \pm 0.03\,\mu$g/ml (Table 2). Similarly, in case of PHT the plasma levels remained unchanged in the presence of L-histidine; respective values were 0.61 ± 0.03 and $0.58 \pm 0.02\,\mu$g/ml (Table 2).

Discussion

In the present study, we have found that L-histidine significantly enhances the protective effects of CBZ and PHT against MES. This enhancement was not associated with concomitant impartment of motor or memory function. Moreover, a pharmacokinetic interaction was not responsible for this effect since the plasma levels of studied AEDs remained unchanged in the presence of L-histidine free. Finally, the observed effect was stereoselective, because metabolically inactive isomer of histidine, D-histidine, did not enhance the protective effects of tested AEDs against MES.

As it was mentioned earlier, L-histidine is the substrate for HDC, the enzyme responsible for histamine synthesis. However, it was argued that following peripheral administration of L-histidine, other non-specific pathways of histamine synthesis and metabolism in the brain could be recruited (Prell et al., 1996). Moreover, some centrally active substances, other that histamine and its metabolites, have been reported to increase after L-histidine load (Prell et al., 1996). Nevertheless, numerous studies have consistently shown that peripheral injection of L-histidine results in prolonged build up of histamine level in the brain, which was associated with an anticonvulsant effect (Yokoyama et al., 1992; Kamei et al., 1998; Kamei, 2001). Namely, there was an inverse correlation between histamine level and severity of seizures in kindled rats (Kamei et al., 1998) and an intracerebroventricular (i.c.v.) injection of histamine mimicked the anticonvulsant effect of L-histidine (Kamei, 2001). It should be underlined, that L-histidine at the highest dose of 500 mg/kg, which enhanced the protective effects of AEDs in the present study, was without any effect upon the threshold for electroconvulsions (Borowicz et al., 2000). Nevertheless, at this dose L-histidine has been reported to increase histamine brain levels by 100%, while a higher dose of 1000 mg/kg, increased histamine levels by more than 200% (Prell et al., 1996). We have shown that higher doses of L-histidine (1000–3000 mg/kg), but not D-histidine (1000 mg/kg) showed anticonvulsant activity in several seizure models, including electroconvulsions (Borowicz et al., 2000). In the present study, D-histidine was also without effects on the protective activity of antiepileptic drugs against MES. This important stereoselective effect strongly supports the involvement of histamine synthesis pathway in the present findings. As such, it could be suggested that the increase in histamine level in the brain, following injection of L-histidine, is primarily responsible for the observed enhancement of the anticonvulsant activity of studied AEDs.

It seems clear, based on pharmacological and genetic evidence, that anticonvulsant effects of histamine are mediated by H_1 receptors. Firstly, centrally acting H_1 receptor antagonists, but not H_2 or H_3 receptor antagonists, diminish the threshold for pentylenetetrazol-induced convulsions (Scherkl et al., 1991). Secondly, i.c.v. injection of H_1 receptor agonists attenuate amygdala-kindled seizures in rats (Kamei, 2001). Thirdly, animals with genetic knock-out of H_1 receptors appear to be more susceptible to convulsant stimuli (Watanabe and Yanai, 2001). Finally, H_1 receptor antagonists reduce the protective effects of AEDs against MES (Swiader et al., 2001). In fact, current study is in a good correlation with lastly mentioned report, where decreased anticonvulsant effects of CBZ and PHT after chronic treatment with H_1 receptor antagonists were observed.

Although only two AEDs have been utilized in the present study it could be suggested that L-histidine may also be a favorable adjuvant for other AEDs. Generally speaking, when the protective activity of CBZ and PHT is enhanced by an adjuvant the likelihood of such beneficial

interaction with other conventional AEDs is very high (Czuczwar and Borowicz, 2002; and the references cited therein). However, it will be very interesting to explore the possible interactions of L-histidine with other conventional and recently introduced AEDs.

Sedative and muscle relaxing actions of AEDs often account for their undesired effects (Vining, 1987; Rogvi-Hansen and Gram, 1995). Histamine is also involved in modulation of locomotion and memory processes (Brown et al., 2001; Philippu and Prast, 2001). However, our study has revealed that L-histidine remained without effect upon motor and memory performance of mice; likewise it did not influence these parameters in combined treatments with CBZ and PHT. This is an important finding, because 25–30% lower doses of AEDs could be used to achieve the same degree of protection against MES in these combined treatments. It could be suggested then, that co-administration of L-histidine with these AEDs may allow reducing their doses without losing efficacy or increasing toxicity.

In recent years several new AEDs have been introduced, but still approximately 20 to 30% of epileptic patients remain resistant to currently available therapies. Moreover, polytherapy with two or more AEDs often does not substantially improve this figure, indicating an urgent need for the development of better antiepileptic treatments (Jacobs et al., 2001). Thus, the present findings underscoring the favorable interaction of L-histidine with selected AEDs may bear some therapeutic value that requires further preclinical and clinical scrutiny.

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Authors' address: Dr. Rafał M. Kamiński, Epilepsy Research Section, NINDS/NIH, 49 Convent Drive, MSC 4457, Bldg. 49 Rm. 5A75, Bethesda, MD 20892, U.S.A., E-mail: kaminskr@ninds.nih.gov