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Blockers for excitatory effects of achatin-I, a tetrapeptide having a D-phenylalanine residue, on a snail neurone

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Abstract

Some histamine H_1 receptor antagonists suppressed the inward current (I_{in}) of an *Achatina* identifiable neurone type, PON (periodically oscillating neurone), caused by an *Achatina* endogenous tetrapeptide having a D-phenylalanine residue, achatin-I (Gly-D-Phe-Ala-Asp), under voltage clamp. Achatin-I was applied locally to the neurone by brief pneumatic pressure ejection and antagonists were administered by perfusion. The dose-response curves of the effective histamine H_1 antagonists indicated their potency order to suppress the I_{in} as follows: chlorcyclizine, promethazine, triprolidine and homochlorcyclizine > trimeprazine and clemastine > diphenylpyraline. The potent drugs were mostly piperazine and phenothiazine types. The effects of chlorcyclizine, promethazine and triprolidine on the dose (the duration of the pressure ejection)-response curve of achatin-I indicated that these drugs affected the I_{in} caused by achatin-I in a non-competitive manner. The antagonists for the receptors of the small-molecule neurotransmitters other than histamine H_1, such as histamine H_2, acetylcholine, γ-aminobutyric acid (GABA), L-glutamic acid, dopamine, α- and β-adrenalin and 5-hydroxytryptamine, had no effect on the I_{in} caused by achatin-I.

Keywords: Histamine H_1 receptor antagonist, blocking effect; Neuropeptide; Achatin-I; (Snail)

1. Introduction

A neuroexcitatory tetrapeptide having a D-phenylalanine residue (Gly-D-Phe-Ala-Asp), termed achatin-I, was isolated from the ganglia of an African giant snail (*Achatina fulica* Férussac). Among achatin-I and its stereoisomers, only achatin-I produced an inward current (I_{in}) of the *Achatina* identifiable giant neurones, indicating that the excitatory effects are stereo-specific for this peptide (Kamatani et al., 1989). Also, among achatin-I and more than 20 compounds related to this peptide, only achatin-I caused markedly the I_{in} of these neurones, indicating the effects to be structure-specific for this peptide (Kim et al., 1991a). Ten of the 23 *Achatina* giant neurone types tested were excited by achatin-I, but none was inhibited. We proposed that this peptide is an excitatory neurotransmitter for these neurones. The I_{in} caused by this peptide was due to an increase in the membrane permeability to Na^+ (Kim et al., 1991b).

Achatin-I in a low concentration enhanced the I_{in} caused by 5-hydroxytryptamine on the *Achatina* giant neurones, and suppressed the I_{in} caused by oxytocin and the outward current (I_{out}) caused by APGW-amide (Ala-Pro-Gly-Trp-NH_2), a tetrapeptide isolated from the ganglia of a prosobranchia (*Fusinus ferrugineus*) (Kuroki et al., 1990) and *Achatina fulica* (Liu et al., 1991b). These findings suggested that achatin-I acts not only as a neurotransmitter but also as a neuromodulator for the *Achatina* giant neurones (Liu and Takeuchi, 1993a,b).

The aim of the present study was to determine the blockers for the I_{in} caused by achatin-I, using an *Achatina* giant neurone type, PON (periodically oscillating neurone). After testing the effects of the drugs, which have been considered to be the receptor antagonists for the small molecule neurotransmitters, such as

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histamine H_1 and H_2, acetylcholine, γ-aminobutyric acid (GABA), L-glutamic acid, dopamine, α- and β-adrenalin and 5-hydroxytryptamine, we found that some histamine H_1 receptor antagonists inhibited the I_{in} of this neurone type caused by achatin-I.

2. Materials and methods

2.1. Preparations

African giant snails (*Achatina fulica* Férussac) were brought by air from Cebu and Manila (Philippines). A giant neurone type identified in the suboesophageal ganglia, PON (periodically oscillating neurone), was used throughout the present experiments. Localization in the ganglia and sensitivities to the small-molecule putative neurotransmitters and the neuroactive peptides of this neurone type were previously reported (Takeuchi et al., 1985, 1987; Liu et al., 1991a). Suboesophageal ganglia containing PON were dissected, and incubated with 0.67% trypsin (type III, Sigma Chemical, USA) for 5–10 min at room temperature (21 ± 1°C). Connective tissue covering the ganglia was removed with fine forceps to denude PON. The ganglia were then fixed on a Sylgard layer by means of a suction pipette and fine tungsten wires in an experimental chamber of about 0.3 ml volume.

2.2. Electrophysiological arrangements

The conventional voltage clamp technique using two microelectrodes implanted into a neurone soma (Okamoto et al., 1976) was adopted. Electrical resistances of the microelectrodes filled with 2 M potassium acetate were $2–5 \times 10^6$ Ω. Membrane voltage was kept at −55 mV (holding voltage (V_h)), near the resting potential of the neurone type. The inward current (I_{in}) caused by achatin-I was recorded with a pen-writing lecticorder, and stored on video tape using a videocorder via a signal converter.

2.3. Compounds used

Synthetic achatin-I (Gly-D-Phe-Ala-Asp) was donated by Dr. K. Nomoto, of the Suntory Institute for Bioorganic Research, Japan. The following drugs were also donated: doxepin hydrochloride (Pfizer Pharmaceutical, Japan), promethazine hydrochloride (Shionogi, Japan), cyproheptadine hydrochloride (Banyu Pharmaceutical, Japan), cimetidine (SmithKline Beecham Seiyaku, Japan), ranitidine hydrochloride (Nippon Glaxo, Japan), famotidine (Yamanouchi Pharmaceutical, Japan), nizatidine (Eli Lilly, USA), roxatidine acetate (Teikoku Hormone Mfg., Japan), pitrazepin, pizotifen hydrogenmaleate and methysergide hydrogenmaleate (Sandoz, Switzerland), sulpiride, alprenorol hydrochloride and metoprolol tartrate (Fujisawa Pharmaceutical, Japan), domperidone (Kyowa Hakko Kogyo, Japan), mianserine hydrochloride (Organon International, Netherlands) and propranolol hydrochloride (ICI, UK). The following drugs were obtained commercially: diphenhydramine hydrochloride, carbinoxamine maleate, tripelennamine hydrochloride, pyrilamine maleate, antazoline hydrochloride, (+)-chlorpheniramine maleate, (±)-brompheniramine maleate, trimeprazine hemi-(+)-tartrate, cyclizine hydrochloride, chlorcyclizine hydrochloride, homochlorcyclizine dihydrochloride, triprolidine hydrochloride, atropine sulfate, bicuculline methiodide, phentolamine hydrochloride and yohimbine hydrochloride (Sigma Chemical), diphenylpyraline teoclate and clemastine fumarate (Wako Pure Chemical, Japan), *d*-tubocurarine chloride (Nacalai Tesque, Japan), 3-((±)-2-carboxypiperazin-4-yl)-propyl-1-phosphonic acid (CPP) and D-(−)-2-amino-5-phosphonopentanoic acid (D-AP5) (Tocris Neuramin, UK).

2.4. Application methods

Achatin-I was dissolved in snail physiological solution (Takeuchi et al., 1973), and filled into a micropipette at 3×10^{-4} M together with 0.5% Fast Green (Sigma Chemical). The tip of the micropipette was placed near the neurone to be tested, and the root was connected with polyethylene tubes to a nitrogen cylinder via an electromagnetic valve. Brief pneumatic pressure ejection (mainly 2×10^5 Pa and 400 ms in duration) of the achatin-I solution, locally to the neurone to be tested, was performed by opening the valve by current application.

To examine their suppressing effects on the I_{in} caused by achatin-I, these drugs were dissolved in physiological solution at 10^{-4} M for the screening trials, and applied by perfusion into the experimental chamber at a constant speed of 2.2 ml/min.

2.5. Statistics

The data obtained in the present study were expressed as means ± S.E.M. in n trials. To compare the two results obtained from a neurone, the two-tailed Student's t-test for paired data was performed. Data from repeated measurements on a neurone were analyzed by analysis of variance (ANOVA) followed by Bonferroni's t-test (Glantz, 1987). Values were considered to be significantly different at $P < 0.05$.

The dose-response curves of a compound were analyzed by the probit method (Litchfield and Wilcoxon, 1949) using a computer program in order to obtain the ED_{50} (the confidence limit at 95%), the ideal sigmoidal curve (r value) and the Hill coefficient (r value).

3. Results

3.1. Inward current (I_{in}) caused by achatin-I

Local application of the endogenous tetrapeptide, achatin-I, to an *Achatina* neurone type, PON (periodically oscillating neurone), by brief pneumatic pressure ejection (2×10^5 Pa, 400 ms in duration, 3×10^{-4} M and 10 min intervals) produced an inward current (I_{in}) of the neurone, 0.82 ± 0.05 nA (mean ± S.E.M.) ($n = 60$), in the physiological solution under voltage clamp. The synaptic influences of the achatin-I effects, which were observed sometimes after its bath application, were prevented as far as possible with this way of application.

The I_{in} values caused by the achatin-I ejection in the same manner were stable for at least 50 min. The relation between the time course (min) and the current (nA) was obtained from the linear regression as follows ($n = 10$): Y (nA) = 0.88095 − 0.00257 X (min). None of the six results obtained for this time course was significantly different from the others according to the analysis of variance (ANOVA) for repeated measurements followed by Bonferroni's t-test (Fig. 1A).

3.2. Suppression of achatin-I-induced I_{in} by histamine H_1 receptor antagonists

Among the compounds recognized as receptor antagonists for the small-molecule neurotransmitters, some histamine H_1 receptor antagonists, perfused in the experimental chamber at 10^{-4} M for the screening trials, suppressed the I_{in} of PON caused by brief pressure ejection of achatin-I. Further, effective histamine H_1 receptor antagonists were examined at various concentrations, 3×10^{-5} M, 3×10^{-4} M and 10^{-3} M, in order to establish their dose-response curves. The classification of the histamine H_1 receptor antagonists tested and their effects obtained in the present study are summarized in Table 1.

A histamine H_1 receptor antagonist, chlorcyclizine, at 10^{-4} M suppressed the I_{in} caused by achatin-I

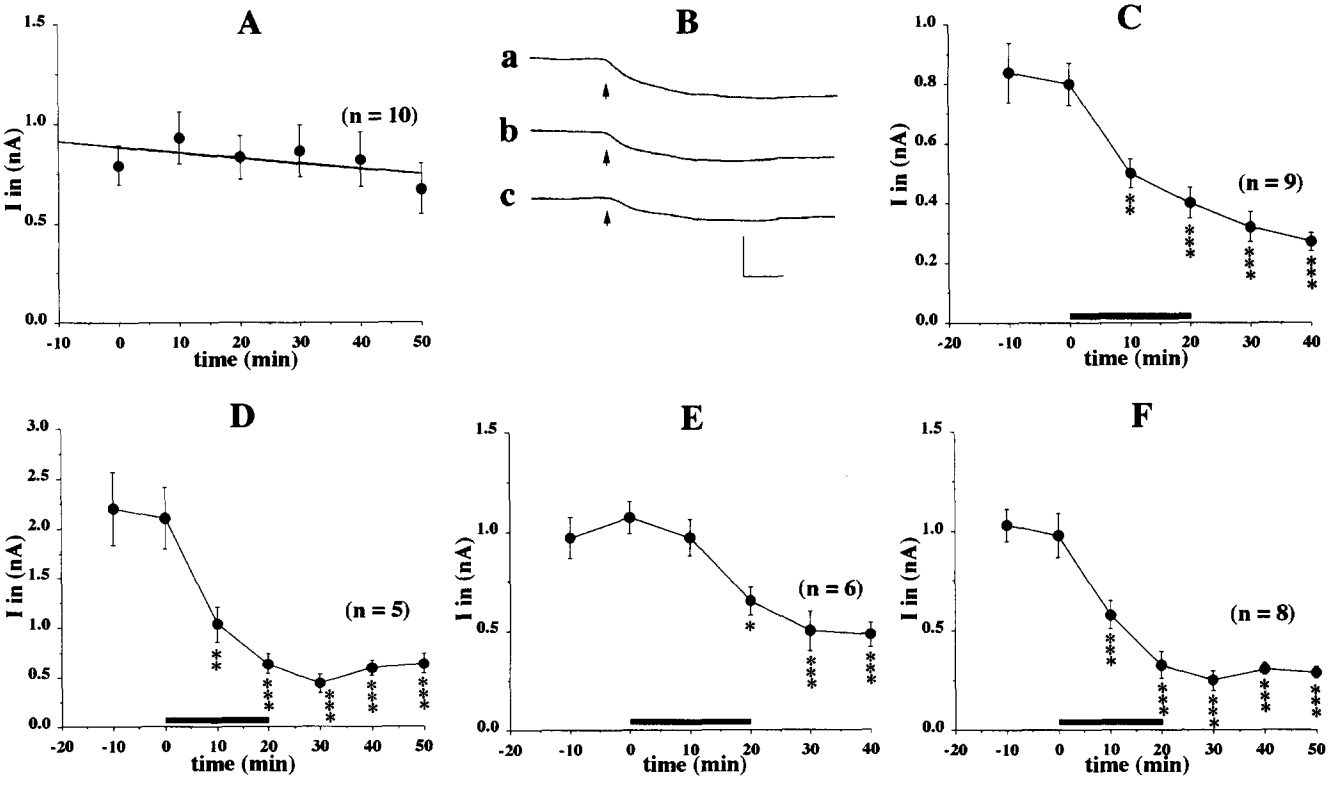

Fig. 1. Suppression by histamine H_1 receptor antagonists of the inward current (I_{in}) of PON (periodically oscillating neurone) caused by achatin-I, applied locally to the neurone by brief pneumatic pressure ejection (2×10^5 Pa, 400 ms, 3×10^{-4} M and 10 min interval), I. (A) Repetitive application of achatin-I in the physiological solution ($n = 10$). The straight line was drawn by linear regression. (B) Effects of chlorcyclizine at 10^{-4} M on the I_{in} produced by achatin-I. (a) In the physiological solution (control), (b) 10 min after drug perfusion, and (c) 20 min after. Arrows indicate achatin-I ejection. Vertical bar, calibration (2 nA); horizontal bar, time course (10 s). (C) Perfusion of chlorcyclizine at 10^{-4} M ($n = 9$) and (D) at 10^{-3} M ($n = 5$). (E) Promethazine at 10^{-4} M ($n = 6$) and (F) at 10^{-3} M ($n = 8$). In (A), (C), (D), (E) and (F): Abscissa, time course (min); ordinate, I_{in} caused by achatin-I (nA) (small vertical bar: S.E.M.). The analysis of variance (ANOVA) for repeated measurements followed by Bonferroni's t-test was performed against the mean I_{in} caused by achatin-I before drug perfusion (the control): * $P < 0.05$; ** < 0.01; *** < 0.001. In (C), (D), (E) and (F): horizontal bar shows the time of drug perfusion.

($n = 9$): 0.82 ± 0.09 nA (mean ± S.E.M.) for the mean value of two control results obtained before drug perfusion, 0.50 ± 0.05 nA ($P < 0.01$; by analysis of variance (ANOVA) for the repeated measurements followed by Bonferroni's t-test against the mean value of control data) for 10 min after perfusion, and 0.40 ± 0.05 nA ($P < 0.001$) for 20 min after. No recovery of the I_{in} suppressed by chlorcyclizine was observed up to 20 min after washing out of the drug. At 10^{-3} M ($n = 5$) this drug inhibited more markedly the I_{in} ($P < 0.01$ for 10 min after perfusion, and $P < 0.001$ for 20 min after). The drug also suppressed the I_{in} slowly after its perfusion; the suppression was sometimes almost irreversible and sometimes semi-reversible after washing out of the drug (Fig. 1B–D).

Another histamine H_1 receptor antagonist, promethazine, at 10^{-4} M also suppressed the I_{in} caused by achatin-I ($n = 6$): 1.03 ± 0.06 nA for the mean value of control data, 0.97 ± 0.09 nA (NS, not significantly different from the mean of the control) for 10 min after drug perfusion, and 0.65 ± 0.07 nA ($P < 0.05$) for 20 min after. The same drug at 10^{-3} M inhibited the I_{in} more markedly ($n = 8$) ($P < 0.001$ for 10 min and 20 min after perfusion) (Fig. 1E and F).

Triprolidine at 10^{-4} M also suppressed the I_{in} of PON caused by achatin-I ($n = 4$): 1.47 ± 0.18 nA for the mean value of control data, 1.23 ± 0.25 nA (NS) for 10 min after drug perfusion, and 0.90 ± 0.22 nA ($P < 0.05$) for 20 min after. The same drug at 3×10^{-4} M inhibited the I_{in} more markedly (Fig. 2A and B).

The I_{in} caused by achatin-I was suppressed by homochlorcyclizine at 10^{-4} M (NS and $P < 0.05$ for 10 and 20 min after perfusion, respectively), trimeprazine at 3×10^{-4} M ($P < 0.05$ and $P < 0.01$), and clemastine ($P < 0.01$) and diphenylpyraline (NS and $P < 0.05$) at 10^{-3} M, according to the drug perfusion time (Fig. 2C–F).

The other histamine H_1 receptor antagonists at 10^{-4} M for the screening trials, listed in Table 1, showed no effect on the I_{in} caused by achatin-I.

3.3. Dose-response curves of effective histamine H_1 receptor antagonists

The dose-response curve of the drug for suppression of I_{in} was established in accordance with the I_{in} levels caused by achatin-I, suppressed 20 min after drug perfusion at each of the various concentrations, in

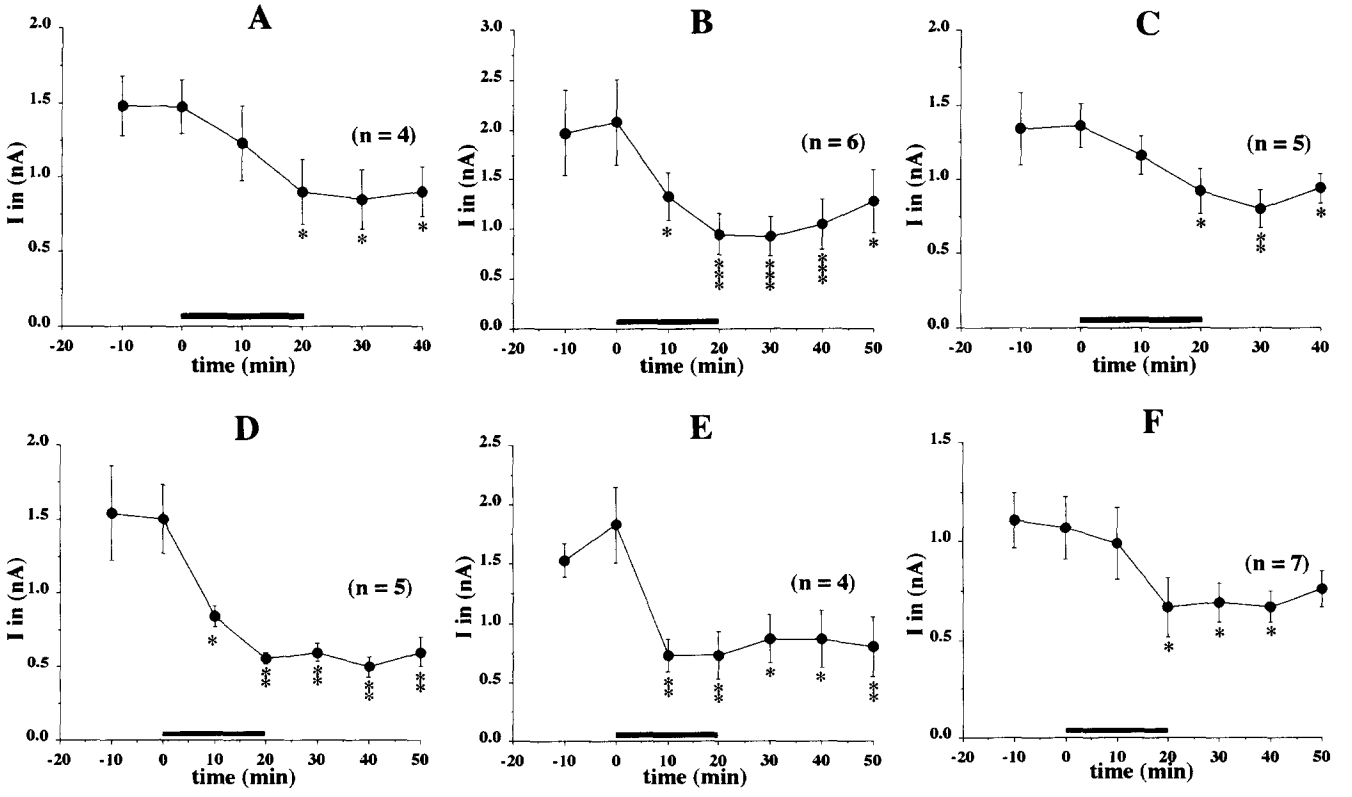

Fig. 2. Suppression by histamine H_1 receptor antagonists of the I_{in} of PON caused by achatin-I, ejected by brief pressure (2×10^5 Pa, 400 ms, 3×10^{-4} M and 10 min interval), II. (A) Triprolidine perfusion at 10^{-4} M ($n = 4$) and (B) at 3×10^{-4} M ($n = 6$). (C) Homochlorcyclizine at 10^{-4} M ($n = 5$). (D) Trimeprazine at 3×10^{-4} M ($n = 5$). (E) Clemastine at 10^{-3} M ($n = 4$). (F) Diphenylpyraline at 10^{-3} M ($n = 7$). Abscissa, time course (min); ordinate, I_{in} caused by achatin-I (nA) (small vertical bar: S.E.M.). The horizontal bar shows the perfusion time of the drug. ANOVA for repeated measurements followed by Bonferroni's t-test was performed against the mean I_{in} caused by achatin-I before drug application (control).

relation to those before drug perfusion (the control) (Fig. 3). The effective histamine H_1 receptor antagonists suppressed the I_{in} in a dose-dependent manner. The curves obtained were analyzed by the probit method using a computer program. The ED_{50} of these drugs and their confidence limits at 95% obtained in this way are described in Table 1.

The Hill coefficient values of the drugs were also calculated from these curves and were as follows: 0.3407 ($r = 0.9992$) for chlorcyclizine, 0.5384 ($r = 0.9428$) for promethazine, 0.9382 ($r = 0.9822$) for triprolidine, 0.9099 ($r = 0.9980$) for homochlorcyclizine, 0.4974 ($r = 0.9480$) for trimeprazine, 0.7632 ($r = 0.9872$) for clemastine and 0.5308 ($r = 0.9828$) for diphenylpyraline.

3.4. Effects of histamine H_1 receptor antagonists on dose (duration of pressure ejection)-response curve of achatin-I

The dose (duration of pressure ejection)-response curve of achatin-I was measured by varying the duration of the brief pressure ejection (10^5 Pa, 3×10^{-4} M, 5 min intervals), from 30 ms to 500 ms, instead of changing the concentration. The synaptic influences of the achatin-I effects were thus minimized. In the control experiments, the dose (pressure duration)-response curves of achatin-I were measured in this manner in the physiological solution twice (first and second control curves) from one PON without any drug, using a 30-min interval between the end of the first control curve and the start of the second control curve ($n = 8$). The ED_{50} (confidence limit at 95%), the Hill coefficient (r value) and the E_{max} (measured at 400 ms in duration) (mean ± S.E.M.) were 51.2 ms (25.0–78.9 ms), 1.5466 ($r = 0.9580$) and 0.95 ± 0.12 nA, respectively, for the first control curve; and 56.6 ms (2.7–131.1 ms), 1.2117 ($r = 0.8952$) and 0.94 ± 0.14 nA for the second control curve. It was confirmed by Student's t-test for paired data that no I_{in} of the second control curve was significantly different (NS) from those obtained with the same pressure durations in the first control curve (Fig. 4A). Therefore, it was concluded that the two dose (pressure duration)-response curves of achatin-I, the one in the physiological solution (control curve) and the other with a drug (test curve), could be measured from one PON.

The effects of the three effective histamine H_1 receptor antagonists, chlorcyclizine, promethazine and triprolidine, on the dose (pressure duration)-response curves of achatin-I were examined in the manner de-

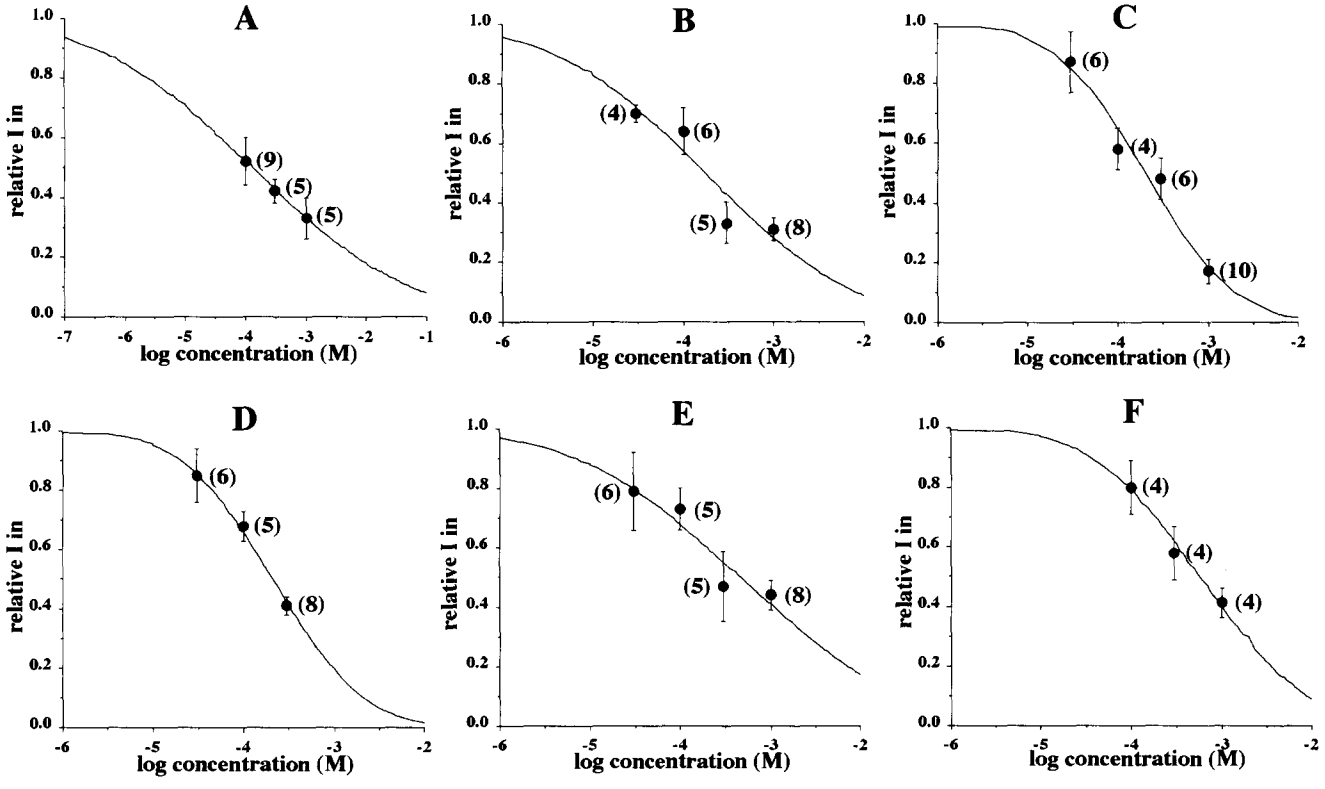

Fig. 3. Dose-response curves for the effective histamine H_1 receptor antagonist suppression of the I_{in} of PON caused by brief pressure ejection (2×10^5 Pa, 400 ms, 3×10^{-4} M and 10 min interval) of achatin-I. (A) Chlorcyclizine perfusion. (B) Promethazine. (C) Triprolidine. (D) Homochlorcyclizine. (E) Trimeprazine. (F) Clemastine. Abscissa, drug concentration on logarithmic scale (M); ordinate, relative value of I_{in} suppressed 20 min after drug perfusion, to that obtained before drug perfusion (control) (small vertical bar: S.E.M.). Numbers of trials are given in parentheses. Curves were drawn by fitting the ideal sigmoidal curves calculated with a computer program.

scribed above. Test curve measurement was begun 30 min after drug perfusion, which commenced immediately after the control curve was established.

In the case of chlorcyclizine at 10^{-5} M ($n = 7$), the ED_{50} (confidence limit at 95%), the Hill coefficient (r value) and the E_{max} (at 300 ms) (mean ± S.E.M.) were 47.5 ms (7.5–71.9 ms), 1.5654 ($r = 0.9989$) and 1.99 ± 0.32 ± nA, respectively, for the control curve, against 34.5 ms (5.8–55.4 ms), 1.1596 ($r = 0.9982$) and 1.11 ± 0.11 nA ($P < 0.05$ against control curve datum), respectively, for the test curve. The ED_{50} of the test curve was somewhat shorter (not significantly) as to pressure duration than for the control curve, possibly because the drug effects were not yet saturated 30 min after perfusion. The I_{in} obtained with the four pressure durations of the test curve were significantly lower than for the control curve, according to Student's t-test for paired data ($P < 0.05$).

In the case of promethazine at 10^{-5} M ($n = 5$), the ED_{50}, the Hill coefficient and the E_{max} (at 400 ms) were 59.7 ms (49.2–72.4 ms), 1.0991 ($r = 0.9969$) and 1.76 ± 0.22 nA, respectively, for the control curve, against 42.6 ms (12.0–71.1 ms), 0.9070 ($r = 0.9809$) and 0.92 ± 0.23 nA ($P < 0.01$), respectively, for the test curve. The ED_{50} of the test curve was slightly (but not significantly) shorter than that of the control curve. The I_{in} obtained with the four pressure durations of the test curve were significantly lower than those of the control curve ($P < 0.05$ at 50 and 500 ms; $P < 0.01$ at 300 and 400 ms) (Fig. 4C and E).

In the case of triprolidine at 10^{-5} M ($n = 5$), the ED_{50}, the Hill coefficient and the E_{max} were 35.1 ms

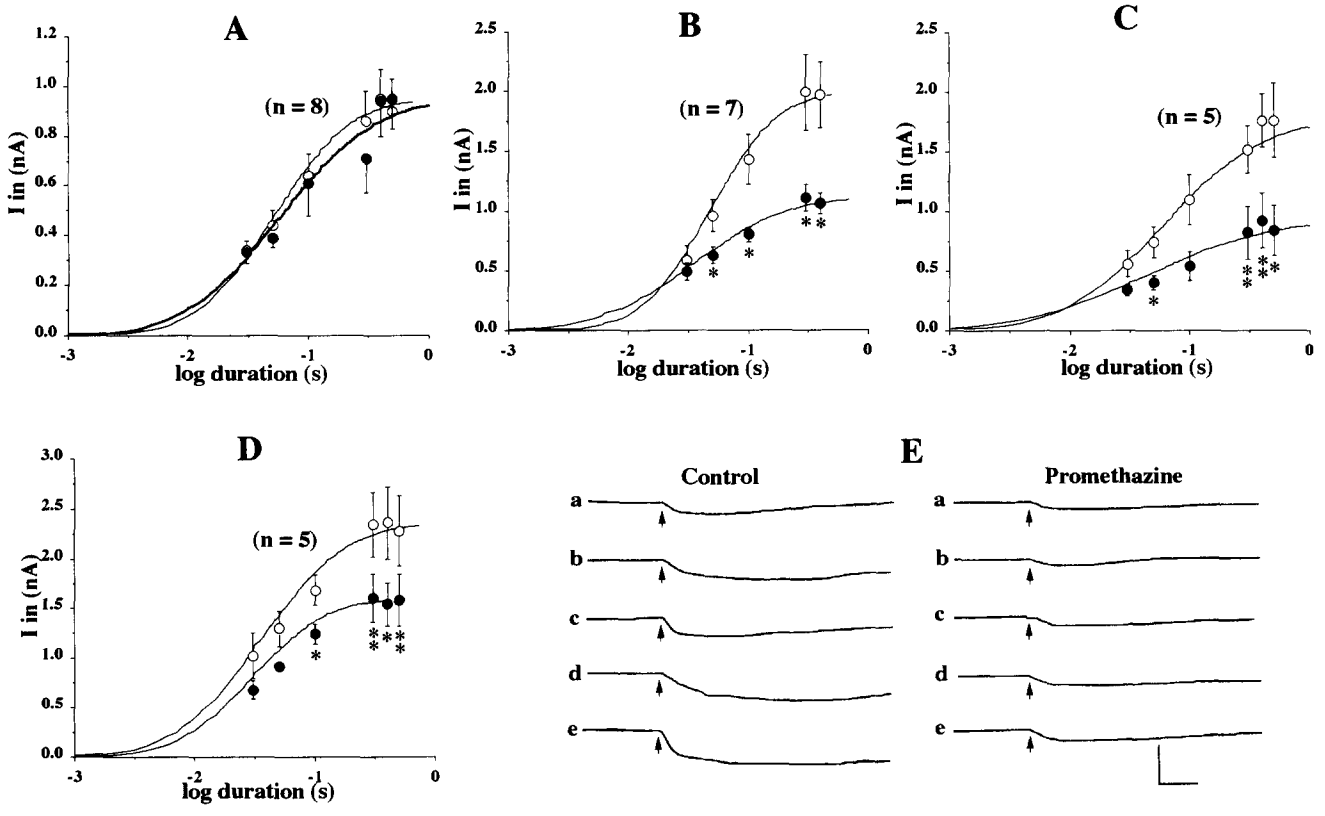

Fig. 4. Effects of the three histamine H_1 receptor antagonists at 10^{-5} M on the dose (duration of pressure ejection)-response curves of achatin-I for PON, obtained by varying the brief pneumatic pressure duration (10^5 Pa, 3×10^{-4} M and 5 min interval). (A) Two dose (pressure duration)-response curves of achatin-I (first and second control curves) were measured in the physiological solution from one PON ($n = 8$). Interval between the end of the first control curve measurement (○) and the start of the second control curve (●) (shown in a thick line) was 30 min. Small upward bar, S.E.M. for first control curve; small downward bar, S.E.M. for second control curve. (B) Effects of chlorcyclizine on the dose (pressure duration)-response curve of achatin-I ($n = 7$). (C) Promethazine ($n = 5$). (D) Triprolidine ($n = 5$). In (B), (C) and (D): interval between the end of measurement of first curve obtained in the physiological solution (control curve) (○) and the start of the second curve with a drug (test curve) (●) was 30 min. Small vertical bar, S.E.M. In (A), (B), (C) and (D): abscissa, pressure duration of the pneumatic ejection of achatin-I on logarithmic scale (s); ordinate, I_{in} caused by achatin-I. The I_{in} values of the test curve were compared with those of the corresponding pressure duration of control curve by Student's t-test for paired data. Curves were drawn by fitting the ideal sigmoidal curves calculated with a computer program. (E) Effects of promethazine at 10^{-5} M on the I_{in} caused by achatin-I, ejected with the various pressure durations. Left column, in the physiological solution (control); right column, with promethazine perfusion (test). (a) 30 ms of pressure duration, (b) 50 ms, (c) 100 ms, (d) 300 ms and (e) 400 ms. Arrows indicate achatin-I ejection. Vertical bar, calibration (2 nA); horizontal bar, time course (10 s).

Table 1
Classification of the histamine H_1 receptor antagonists tested and their suppressing effects on the inward current (I_{in}) of PON (periodically oscillating neurone) caused by achatin-I

A. Aminoalkylethers	
1. Diphenhydramine	Ineffective
2. Diphenylpyraline	Sup: 26.86×10^{-4} M ($1.67-\times10^{-4}$ M)
3. Carbinoxamine	Ineffective
4. Clemastine	Sup: 5.67×10^{-4} M ($0.92-\times10^{-4}$ M)
B. Ethylenediamines	
1. Tripelenamine	Ineffective
2. Pyrilamine	Ineffective
3. Antazoline	Ineffective
C. Alkylamines	
1. (+)-Chlorphenylamine	Ineffective
2. (±)-Bromophenylamine	Ineffective
3. Doxepin	Ineffective
D. Phenothiazines	
1. *Promethazine*	Sup: 1.73×10^{-4} M ($0.08-\times10^{-4}$ M)
2. Trimeprazine	Sup: 4.58×10^{-4} M ($1.18-\times10^{-4}$ M)
E. Piperazines	
1. Cyclizine	Ineffective
2. *Chlorcyclizine*	Sup: 1.28×10^{-4} M ($0.73-2.83\times10^{-4}$ M)
3. *Homochlorcyclizine*	Sup: 2.10×10^{-4} M ($0.70-\times10^{-4}$ M)
F. Others	
1. *Triprolidine*	Sup: 2.01×10^{-4} M ($0.96-8.10\times10^{-4}$ M)
2. Cyproheptadine	Ineffective

The four drugs showing marked effects are italicized. Sup, suppressing effects: ED_{50} (confidence limit at 95%). Ineffective, at 10^{-4} M in the screening trials.

(−80.8 ms), 1.2527 ($r = 0.9913$) and 2.36 ± 0.36 nA (at 400 ms), respectively, for the control curve, against 29.6 ms (22.7–38.7 ms), 1.4730 ($r = 0.9999$) and 1.60 ± 0.24 nA (at 300 ms) ($P < 0.05$), respectively, for the test curve. The I_{in} obtained with the four pressure durations of the test curve were significantly lower than those of the control curve ($P < 0.05$ at 100 and 400 ms; $P < 0.01$ at 300 and 500 ms) (Fig. 4D).

3.5. Effects of drugs other than histamine H_1 receptor antagonists

The effects of the following drugs, which were recognized as the receptor antagonists for the small molecule neurotransmitters, other than histamine H_1 receptor antagonists, on the I_{in} of PON caused by achatin-I, were also examined: histamine H_2 receptor antagonists (cimetidine, ranitidine, famotidine, nizatidine and roxatidine), acetylcholine receptor antagonists (atropine and *d*-tubocurarine), GABA receptor antagonists (bicuculline and pitrazepin), L-glutamate receptor antagonists (3-((±)-2-carboxypiperazin-4-yl)-propyl-1-phosphonic acid (CPP) and D-(−)-2-amino-5-phosphonopentanoic acid (D-AP5)), dopamine receptor antagonists (sulpiride and domperidone), α-adrenoceptor antagonists (phentolamine and yohimbine), β-adrenoceptor antagonists (alprenorol, propranolol and metoprolol) and 5-hydroxytryptamine receptor antagonists (pizotifen, methysergide and mianserine). These drugs at 10^{-4} M ($n \geq 4$) did not affect the I_{in} of the neurone caused by achatin-I.

4. Discussion

The present study forms part of an ongoing research program of serial investigations on the effects of achatin-I, an *Achatina* endogenous tetrapeptide having a D-phenylalanine residue (Kamatani et al., 1989; Kim et al., 1991a,b; Liu and Takeuchi, 1993a,b). In the course of these investigations, some histamine H_1 receptor antagonists were found to suppress the I_{in} of an *Achatina* neurone type, PON, caused by achatin-I, though the receptor antagonists for other small molecule neurotransmitters showed no effect. The ED_{50} of the effective histamine H_1 receptor antagonists, found from their dose-response curves to suppress the I_{in} caused by achatin-I, indicated a potency order as follows: chlorcyclizine (piperazine type), promethazine (phenothiazine type), triprolidine (other type) and homochlorcyclizine (piperazine type) > trimeprazine (phenothiazine type) and clemastine (aminoalkylether type) > diphenylpyraline (aminoalkylether type). The histamine H_1 receptor antagonists showing these marked effects were mostly piperazine and phenothiazine types.

Less than half the histamine H_1 receptor antagonists tested suppressed the I_{in} caused by achatin-I, making it unlikely that these drugs affected the achatin-I receptors which were pharmacologically similar to those of histamine H_1. Further, achatin-I produced markedly the I_{in} of PON, whereas histamine had almost no effect on this neurone type (Takeuchi et al., 1985, 1987). Thus, histamine cannot be an agonist of achatin-I for PON.

The dose (duration of pressure ejection)-response curves of achatin-I showed that the three effective histamine H_1 receptor antagonists, chlorcyclizine, promethazine and triprolidine, markedly lowered the E_{max} of this peptide, but did not shift significantly its ED_{50}. This indicates that these drugs affected the I_{in} caused by achatin-I in a non-competitive manner. Based on these findings, it is considered that these drugs suppressed the achatin-I-induced I_{in} by modifying the features of the achatin-I receptors, by blocking the Na^+ channels for the achatin-I effects (Kim et al., 1991a), or by affecting the intracellular signalling mechanisms linked with the peptide effects. It was

previously demonstrated that the intracellular injection of adenosine 3′5′-cyclic monophosphate (cyclic AMP) and guanosine 3′5′-cyclic monophosphate (cyclic GMP) excited *Achatina* giant neurones including PON, whereas that of inositol 1,4,5-triphosphate (IP_3) inhibited these neurones (Liu and Takeuchi, 1993c).

In vertebrates, the histamine H_1 receptor antagonists, including promethazine, reportedly suppress post-synaptically the end plate potentials of the frog sartorius muscle (Katayama and Tasaka, 1985). Promethazine reduces the inositol phosphate accumulation caused by carbachol, a muscarinic acetylcholine agonist, in the longitudinal smooth muscle of the guinea-pig ileum (Donaldson and Hill, 1985). Histamine H_1 receptor antagonists, including promethazine, homochlorcyclizine and clemastine, have a high affinity for binding with muscarinic receptors in the bovine cerebral cortex (Kubo et al., 1987). The histamine H_1 receptor antagonists, including promethazine, inhibit agonist binding to σ-opioid and muscarinic receptors in the rat brain (Gray et al., 1990). These findings indicate that the histamine H_1 receptor antagonists affect systems other than the histamine transmission system. Also, the histamine H_1 receptor antagonists, including homochlorcyclizine, clemastine and triprolidine, inhibited in human neutrophils the release of arachidonic acid (Taniguchi et al., 1991), which is a second messenger in the nervous system.

In invertebrates, promethazine has a high affinity for binding with the octopamine receptors in the neurones of a locust (*Locusta migratoria, L.*) (Roeder, 1990).

Our ongoing research on achatin-I effects using *Achatina* giant neurones will attempt to elucidate in more detail the structure needed to suppress the I_{in} caused by achatin-I through tests on the effects of derivatives of the effective histamine H_1 receptor antagonists. The effects of these receptor antagonists on the I_{in} caused by other compounds, such as 5-hydroxytryptamine and oxytocin, will be examined. Signalling mechanisms of achatin-I excitation will also be studied in a fundamental investigation on the effects of this peptide.

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