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Lack of substantial effect of the H_3-antagonist thioperamide and of the non-selective mixed H_3-antagonist/H_1-agonist betahistine on amygdaloid kindled seizures

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Abstract

We investigated whether some histamine H_3-antagonists would attenuate amygdaloid kindled seizures in rats. Thioperamide, a standard H_3-antagonist, did not significantly reduce either seizure ranks or afterdischarge duration (ADD). Betahistine which has both H_3-antagonistic activity and H_1-agonistic activity significantly reduced ADD, albeit mild at a toxic dose, though seizure ranks were not affected. In addition, L-histidine, the precursor of histamine, affected neither seizure ranks, nor ADD. It was shown that H_3-antagonists have no significant inhibitory action against amygdaloid kindled seizures, probably because released histamine was unable to inhibit those seizures.

Keywords: Thioperamide; Betahistine; H_3-antagonist; H_1-agonist; L-histidine Kindled seizures; Rats

1. Introduction

Exogeneously administered histamine or H_1-agonists and an increase in brain histamine content produced by either metoprine or histidine have been reported to show an anticonvulsant action against electroshock seizures or chemically-induced seizures of animals (Tuomisto and Tacke, 1986; Freeman et al., 1990; Scherkl et al., 1991; Yokoyama et al., 1992, 1994). Furthermore, histamine or H_1-agonist has been reported to suppress kindled seizures (Kakinoki et al., 1995). H_3-antagonists have been reported to release endogeneous histamine from presynaptic nerve terminals (Schwartz et al., 1991). However, effects of H_3-antagonists on kindled seizures have not been reported. H_3-receptors have been reported to be rich at a basolateral amygdaloid nucleus (Schwartz et al., 1991). We investigated whether thioperamide, a selective H_3-antagonist, would inhibit amygdaloid-kindled seizures by releasing endogenous histamine. Secondly, we investigated whether betahistine, which has a weak H_1-agonis-

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tic activity as well as H_3-antagonistic activity, would show an inhibitory action against such seizures. Lastly, we investigated whether L-histidine, the precursor aminoacid of histamine, would protect amygdaloid-kindled seizures.

2. Materials and methods

2.1. General procedures

Male Wistar rats weighing 300 g were used. All rats were housed with 12 h light–12 h dark cycle and were supplied with food and water ad libitum.

A bipolar stainless steel enamel-coated electrode (0.2 mm in diameter) was implanted into the right amygdaloid nucleus (coordinate A 6.0, L 5.0, H 8.5) using the stereotaxic atlas of Praxinos and Watson. Tip separation of the electrode was 0.5 mm.

Carvarial screw electrodes were placed at the left frontal region and the anion. All implantations were carried out under sodium pentobarbital anaesthesia.

After a recovery period of 10 days, kindling stimulation (200 μA, 1 ms, 60 Hz) for 2 s was delivered at the right amygdaloid nucleus once a day.

Behavioral manifestations were divided into the five-class scale devised by Racine (1972). These were (1) facial movement; (2) facial movement and head nodding; (3) facial movement, head nodding and forelimb clonus; (4) facial movement, head nodding, forelimb clonus and rearing; and (5) facial movement, head nodding, forelimb clonus, rearing and falling. A rat, which exhibited consecutively five class 5 seizures, was regarded as fully kindled.

EEG was recorded for 2 min before stimulation and for 5–10 min after stimulation. The electrode at the anion was used as a reference. The duration of afterdischarge (ADD) at the amygdala was measured.

The day after completion of kindling, the threshold for generalised seizures (GST) was tentatively determined by the application of stimulus that was increased 20 μA stepwise starting from 60 μA. Each stimulation was separated by an interval of 10 min. From the next day, the rats were stimulated at the tentative GST, or 20 μA lower or higher than this GST once a day. In this way, the least current to induce class 5 seizures (GST) was determined within 4 days.

All substances were administered intraperitonially.

Thioperamide maleate and betahistine hydrochloride were administered in a volume of 1 ml/kg and L-histidine monohydrochloride monohydrate was in a volume of 4–6 ml/kg. Thioperamide maleate was suspended in physiological saline containing 0.5% CMC. It was administered 60 min prior to the stimulation at GST, since, the duration time of effectiveness has been described to be 0.5–4 h after the administration in rats (Clapham and Kilpatrick, 1993) or according to the previous report where the drug was administered 60 min prior to tests (Imaizumi and Onodera, 1993). Betahistine hydrochloride was dissolved in physiological saline and administered 30 min before the stimulation according to previous descriptions (Palitzsch et al., 1995; Imaizumi et al., 1996). L-histidine was dissolved in warm saline and administered 5 h before the stimulation (Kamei et al., 1998).

On the first day, rats were stimulated at GST after the administration of control vehicles. The next day, rats were stimulated at GST after the administration of test substances. When two doses were tested in the same rat, tests were separated by 1 week at least and performed using GSTs, which were determined again.

Betahistine hydrochloride and thioperamide maleate were purchased from Sigma. L-histidine was purchased from Nacalai Tesque Inc.

2.2. Histology

Animals were overdosed with sodium pentobarbital and perfused through the heart with 10% formalin and saline.

The brains were removed, stored in 10% formalin and prepared for histological analysis using standard techniques. Coronal sections were cut at a thickness of 50 μm on a freezing microtome.

They were mounted and stained with cresyl violet to verify electrode placement.

Only animals with electrodes lying within the amygdaloid nuclei were included in the analysis.

2.3. Statistics

ADD was analysed with one-way repeated ANOVA. Seizure rank scores were not analysed since both control animals and test animals showed class 5 seizures in all tests.

Table 1
Effects of thioperamide on amygdaloid kindled seizures[a]

Drug (mg/kg)	*N*	Seizure rank	ADD (s)
Vehicle	7	5 ± 0	96.4 ± 4.7
Thioperamide 20	7	5 ± 0	92.5 ± 10.8
Vehicle	6	5 ± 0	115.2 ± 34.9
Thioperamide 30	6	5 ± 0	100.7 ± 14.7

[a] Values are mean $\pm$ S.D.; ADD, afterdischarge duration; vehicle was saline containing 0.5% CMC.

Table 2
Effects of betahistine on amygdaloid kindled seizures[a]

Drug (mg/kg)	*N*	Seizure rank	ADD (s)
Vehicle	5	5 ± 0	89.2 ± 8.6
Betahistine 200	5	5 ± 0	86.0 ± 4.3
Vehicle	5	5 ± 0	82.6 ± 11.1
Betahistine 400	5	5 ± 0	73.2 ± 6.5*

[a] Values and ADD are identical to Table 1.
* $P < 0.05$; vehicle was saline.

Table 3
Effects of L-histidine on amygdaloid kindled seizures[a]

Drug (g/kg)	*N*	Seizure rank	ADD (s)
Vehicle	6	5 ± 0	83.1 ± 9.1
L-histidine 1.0	6	5 ± 0	84.9 ± 9.6
Vehicle	6	5 ± 0	93.0 ± 11.0
L-histidine 1.5	6	5 ± 0	96.6 ± 12.7

[a] Values and ADD were identical to Table 1. Vehicle was saline.

3. Results

3.1. Effects of thioperamide on kindled seizures (Table 1)

Rats treated with thioperamide showed class 5 seizures at both doses of 20 and 30 mg/kg without significant difference from rats treated with control vehicle. ADDs in rats treated with both doses were also not significantly different from those in rats treated with control vehicle.

In a rat treated with the larger dose, AD was followed by bursts of high amplitude sharp waves.

In another rat treated with the larger dose, AD was followed by many afterspikes.

3.2. Effects of betahistine on kindled seizures (Table 2)

Betahistine induced hypotonia and leg splaying.

Rats treated with betahistine showed class 5 seizures at both doses of 200 and 400 mg/kg without significant difference from those with control vehicle.

ADDs in rats treated with a dose of 200 mg/kg were not significantly different from those with control vehicle.

On the other hand, ADDs in rats treated with a dose of 400 mg/kg were significantly reduced as compared with those with control vehicle ($P < 0.05$).

3.3. Effects of L-histidine on kindled seizures (Table 3)

Rats treated with L-histidine showed class 5 seizures at both 1.0 and 1.5 g/kg without significant difference from those with control vehicle.

ADDs in rats treated with both 1.0 and 1.5 g/kg were also not significantly different from those with control vehicle.

4. Discussion

Exogeneously administered histamine, its precursor aminoacid histidine or H_1-agonistic substances, has been reported to show inhibitory

effects against electroshock seizures or chemically-induced seizures.

It has been reported that thioperamide, a selective H_3-antagonist, has both actions of releasing histamine and synthesising histamine (Schwartz et al., 1991).

Thioperamide has been reported to increase wakefulness in rats, differing from clinically-used antiepileptic drugs, which commonly induce sleepiness as untoward effects (Lin et al., 1990; Monti et al., 1991).

Therefore, if this substance would show an anticonvulsant effect, it might become a useful antiepileptic drug.

Thioperamide has been reported to reduce each phase of electrically-induced convulsions and elevate the seizure threshold in mice, though it was not so effective as to abolish convulsions (Yokoyama et al., 1993).

On the other hand, thioperamide has not been reported to alter either pentetrazole seizure threshold or electroconvulsive threshold in mice (Scherkl et al., 1991). Thioperamide has been reported to rather increase the severity of clonic convulsions induced by picrotoxin in mice (Sturman et al., 1994).

Amygdaloid kindling has been used as an experimental model of temporal lobe epilepsy.

High densities of H_3-receptors in amygdaloid complex have been demonstrated at central, lateral and basolateral nuclei (Schwartz et al., 1991).

Nevertheless, it has not been reported, whether H_3-antagonists would show an inhibitory effect against amygdaloid kindled seizures until now.

The present study revealed that thioperamide, a standard H_3-antagonist, showed no anticonvulsant activity against either behavioural seizures or ADDs in amygdaloid kindled seizures.

Thioperamide at the higher dose seemed to rather intensify seizure discharges in some of rats.

Intraventriclarly-administered histamine or H_1-agonists has been reported to inhibit amygdaloid-kindled seizures (Kakinoki et al., 1995). Enough histamine to attenuate kindled seizures might not be released by thioperamide from presynaptic histaminergic nerve terminals in the present study. However, L-histidine, the precursor aminoacid of histamine, at a dose of 1.0 or 1.5 g/kg, was not shown to attenuate kindled seizures in the present study. Though our findings did not conform to those described by Kamei et al. (1998), they conformed to those reported by Wada et al. (1996). Furthermore, Wada et al. showed that L-histidine facilitated the development of kindling. These findings were also contrary to the facilitating effects of H_1-antagonists such as pyrilamine or ketotifen on the development of amygdaloid kindling in rats (Yokoyama et al., 1996).

Somewhat proconvulsant effects observed on EEG in some of rats might be attributed to special features of H_3-receptors as heteroreceptors. H_3-receptors have been reported to modulate the release of other neurotransmitters, such as, dopamine, noradrenaline, serotonin, acetylcholine and recently that of GABA or glutamate (Schlicker et al., 1994). Alternatively, histamine might have no anticonvulsant action against this epilepsy model. Actually, Haas et al. have reported that histamine increased the burst activity of pyramidal cells in the CA3 region and potentiated NMDA responses in hippocampal neurons (Vorobjev et al., 1993; Yanovsky and Haas, 1998). Becker (1993) has reported that thioperamide was unable to block the histamine enhancement of NMDA-mediated synaptic transmission. Selbach et al. (1997) suggested that biogenic amines including histamine could play a role in epileptic foci.

Betahistine has been reported to possess not only H_3-antagonistic action but also H_1-agonistic action (Arrang et al., 1985).

Betahistine at a high dose significantly reduced ADD, albeit mildly, though behavioural seizures were not affected. However, this inhibitory effect of betahistine against kindled seizures was questionable, since this effect was induced by the toxic dose.

In conclusion, not only thioperamide, a standard H_3-antagonist, but also betahistine, a H_3-antagonist with H_1-agonistic action, did not suppress amygdaloid kindled seizures in rats. In addition, L-histidine, the precursor aminoacid of histamine, did not suppress seizures of this kind.

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