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Histamine and selective H_3-receptor ligands: a possible role in the mechanism and management of epilepsy

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Abstract

The interaction of selective histamine H_3-receptor agonist *R*(α)-methyl-histamine (RAMH) and antagonist thioperamide (THP) with some antiepileptic drugs [AED; phenytoin (PHT), carbamazepine (CBZ), sodium valproate (SVP), and gabapentin (GBP)] was studied on seizures induced by maximal electroshock (MES) and pentylenetetrazole (PTZ) in mice. It was found that subeffective dose of THP in combination with the subeffective doses of PHT and GBP provided protection against MES and/or PTZ-induced seizures. Further, RAMH reversed the protection afforded by either PHT or GBP on MES and/or PTZ seizures. In another set of experiments, the histamine content was measured in the whole brain and in different brain regions including cerebral cortex, hypothalamus, brain stem and cerebellum following convulsant (MES and PTZ) and AED treatment. It was seen that while MES exhibited a tendency to enhance brain histamine levels, PTZ showed the opposite effect. AEDs either increased (PHT and GBP) or decreased (SVP) brain histamine content in different regions to varying degrees. The results indicate a role for histamine in seizures and in the action of AEDs and suggest that selective H_3-receptor antagonists may prove to be of value as adjuncts to conventional AEDs.

Keywords: Epilepsy; Histamine; H_3-receptors; Thioperamide; *R*(α)methyl-histamine; Antiepileptics; Phenytoin; Carbamazepine; Sodium valproate; Gabapentin; Maximal electroshock; Pentylenetetrazole

1. Introduction

Epilepsy afflicts between 0.5% and 1% of the population (Blum, 1998). Drug therapy is the mainstay of treatment and is largely symptomatic (Vohora and Vohora, 1999). Contemporary antiepileptic therapy is neither universally effective (Vohora and Vohora, 1999) nor invariably safe (Desai et al., 1995; Shin and McNamara, 1994). Advances in the knowledge of mechanisms of epilepsies would allow for more rational therapeutic approaches to this difficult neurological disorder (Shin and McNamara, 1994). So far, research in epilepsy has largely focussed on the GABAergic and glutamatergic neurotransmission as paradigms of inhibitory and excitatory elements of central nervous system (CNS) activity (Rogawski, 1998; Sarro et al., 1998).

However, lately, several lines of evidence have indicated that the central histaminergic neuronal system plays an important role in the inhibition of seizure activity (Kamei et al., 1998; Onodera et al., 1992; Scherkl et al., 1991; Tuomisto and Tacke, 1986; Vohora et al., 2000b; Yokoyama et al., 1992, 1993a,b, 1994). The first early evidence for the involvement of histamine came from animal studies in which drugs that deplete brain histamine were found to potentiate convulsions and vice versa (Tuomisto and Tacke, 1986; Yokoyama et al., 1992). Moreover, direct histamine H_1-receptor activation or modulation of CNS histamine levels by L-histidine loading and inhibition of histamine synthesis or metabolism in rodents have indicated histamine to be an endogenous anticonvulsant (Leurs et al., 1998).

Presynaptic control via histamine H_3-receptors (autoreceptors) is an important mechanism of histamine-mediated neurotransmission (Arrang et al., 1983, 1987). Recently, we have demonstrated that thioperamide (THP), a selective histamine H_3-receptor antagonist, provides protection against pentylenetetrazole (PTZ)-induced convulsions in mice possibly by increasing endogenously released histamine in the brain (Vohora et al., 2000b). Similarly, other

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studies have reported protection with H_3-receptor antagonists in different seizure models such as the maximal electroshock (MES; Yokoyama et al., 1993a) and kindling (Kakinoki et al., 1998) methods. These findings support the notion that H_3-receptor antagonists could represent a novel approach to the development of antiepileptic drugs (AED) and may have an important potential as adjuvant to AEDs, especially in those patients where conventional therapy has been inadequate. With this view, in the present study, we investigated the effect of histamine H_3-receptor ligands in combination with some AEDs against conventional models of experimental convulsions in mice. Further, in an attempt to elucidate the role of histamine in seizures and in the action of AEDs, we measured histamine levels in various brain regions following convulsions and AED treatment.

2. Method

2.1. Animals

Swiss-strain male albino mice (18–25 g), raised at the Central Animal House Facility of our university, were housed in polypropylene cages and maintained on a natural light–dark cycle. They were fed on a standard pellet diet (Amrut rat and mice feed; Chakan Oil Mills, Pune, India) and water ad libitum. All procedures involving animals were conducted in accordance with the guidelines of the Animal Ethics Committee, Hamdard University.

2.2. Induction of seizures

Clonic convulsions were induced by injecting PTZ in a dose of 70 mg/kg ip. Both tonic and clonic convulsions were produced by giving MES (48 mA for 0.2 s) using an electroconvulsiometer (INCO, Ambala) and crocodile earclips. The dose of PTZ used and the current employed in MES produced convulsions in 100% of animals without mortality in our laboratory setup.

2.3. Drugs and their administration

PTZ (Sigma, USA), phenytoin (PHT; Sigma), *R*(α)methylhistamine (RAMH; Sigma), THP (Sigma), carbamazepine suspension (CBZ; Ciba-Geigy, India), sodium valproate (SVP; Torrent, India), and gabapentin (GBP, Intas, India) were used. All drug solutions were prepared in distilled water except for PHT, which was suspended in 1% gum acacia, and RAMH, which was dissolved using pyrogen-free sterile water for injection. All treatments were administered by the intraperitoneal (ip) route in a volume not exceeding 10 ml/kg except RAMH, which was given intracerebroventricularly (icv) in a volume of 5 μl/mouse using a Hamilton's microlitre syringe following the method of Haley and McCormick (1957). The reliability of the technique was confirmed by pilot studies using injection of India ink and examining its diffusion into various parts of the brain by sacrificing the animals. This was further confirmed by observation of symptoms following intracerebroventricular injections of acetylcholine (1 and 10 μg), epinephrine (1 and 10 μg) and atropine (10 and 100 μg) in pilot experiments as described by other workers (Haley and McCormick, 1957). The site of injection was 2 mm from either side of the midline on a line drawn through the anterior base of the ears (Haley and McCormick, 1957). Control groups received the appropriate vehicle (either intraperitoneal or intracerebroventricular) used for making the drug solutions.

Dose–response studies were conducted in order to establish the 100% protective doses (ED_{100}) for each AED used. These doses were subsequently used for studies with RAMH. RAMH was administered 15 min before the AED. The animals were then subjected to MES or PTZ as appropriate. In another set of experiments, subeffective doses (maximum possible dose at which there is no protection) were determined for each AED and were then combined with the subeffective dose of THP in the MES and PTZ tests. PHT, CBZ, and GBP were used for studying drug combinations with THP or RAMH against MES seizures while SVP and GBP were used for PTZ seizures. Accordingly, the animals were divided into 14 groups, seven each for combined study of AEDs with THP or RAMH in the MES model, and into 10 groups, five each for the combined study of AEDs with THP or RAMH in the PTZ model. The effective and the subeffective doses of THP against PTZ and MES seizures were based on our previous findings (Vohora et al., 2000b), pilot experiments, and other reports (Yokoyama et al., 1993a). The dose of RAMH used was as per other studies (Vohora et al., 2000b; Yokoyama et al., 1993a). The pretreatment timings were determined on the basis of their reported time of peak action and our pilot experiments: 1 h for PHT (Gordon et al., 1993), SVP (Loscher and Schmidt, 1988), GBP (Sarro et al., 1998), and THP (Vohora et al., 2000a; Yokoyama et al., 1993a); 30 min for CBZ; and 15 min for RAMH (Vohora et al., 2000b).

2.4. Observational parameters

The animals were observed immediately after MES/PTZ challenge. For MES, the duration of the different phases of convulsions was observed as per the method of Yokoyama et al. (1993a). The tonic phase was regarded as the period between the onset of hind limb extension (HLE) and the start of myoclonic jerks. The duration of myoclonic jerks was considered as the clonic phase. The convulsive coma phase was the period between the end of myoclonic jerks and the recovery of righting reflex. The number of animals showing complete abolition of tonic HLE was also observed and expressed as percent protection.

Observations for PTZ-induced convulsions were made for a period of 30 min. The latency to and the percent protection from myoclonic jerks and clonic generalized seizures with falling were recorded (Vohora et al., 2000b).

2.5. Spectrofluorimetric estimation of regional brain histamine

The animals were sacrificed by decapitation following MES (during tonic extensor phase), PTZ (during clonic generalized seizures), and following AEDs at their 100% protective doses after the specified treatment schedule. Control groups received the appropriate vehicle for each drug used. The animals were divided into 15 groups for studying the effect of MES and PTZ on brain histamine (three groups each for whole brain and four brain regions). The effect of four AEDs on brain histamine employed the use of 25 groups, five each for whole brain and four brain regions for different treatments. The control readings for individual brain regions were pooled together in both experiments. The brains were promptly removed and placed on ice. Different regions of the brain, the cerebral cortex, hypothalamus, brain stem (medulla–pons), and cerebellum were dissected out as described in our earlier study (Pal and Dandiya, 1994) and following the method of Sadasivudu and Lajtha (1970). The hypothalamus was dissected out as a single block including the preoptic area (Pal and Dandiya, 1994). Histamine content in the whole brain and in different regions was measured as per the method of Shore et al. (1959). Briefly, the method is as below:

Extraction of HA from tissues: Tissues were homogenized in 9 volumes of 0.4 N perchloric acid and then centrifuged. A 4-ml aliquot of the supernatant fluid was taken and transferred to a tube containing 0.5 ml of 5 N NaOH, 1.5 g of solid NaCl, and 10 ml of *n*-butanol. The tube was then shaken for 5 min to extract the histamine into the butanol and after centrifugation, the aqueous phase was removed. The organic phase was then shaken for 1 min with 5 ml of 0.1 N NaOH. This wash removed any residual amounts of histidine. The tube was then centrifuged and an 8-ml aliquot of butanol was transferred to a tube containing 2.5 ml of 0.1 N HCl and 15 ml of *n*-heptane. After shaking for about 1 min, the tube was centrifuged and histamine in the aqueous phase was assayed fluorometrically.

Fluorometric assay: A 2 ml aliquot of histamine was taken in a tube and 0.4 ml of 1 N NaOH was added to it followed by 0.1 ml of OPT reagent (*O*-phthalaldehyde in methanol). After 4 min, 0.2 ml of 3 N HCl was added. The fluorescence of the solution was measured at 450 μm, resulting from activation at 360 μm in a spectrofluorometer.

2.6. Statistical analysis

Results are expressed as mean ± S.E.M. and analysed using one-way analysis of variance (ANOVA) followed by Dunnett's *t* test or by Kruskal–Wallis one-way ANOVA followed by multiple range test. For incidence percent, χ^2 test with Yate's correction was used. P values $\leq .05$ were considered to be statistically significant.

3. Results

3.1. Effect of RAMH pretreatment on the protection afforded by AEDs on MES seizures

The intraperitoneal doses of AEDs that protected 100% of animals against MES-induced seizures (ED_{100} doses) were found to be 25 mg/kg for PHT, 10 mg/kg for CBZ, and 200 mg/kg for GBP. RAMH (10 μg/mouse icv) administered 15 min before the ED_{100} doses of AEDs significantly reversed the protection afforded by PHT and GBP on MES seizures, the effect being more marked with GBP ($P < .001$, χ^2 test) (Fig. 1). In the presence of RAMH, the percent protection against tonic HLE was reduced from 100% to 60% in case of PHT and to 16.66% in case of GBP. No effect was, however, observed on the antiepileptic effects of CBZ.

3.2. Effect of RAMH pretreatment on the protective action of AEDs on PTZ seizures

The results are presented in Fig. 2. The ED_{100} doses for SVP and GBP against PTZ-induced seizures were found to be 300 and 400 mg/kg ip, respectively. RAMH (10 μg/mouse icv) significantly reversed the protective action of GBP on myoclonic jerks ($P < .05$, χ^2 test) and only marginally on clonic generalized seizures. The percent protection against myoclonic jerks was reduced from 100% to 28.57% and to 42.85% against clonic generalized seizures. The protective action of SVP, however, remained unaffected in the presence of RAMH.

3.3. Effect of combined treatment with subeffective doses of THP and AEDs on MES-induced convulsions

Preliminary dose–response studies showed that intraperitoneal administration of THP (7.5 mg/kg), PHT (10 mg/kg), CBZ (3.5 mg/kg), and GBP (50 mg/kg) offered no

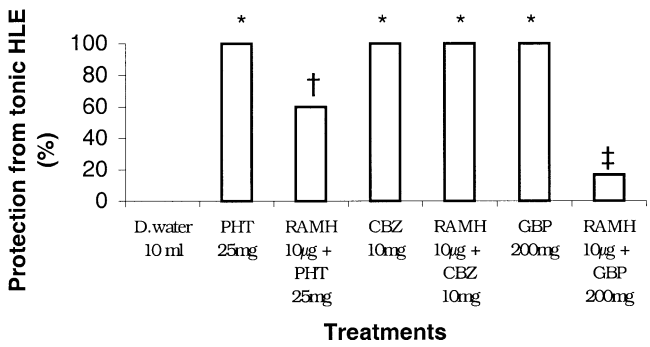

Fig. 1. Effect of RAMH pretreatment on the percent protection of AEDs against MES seizures. All AEDs administered per kg ip and RAMH given per mouse icv. Control groups received distilled water intraperitoneally or intracerebroventricularly, data pooled. Number of animals per group ranged from 5 to 10. * $P < .001$, † $P < .05$ vs. distilled water group; ‡ $P < .01$ vs. groups treated with PHT, CBZ, GBP, and RAMH + CBZ. Significance by χ^2 test with Yate's correction.

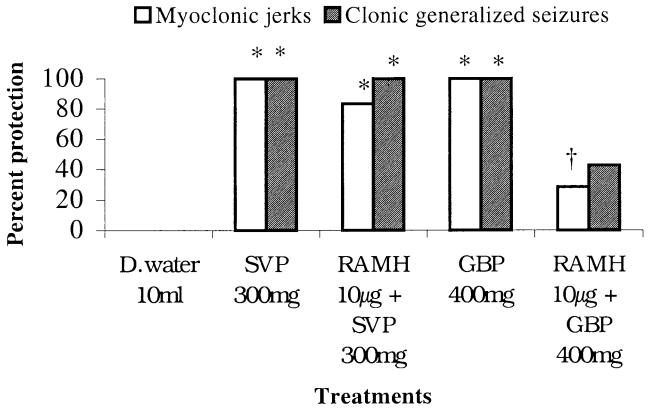

Fig. 2. Effect of RAMH pretreatment on the protective action of AEDs against PTZ-induced seizures. All AEDs administered per kg ip and RAMH given per mouse icv. Control group received distilled water intraperitoneally or intracerebroventricularly, data pooled. Number of animals in each group ranged from 5 to 12. * $P<.001$ vs. distilled water group; † $P<.05$ vs. groups treated with SVP, GBP, and RAMH + SVP. Significant by χ^2 test with Yate's correction.

protection against MES seizures. The combined efficacy with the ineffective doses of THP and AEDs are shown in Table 1. Both PHT and GBP, but not CBZ, when combined with THP and administered 60 min before MES, significantly reduced the duration of tonic HLE phase as compared to control or AED treatment alone [$F(6,33)=6.92$, $P<.01$, one-way ANOVA]. With GBP, the duration of the clonic phase was also significantly reduced [$F(6,53)=2.68$, $P<.05$, one-way ANOVA]. The duration of the convulsive coma phase, however, remained unaffected by all combinations.

3.4. Effect of combined treatment with subeffective doses of THP and AEDs on PTZ-induced convulsions

The subeffective dose for SVP and GBP against PTZ-induced seizures was found to be 75 mg/kg and for THP to be 3.75 mg/kg. The latter, when combined with GBP, significantly prolonged the latency to myoclonic jerks [$H=11.01$, $P<.05$, Kruskal–Wallis one-way ANOVA] and clonic generalized seizures [$H=9.48$, $P<.05$, Kruskal–Wallis one-way ANOVA] as compared to control or GBP treatment alone. No change in latency was observed with the combined treatment of THP and SVP (Table 2).

Table 2
Interaction of subeffective doses of THP and AEDs on PTZ-induced seizures in mice

Group no.	Treatment	Dose/kg ip	Latency (mean ± S.E.M., s) Myoclonic jerks	Clonic generalized seizures
I	Distilled water	10 ml	66.80 ± 4.38	198.25 ± 18.78
II	SVP	75 mg	88.50 ± 17.58	215.16 ± 38.04
III	GBP	100 mg	62.80 ± 6.94	202.20 ± 8.52
IV	THP + SVP	3.75 mg 75 mg	96.83 ± 8.72	232.83 ± 49.13
V	THP + GBP	3.75 mg 100 mg	91.50 ± 2.86*	348.50 ± 17.64*

Number of animals used per group ranged from 5 to 12.
* $P<.05$ vs. I and III. Significant by Kruskal–Wallis one-way ANOVA followed by multiple range test.

3.5. Effect of convulsants (MES and PTZ) on regional and whole brain histamine content

The results for whole brain and regional histamine levels are indicated in Table 3. A significant elevation in the whole brain histamine content was observed in the MES-subjected group [$F(2,21)=4.76$, $P<.05$, one-way ANOVA]. When measured in the different brain regions, a significant rise in the brain stem histamine concentration was observed [$F(2,10)=14.04$, $P<.01$, one-way ANOVA]. The histamine levels, both in the

Table 1
Interaction of subeffective doses of THP and some AEDs on MES-induced convulsions in mice

Group no.	Treatment	Dose/kg ip	Duration of convulsions (mean ± S.E.M., s) Tonic	Clonic	Convulsive coma
I	Distilled water	10 ml	14.00 ± 0.72	5.00 ± 0.66	99.40 ± 11.45
II	PHT	10 mg	13.60 ± 0.77	5.00 ± 0.28	102.60 ± 17.56
III	CBZ	3.5 mg	12.60 ± 0.82	5.80 ± 1.48	100.20 ± 27.16
IV	GBP	50 mg	12.20 ± 0.76	4.60 ± 0.35	90.00 ± 11.79
V	THP + PHT	7.5 mg 10 mg	9.00 ± 0.89*,**	4.20 ± 0.76	84.20 ± 17.94
VI	THP + CBZ	7.5 mg 3.5 mg	13.00 ± 0.63	5.40 ± 0.72	76.80 ± 18.63
VII	THP + GBP	7.5 mg 50 mg	8.40 ± 0.45*,***	1.80 ± 0.17****	79.60 ± 9.61

Results were obtained from a minimum of five animals per group.
* $P<.01$ vs. I. Significance by one-way ANOVA followed by Dunnett's t test.
** $P<.01$ vs. II. Significance by one-way ANOVA followed by Dunnett's t test.
*** $P<.01$ vs. IV. Significance by one-way ANOVA followed by Dunnett's t test.
**** $P<.05$ vs. I. Significance by one-way ANOVA followed by Dunnett's t test.

Table 3
Effect of convulsants (MES/PTZ) on brain histamine levels in mice

Treatment	Concentration (mean ± S.E.M., μg/g)				
	Whole brain	Cerebral cortex	Hypothalamus	Brain stem	Cerebellum
Control	0.238 ± 0.031 (9)	0.411 ± 0.140 (7)	0.947 ± 0.220 (7)	0.182 ± 0.050 (7)	0.381 ± 0.090 (7)
MES (48 mA for 0.2 s)	0.431* ± 0.068 (10)	0.836 ± 0.080 (3)	0.790 ± 0.070 (3)	0.696† ± 0.110 (3)	0.490 ± 0.190 (3)
PTZ (70 mg/kg ip)	0.206 ± 0.013 (5)	0.100 ± 0.010 (3)	0.403 ± 0.060 (3)	0.073 ± 0.016 (3)	0.200 ± 0.012 (3)

Number in parenthesis represents animals used.
* $P < .05$ vs. Control. Significant by one-way ANOVA followed by Dunnett's t test.
† $P < .01$ vs. Control. Significant by one-way ANOVA followed by Dunnett's t test.

whole brain and in the different brain regions in mice subjected to PTZ, were not significantly different from the values for the control group. A tendency towards reduced histamine levels was, however, seen after PTZ.

3.6. Effect of AEDs on regional and whole brain histamine content

The results showing the effect of AEDs on whole brain and regional histamine levels are depicted in Table 4. None of the drugs elicited any appreciable effect on the histamine content in the whole brain except GBP, which significantly increased it [$F(4,43) = 7.58$, $P < .01$, one-way ANOVA]. Both PHT and GBP significantly raised the histamine content in the brain stem [$F(4,18) = 15.85$, $P < .01$, one-way ANOVA] GBP, in addition, also elevated cortical [$F(4,18) = 4.30$, $P < .05$, one-way ANOVA] and hypothalamic [$F(4,18) = 16.67$, $P < .01$, one-way ANOVA] histamine levels. Though the latter effects (increase in the cortical and hypothalamic histamine content) were also seen with PHT and CBZ, the effects were not statistically significant. Thus, all the AEDs (PHT, CBZ, and GBP) exhibited a tendency to increase brain histamine except SVP, which showed an opposite effect. The hypothalamic [$P < .05$, Dunnett's t test] histamine content was found to be significantly reduced after treatment with SVP. A marginal reduction was also observed in the cerebellum region. The levels in the cerebral cortex and brain stem, however, remained unaffected by SVP.

4. Discussion

In the present study, we have shown a protective action against MES-induced seizures by combining THP with PHT and GBP in their respective subeffective doses. Further, this protection was reversed by pretreatment with RAMH. Recently, we demonstrated a dose-dependent protective action of THP against PTZ-induced seizures and its reversal by RAMH (Vohora et al., 2000b). We have now shown that the combination of subeffective doses of THP and GBP elicits a protective action in the PTZ model by prolonging the latencies to myoclonic jerks and clonic generalized seizures. Further, the protective action of GBP was completely countered by RAMH. These findings clearly indicate the involvement of H_3-receptor mechanisms in seizures. This opens up exciting newer approaches to the management of seizures. Thus, an H_3-receptor antagonist can serve as a useful adjunct in epileptic patients not responding to a conventional AED regimen. In a chronic disease like epilepsy that requires long-term treatment, the desirability of using minimal doses can not be overstressed for minimizing iatrogenic effects. Further, the beneficial effects of H_3-receptor antagonists in maintaining wakefulness (Monti et al., 1991) and in improving cognitive function (Meguro et al., 1995) has been suggested by numerous studies. These

Table 4
Effect of some AEDs on histamine levels in the mouse brain

Treatment	Dose/kg ip	Concentration (mean ± S.E.M., μg/g)				
		Whole brain	Cerebral cortex	Hypothalamus	Brain stem	Cerebellum
Distilled water	10 ml	0.250 ± 0.026 (12)	0.411 ± 0.140 (7)	0.947 ± 0.220 (7)	0.182 ± 0.050 (7)	0.381 ± 0.090 (7)
PHT	25 mg	0.311 ± 0.060 (11)	0.842 ± 0.050 (4)	1.215 ± 0.189 (4)	0.662* ± 0.022 (4)	0.416 ± 0.100 (3)
CBZ	10 mg	0.258 ± 0.050 (8)	0.610 ± 0.068 (4)	1.032 ± 0.194 (4)	0.260 ± 0.022 (4)	0.576 ± 0.150 (3)
SVP	300 mg	0.108 ± 0.020 (9)	0.457 ± 0.153 (4)	0.070† ± 0.009 (4)	0.225 ± 0.066 (4)	0.146 ± 0.033 (3)
GBP	200 mg	0.511† ± 0.053 (8)	1.107* ± 0.094 (4)	3.047* ± 1.058 (4)	0.645* ± 0.065 (4)	0.663 ± 0.109 (3)

Number in parentheses represents animals used per group. All treatments administered at their ED_{100} doses.
* $P < .01$ vs. distilled water. Significant by one-way ANOVA followed by Dunnett's t test.
† $P < .05$ vs. distilled water. Significant by one-way ANOVA followed by Dunnett's t test.

effects may be specifically important in the light of sedative and cognitive effects associated with AED therapy. The subeffective doses used in this study are too low to show cognitive impairment or sedative action. Experiments in our laboratory revealed impairment in learning and memory only after 14-day administration of PHT at 25 mg/kg (Vohora et al., 2000a) and after single ED_{100} dose of SVP (300 mg/kg; unpublished observation). Studies on the effect of THP on AED-induced cognitive deficits can form the focus of subsequent studies and will now be taken up in our laboratory.

Varied neurotransmitters and neuropeptide systems have been extensively investigated for their involvement in seizures and epilepsy (Dragunow, 1986). Little is however known about the alterations in histaminergic system during this disease. We found a significant increase in brain stem histamine concentrations following MES seizures. Contrary to the effects in MES model, a tendency towards decrease brain histamine levels (though nonsignificant) was observed following PTZ-induced clonic convulsions. The reduction was seen in the cerebral cortex, hypothalamus, brain stem, and cerebellum. Consistent with our findings, a decrease in the histamine content of amygdala and hypothalamus has been reported following kindling (Kamei et al., 1998). The three models (MES, PTZ, and amygdala kindling) are known to involve different mechanisms and represent different kind of seizures (Loscher and Schmidt, 1988). Therefore, contradictory observations on brain histamine levels following MES and PTZ may not be surprising. It is noteworthy that brain stem (and a number of brain stem structures) has been implicated in seizure arrest (Dragunow, 1986). Elevation of brain histamine following MES may be due to maximum neuronal activity with enhanced neurotransmitter synthesis/release. Seizure arrest and postictal refractory period (PIRP) are associated with corresponding activity or release of endogenous substances in the brain (Dragunow, 1986). The observed increase in histamine following MES may reflect protective physiological mechanisms, supporting the concept of histamine as an endogenous anticonvulsant (Leurs et al., 1998).

It is well known that H_3-receptors are autoreceptors on presynaptic histaminergic terminals with an inhibitory action on the synthesis and release of histamine (Arrang et al., 1983, 1987). Blockade of these receptors by selective H_3-antagonists, e.g. THP, would lead to an enhanced neuronal histamine release in the brain, resulting in anticonvulsant effects (Arrang et al., 1987; Kakinoki et al., 1998; Vohora et al., 2000b; Yokoyama et al., 1993a). Such effects of H_3-receptor antagonists are reported to be reversed either by H_3-receptor agonists or by H_1-receptor antagonists but not by H_2-receptor antagonists, suggesting an interaction of the THP-released histamine with histamine H_1-receptors on the postsynaptic neurons (Kakinoki et al., 1998; Vohora et al., 2000b; Yokoyama et al., 1993a). However, H_3-receptors are now considered as heteroreceptors in both CNS and peripheral nervous system (Schlicker et al., 1996). They regulate not only the release of histamine but also of other neurotransmitter, e.g. 5-HT (Schlicker et al., 1988), NE (Schlicker et al., 1989), DA (Schlicker et al., 1993), ACh (Blandina et al., 1996), and GABA (Meguro et al., 1995). Therefore, in the present study, it is possible that THP influenced seizures through other neuronal systems in the brain. However, the heteroreceptor function of H_3-receptor ligands in the regulation of monoaminergic activity is suggested to be minor in contrast with their function in modulating histaminergic activity (Oishi et al., 1990). Further, the dose of THP used in this study is reported to produce no effect on the brain levels of DA, 3,4-dihydroxypphenyl acetic acid, 5-HT, and 5-hydroxyindole acetic acid (Yokoyama et al., 1993a), ruling out the involvement of these neurotransmitters in causing the observed effects. A role for GABAergic mechanisms appears to be more likely. Recently, THP was shown to increase the release of GABA from the rat hypothalamus (Yamamoto et al., 1997). A disinhibition of GABA was known to be involved in the initiation and generalization of PTZ-induced seizure (Macdonald and Barker, 1977). It may, therefore, be speculated that the protective effect of THP and GBP on PTZ was mediated through an indirect action on GABA. An interplay of NE sites may not be overlooked, as H_3-receptors regulating the release of NE are located on the catecholaminergic nerve terminals (Schlicker et al., 1989).

The AEDs under study did not affect the histamine levels in the whole brain at their ED_{100} doses. An exception to this was GBP, which caused a significant rise in the neurotransmitter. An increase was noted in the brain regions such as the cerebral cortex and brain stem, considered relevant for epilepsy (Gale and Broning, 1988), following treatment with PHT and GBP. One AED, viz. SVP, caused a significant reduction in the hypothalamic histamine content. The pharmacological significance of such differences observed for SVP is difficult to explain at this stage. These findings, however, preclude a role for histaminergic neuronal system in mediating the anticonvulsant effects of SVP and CBZ. Tuomisto and Tacke (1986) reported a linear correlation between the rise in brain histamine levels and protection against MES seizures in rats. Besides, drugs that enhance brain histamine (e.g. L-histidine, metoprine, and histamine itself) are known to act as anticonvulsant in various experimental models (Scherkl et al., 1991; Tuomisto and Tacke, 1986; Yokoyama et al., 1994). These reports coupled with the present PHT–GBP–THP effects on seizures and brain histamine concentrations point to the involvement of the histaminergic neuronal system in the anticonvulsant effects of these drugs.

From a perusal of the results, it may be concluded that (a) histaminergic mechanisms play a significant role in seizure generation and its control; (b) H_3-receptor antagonists may represent a novel class of AED with therapeutic potential as adjuncts to conventional drugs; and (c) designing AEDs with a mechanism-specific approach targeting histamine deserves more scientific attention.

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