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ORIGINAL PAPER

Histamine H_3 Receptor Agonists Decrease Hypothalamic Histamine Levels and Increase Stereotypical Biting in Mice Challenged with Methamphetamine

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Abstract The effects of the histamine H_3 receptor agonists (*R*)-α-methylhistamine, imetit and immepip on methamphetamine (METH)-induced stereotypical behavior were examined in mice. The administration of METH (10 mg/kg, i.p.) to male ddY mice induced behaviors including persistent locomotion and stereotypical behaviors, which were classified into four categories: stereotypical head-bobbing (1.9%), circling (1.7%), sniffing (14.3%), and biting (82.1%). Pretreatment with (*R*)-α-methylhistamine (3 and 10 mg/kg, i.p.) significantly decreased stereotypical sniffing, but increased stereotypical biting induced by METH, in a dose-dependent manner. This effect of (*R*)-α-methylhistamine on behavior was mimicked by imetit or immepip (brain-penetrating selective histamine H_3 receptor agonists; 10 mg/kg, i.p. for each drug). Hypothalamic histamine levels 1 h after METH challenge were significantly increased in mice pretreated with saline. These increases in histamine levels were significantly decreased by pretreatment with histamine H_3 receptor agonists, effects which would appear to underlie the shift from METH-induced stereotypical sniffing to biting.

Keywords Methamphetamine · Histamine · Hypothalamus · Behavior

Abbreviations

ANOVA	Analysis of variance.
HPLC	High-performance liquid chromatography.
METH	Methamphetamine.
SEM	Standard error of the mean.

Introduction

In rodents, amphetamines produce locomotor hyperactivity at lower doses that is replaced by repetitive, compulsive behaviors termed "stereotypical behavior" or "stereotypies" at higher doses [1–3]. A single administration of methamphetamine (METH) in rats or mice induces stereotypical behavior including sniffing, biting, head-bobbing, and circling. Once initiated, the stereotypical behavior persists for several hours [1–6]. Overall frequency of stereotypical behavior is reduced by dopamine receptor antagonists [7, 8],

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suggesting that METH-induced stereotypy depends mainly on the activity of dopaminergic neurons in the brain. However, the composition of individual behavioral components of METH-induced stereotypy can be regulated by agents other than dopamine receptor antagonists. For instance, we examined the effects of metoprine and SKF 91488, inhibitors of histamine *N*-methyltransferase [9, 10], the sole enzyme catabolizing histamine in the mammalian brain [11, 12], on METH-induced stereotypical behavior in mice. These findings provided us with an approach to evaluate agents by their ability to modulate two independent components of METH-induced stereotypy: the overall frequency (i.e. intensity) of stereotyped behavior and the distribution of distinct behavioral subcomponents (i.e. expression pattern).

Our previous results indicated that these histamine *N*-methyltransferase inhibitors alter the pattern of METH-induced stereotypies, producing a shift from biting to sniffing, without affecting the overall frequency of stereotypies [13]. Increases in hypothalamic histamine levels may mediate this phenomenon [13]. These observations suggest an approach to examining these phenomena by using histamine H_3 receptor agonists that inhibit release and synthesis of histamine at the presynaptic level [14, 15]. In the present study, we investigated the effects of pretreatment with histamine H_3 receptor agonists on METH-induced stereotypies and hypothalamic histamine levels in mice, in order to understand a possible contribution of histamine H_3 receptor signaling to METH-induced stereotypical behavior, especially the expression pattern.

Experimental Procedure

Subjects

Male ICR mice (10–12 weeks old; Japan SLC, Shizuoka, Japan) were housed in groups of eight (cage size: 37 × 22 × 15 cm) in a temperature—(22 ± 2°C) and humidity—(50 ± 10%) controlled environment under a 12 h light/dark cycle (lights on at 07:00) with food and water available ad libitum except during testing. The observation of stereotypical behavior was performed by trained observers (see Rating of stereotypical behavior). Animal handling and care were conducted according to the *Guide for the Care and Use of Laboratory Animals* (7th edition, Institute of Laboratory Animal Resources-National Research Council, National Academy Press 1996), and all experiments were reviewed and approved by our Institutional Animal Research Committee. Mice were used only once (body weight on experimental day: 35–48 g, $n = 84$ total) after at least one-week habituation in the facility.

Reagents

METH hydrochloride was purchased from Dainippon Sumitomo Pharma Co., Ltd (Osaka, Japan). (*R*)-α-Methylhistamine dihydrochloride, imetit dihydrobromide (5-[2-(Imidazol-4-yl)ethyl]isothiourea dihydrobromide), and immepip dihydrobromide (4-(1H-Imidazol-4-ylmethyl) piperidine dihydrobromide) were obtained from Sigma–Aldrich (St. Louis, MO, USA). All other chemicals used were of the highest purity commercially available.

Treatment Protocols

Preparation of Reagents

METH and all histamine H_3 receptor agonists were dissolved in sterile saline on the day of the experiment. The drug solutions were prepared in such a way that the necessary dose could be injected intraperitoneally (i.p.) in a volume of 0.1 ml/10 g of body weight. The doses of the reagents refer to the weight of salt. The doses of METH and the histamine H_3 receptor agonists were chosen based on previous literature [13, 16–19]. Regarding the METH dose, METH-induced stereotypy is dose-dependent; lower doses stimulate locomotion but not lead to stereotypical behavior. For instance, about only 30% of mice exhibited stereotypy when 5 mg/kg of METH was used (authors' unpublished observation). Since the intention of this study was to examine individual components of METH-induced stereotypy, the higher dose (i.e. 10 mg/kg) was chosen so that all subjects would exhibit stereotypy.

Effect of (R)-α-Methylhistamine Pretreatment on METH-Induced Stereotypy

On the day of the experiment, mice ($n = 48$) were weighed and randomly divided into six groups ($n = 8$ per group). The subjects were pretreated with saline or (*R*)-α-methylhistamine (3 or 10 mg/kg, i.p.) 1 h prior to METH (or saline control) treatment. This period of pretreatment was chosen because a significant alteration in histamine metabolism was reported at this time point [16]. After the pretreatment period, mice were challenged with saline or 10 mg/kg METH and then placed in the test apparatus immediately after the injection to assess stereotypic behavior for 1 h as described below.

Effect of Imetit or Immepip Pretreatment on METH-Induced Stereotypy

On the day of the experiment, mice ($n = 36$) were weighed and randomly divided into six groups ($n = 6$ per group). The subjects were pretreated with saline, 10 mg/kg (i.p.)

imetit, or 10 mg/kg (i.p.) immepip, followed by saline or 10 mg/kg METH 1 h later. After the challenge injection, all mice were placed in the test apparatus in order to assess stereotypic behavior for 1 h as described below.

Rating of Stereotypical Behavior

Test subjects were placed in a transparent acrylic test box and observed for stereotypy for 1 h after drug administration by observers unaware of the treatments. Behavior was assessed in 30-s intervals, and the predominant behavior observed during each interval was recorded. Since individual stereotypical behaviors were unchanged for long periods (>30 s) after drug treatment, it was possible to record the observations by hand. The behaviors scored were inactive (awake and inactive, or sleeping), ambulating, rearing, persistent locomotion, head bobbing (up-and-down movements of the head), continuous sniffing, circling, and continuous nail and/or wood chip biting or licking, according to a method described previously [20]. Ambulating, rearing, and persistent locomotion were considered locomotor and exploratory behaviors, and the last four categories were considered stereotypies. Persistent locomotion was not classified as stereotypy because the mice scored as having "persistent locomotion" showed horizontal locomotor activity less than or equal to that displayed by mice showing "hyperlocomotion" (which is not generally defined as a stereotypy) measured by Animex Auto [20, 21]. The cumulative number of intervals within each 5 min period in which stereotypies were rated is shown as a time course below (maximal value = 10).

Measurement of Histamine and N^{τ}-Methylhistamine Contents

After the behavioral analyses, mice were sacrificed by cervical dislocation. Their brains were immediately removed, and the region of hypothalamus was dissected, weighed, and frozen in liquid nitrogen. Histamine and N^{τ}-methylhistamine contents were measured by the high-performance liquid chromatography (HPLC)-fluorescence method using *o*-phthalaldehyde as described previously [13]. In brief, each frozen hypothalamic sample was homogenized with a Teflon/glass homogenizer in 5 volumes (wt/vol) of ice-cold 0.1 N perchloric acid/30 μM Na_2EDTA, containing N^{π}-methylhistamine as an internal standard. After boiling at 100°C for 5 min, the homogenates were centrifuged at 10,000 × *g* for 10 min at 4°C, and the supernatants were filtered through a 0.20-μm membrane filter (Millipore Co., Bedford, MA, USA). The mobile phase was a 131:100 (vol/vol) mixture of buffer (60 mM KH_2PO_4 and 0.4% triethylamine) and acetonitrile-methanol (2:3, vol/vol), and the flow rate was set at 0.9 ml/min. The HPLC column was a 5-μm Ultrasphere ODS high-resolution end-capped column (internal diameter = 4.0 mm; length = 150 mm; Chemco Scientific Co., Ltd., Osaka, Japan). The filtrates (20 μl) were reacted with *o*-phthalaldehyde in an alkaline medium to form an unstable fluorescent adduct and injected directly into the HPLC system. The fluorescence of samples was determined using a spectrofluorometer (type FP-210, JASCO, Tokyo, Japan) at an excitation wavelength of 310 nm and a detection wavelength of 375 nm. Data were analyzed using the Chromatopac C-R4A (operation system version 2.7) (Shimadzu Co., Kyoto, Japan).

Statistics

Data are presented as mean ± the standard error of the mean (SEM). Statistical analysis was performed using mixed factor analysis of variance (ANOVA) with or without repeated measures followed by Bonferroni/Dunn *post-hoc* tests (Statview 5.0 for Apple Macintosh, SAS Institute, Inc., Cary, NC, USA). For the HPLC analysis, data were analyzed by Fischer's PLSD tests when ANOVA showed significant main effect(s). $P < 0.05$ was considered statistically significant.

Results

The Effect of (*R*)-α-Methylhistamine on METH-Induced Stereotypy

Figure 1 shows the time course of the frequency of all types of stereotypical behavior after METH (or saline vehicle) challenge in mice. There was an increase in the overall frequency of stereotypy in the mice after METH challenge, as compared to that after saline challenge, beginning at 10 min post-injection, reaching a maximum at 20 min post-injection, and continuing unabated for the duration of the test session. Pretreatment with 3 or 10 mg/kg (*R*)-α-methylhistamine did not affect the overall frequency of stereotypical behaviors displayed by METH- or saline-challenged mice. A repeated-measures ANOVA (challenge × pretreatment × time) applied to the data represented in Fig 1 yielded significant main effects of METH challenge ($F(1,42) = 27{,}869$, $P < 0.0001$) and time ($F(12,546) = 669$, $P < 0.0001$), but no significant main effect of (*R*)-α-methylhistamine pretreatment ($F(2,42) = 0.816$, $P = 0.4490$). This analysis also yielded significant METH challenge × time interaction ($F(12,546) = 664$, $P < 0.0001$), but no significant (*R*)-α-methylhistamine pretreatment × time ($F(24,546) = 0.507$, $P = 0.9765$), (*R*)-α-methylhistamine pretreatment × METH challenge ($F(2,42) = 0.117$, $P = 0.8902$), or (*R*)-α-methylhistamine pretreatment × METH

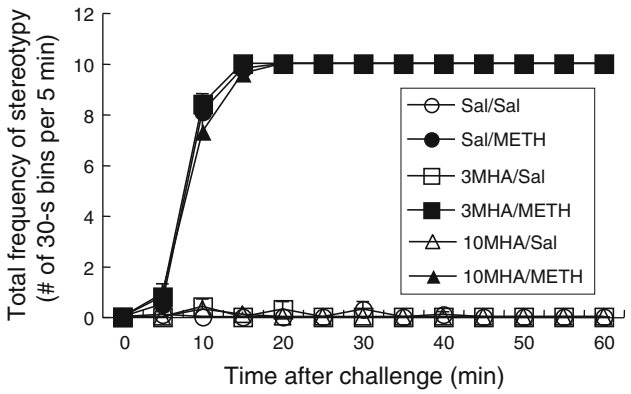

Fig. 1 Frequencies of stereotypy after a single administration of methamphetamine in mice pretreated with (*R*)-α-methylhistamine or vehicle. Values are shown as the mean ± SEM ($n = 8$). METH: methamphetamine (10 mg/kg, i.p.); 3MHA: 3 mg/kg (i.p.) (*R*)-α-methylhistamine; 10MHA: 10 mg/kg (i.p.) (*R*)-α-methylhistamine; Sal: saline (vehicle)

challenge × time interactions ($F(24,546) = 0.813$, $P = 0.7222$). *Post-hoc* pair-wise comparisons showed significant differences in time course between 5 min and 10-60 min and between 10 min and 15–60 min (Bonferroni/Dunn test, $P < 0.05$).

Four categories of stereotypical behaviors were observed, and the frequency of each behavior was measured for 1 h (Fig. 2a–d). The total count of all observed stereotypical behaviors (i.e., stereotyped head-bobbing + circling + sniffing + biting) is shown in Fig. 2e. The count of persistent locomotion is shown in Fig. 2f. METH challenge increased the frequency of each category of stereotypical behavior and persistent locomotion, compared with saline treatment. Two-way ANOVA ((*R*)-α-methylhistamine pretreatment x METH challenge) was applied separately for each behavior shown in Fig. 2. ANOVA showed significant main effects of METH challenge for stereotypical head-bobbing ($F(1,42) = 25.110, P < 0.0001$), circling ($F(1,42) = 60.632$, $P < 0.0001$), sniffing ($F(1,42) = 6.946$, $P < 0.05$), biting ($F(1,42) = 13{,}045$, $P < 0.0001$), and persistent locomotion ($F(1,42) = 66.964$, $P < 0.0001$). Regarding the METH challenge, *post-hoc* comparisons indicated significant differences in the frequencies of the four stereotypical behavior components and persistent locomotion between the METH-challenged and saline-challenged mice (Bonferroni/Dunn test, $P < 0.05$). Furthermore, pretreatment with (*R*)-α-methylhistamine (3 or 10 mg/kg) significantly affected the expression of biting and sniffing, but not head-bobbing, circling, or persistent locomotion. Thus, (*R*)-α-methylhistamine pretreatment significantly increased the incidence of stereotypical biting ($F(2,42) = 22.265$, $P < 0.0001$) and significantly reduced the incidence of stereotypical sniffing ($F(2,42) = 42.861$, $P < 0.0001$), but there was no significant main effect of (*R*)-α-methylhistamine pretreatment on stereotypical head-bobbing ($F(2,42) = 0.916$, $P = 0.4079$), circling ($F(2,42) = 0.237$, $P = 0.7902$), or persistent locomotion ($F(2,42) = 0.480$, $P = 0.6223$). As shown in Fig. 2e, the total incidence of stereotypy was increased by METH challenge, compared with that in the saline-treated mice. ANOVA yielded a significant main effect of METH challenge ($F(1,42) = 27{,}846$, $P < 0.0001$), but no significant effect of (*R*)-α-methylhistamine pretreatment ($F(2,42) = 0.087$, $P = 0.9166$) or METH challenge x (*R*)-α-methylhistamine pretreatment interaction ($F(2,42) = 0.012$, $P = 0.9876$) on the overall incidence of stereotypy.

Effect of Imetit or Immepip Pretreatment on METH-Induced Stereotypy

The effects of the selective histamine H_3 receptor agonists imetit and immepip on METH-induced stereotypies and persistent locomotion were examined.

Figure 3 shows the time course of the frequency of all types of stereotypical behavior after METH (or saline vehicle) challenge in mice. As before, there was an increase in the overall frequency of stereotypy in mice after METH challenge, as compared to that after saline challenge, beginning at 10 min post-injection, reaching a maximum at 20 min post-injection, and continuing unabated for the duration of the test session. Pretreatment with 10 mg/kg imetit did not affect the overall incidence of stereotypical behaviors displayed by METH- or saline-challenged mice. Pretreatment with 10 mg/kg immepip did have some small effect on the overall incidence of stereotypical behavior displayed by the METH- and saline-challenged mice at a time point of 10 min after drug challenge. In the case of both drugs, once the maximal effects of METH had been reached, there was no affect of the H_3 histamine agonists on the overall incidence of stereotypy. A repeated-measures ANOVA (challenge × pretreatment, with time as a repeated measure) applied to the data represented in Fig 3 yielded significant main effects of METH challenge ($F(1,30) = 30{,}939$, $P < 0.0001$) and time ($F(12,390) = 569$, $P < 0.0001$), but no significant main effect of imetit/immepip pretreatment ($F(2,30) = 0.842$, $P = 0.4410$). This analysis also yielded significant METH challenge × time ($F(12,390) = 588$, $P < 0.0001$) and imetit/immepip pretreatment × time ($F(24,390) = 1.579$, $P < 0.05$), and imetit/immepip pretreatment × METH challenge × time interactions ($F(24,390) = 2.843$, $P < 0.0001$), but no significant imetit/immepip pretreatment × METH challenge interaction ($F(2,30) = 1.381$, $P = 0.2669$). *Post-hoc* pair-wise comparisons showed significant differences in time course between 5 min and 10–60 min and between 10 min and 15–60 min (Bonferroni/Dunn test, $P < 0.05$). At a time point of 10 min after

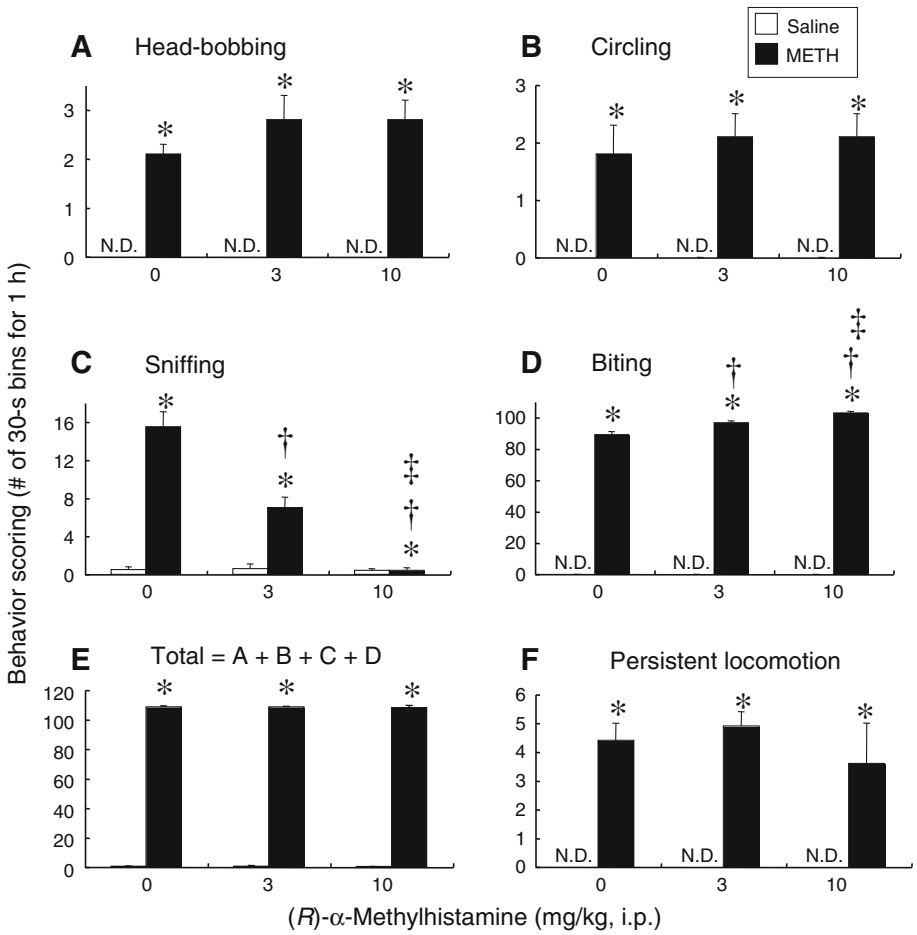

Fig. 2 Different types of stereotypical behavior in response to saline or methamphetamine in mice pretreated with (*R*)-α-methylhistamine or vehicle. Values are shown as the mean ± SEM ($n = 8$). N.D., not detected. METH: methamphetamine (10 mg/kg, i.p.). $^{*}P < 0.05$, compared with the Saline challenge group (open column; Bonferroni/Dunn test). $^{\dagger}P < 0.05$, compared with the METH challenge group pretreated with 0 mg/kg (*R*)-α-methylhistamine (Bonferroni/Dunn test). $^{\ddagger}P < 0.05$, compared with the METH challenge group pretreated with 3 mg/kg (*R*)-α-methylhistamine (Bonferroni/Dunn test)

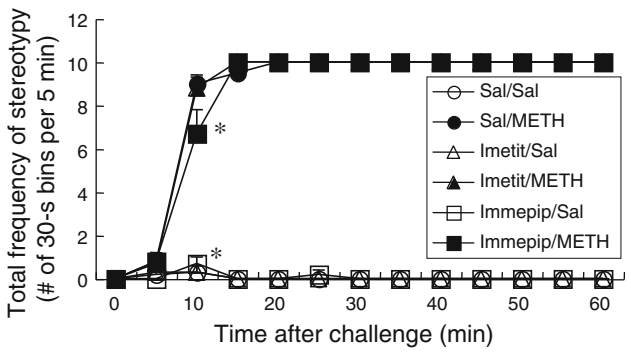

Fig. 3 Frequencies of stereotypy after a single administration of methamphetamine in mice pretreated with 10 mg/kg imetit, 10 mg/kg immepip, or vehicle. Values are shown as the mean ± SEM ($n = 6$). METH: methamphetamine (10 mg/kg, i.p.); Sal: saline (vehicle). $^{*}P < 0.05$, compared with corresponding group pretreated with saline or imetit

drug challenge, total stereotypy was decreased and increased significantly in METH- and saline-challenged mice pretreated with immepip, respectively (Bonferroni/Dunn test, $P < 0.05$).

Four categories of stereotypical behaviors were observed, and the frequency of each behavior was measured for 1 h (Fig. 4a–d). The total count of all observed stereotypical behaviors is shown in Fig. 4e. The count of bins showing persistent locomotion is shown in Fig. 4f. METH challenge increased the frequency of each category of stereotypy and persistent locomotion, compared with saline treatment. Two-way ANOVA (selective histamine H_3 receptor agonist pretreatment × METH challenge) was applied separately for each behavior shown in Fig. 4. ANOVA showed significant main effects of METH challenge for stereotypical head-bobbing ($F(1,30) = 74.128$, $P < 0.0001$), circling ($F(1,30) = 23.217$, $P < 0.0001$), sniffing ($F(1,30) = 270.474$, $P < 0.0001$), biting ($F(1,30) = 15{,}698$, $P < 0.0001$), and persistent locomotion ($F(1,30) = 95.369$, $P < 0.0001$). Regarding the METH challenge, *post-hoc* comparisons indicated significant differences in the frequencies of the four stereotypical behavior components and persistent locomotion between the METH-challenged and saline-challenged mice (Bonferroni/Dunn test, $P < 0.05$). Pretreatment with imetit and immepip significantly affected the incidence of biting and sniffing, but not head-bobbing, circling, or persistent locomotion. Thus, imetit/immepip pretreatment significantly increased stereotypical biting ($F(2,30) = 59.017$,

$P < 0.0001$) but significantly reduced stereotypical sniffing ($F(2,30) = 172.951$, $P < 0.0001$), while having no significant effects on stereotypical head-bobbing ($F(2,30) = 0.436$, $P = 0.6505$), circling ($F(2,30) = 0.096$, $P = 0.9092$), or persistent locomotion ($F(2,30) = 3.528$, $P = 0.2259$). *Post-hoc* comparisons showed that both imetit and immepip significantly reduced stereotypical sniffing and increased stereotypical biting. However, as shown in Fig. 2e, although the total incidence of stereotypy was increased by METH challenge, compared with that observed in saline-treated mice there were no effects of either H_3 agonist on the overall amount of stereotypy. ANOVA yielded a significant main effect of METH challenge ($F(1,30) = 30{,}674$, $P < 0.0001$), but no significant effect of imetit/immepip pretreatment ($F(2,30) = 0.755$, $P = 0.4788$) or METH challenge x imetit/immepip pretreatment interaction ($F(2,30) = 1.193$, $P = 0.3172$).

Hypothalamic Histamine and N^{τ}-Methylhistamine Contents

A significant alteration in histamine metabolism has been reported in mice 1–2 h after (*R*)-α-methylhistamine treatment [16]. Therefore, histamine and N^{τ}-methylhistamine contents were determined in mice 1 h after METH challenge (i.e. 2 h after (*R*)-α-methylhistamine pretreatment) (Fig. 5).

ANOVA applied to the data represented in Fig. 5a identified significant main effects of (*R*)-α-methylhistamine pretreatment ($F(2,42) = 9.899$, $P < 0.001$) and METH challenge ($F(1,42) = 10.601$, $P < 0.01$) and a significant (*R*)-α-methylhistamine pretreatment × METH challenge interaction on hypothalamic histamine content ($F(2,42) = 5.016$, $P < 0.05$). *Post-hoc* comparisons showed that METH challenge significantly increased histamine content compared with saline-challenged subjects ($P < 0.01$) and that 3 and 10 mg/kg (*R*)-α-methylhistamine pretreatment significantly decreased the hypothalamic histamine content of mice compared with saline pretreatment prior to METH challenge (Fischer's PLSD test, $P < 0.05$). However, (*R*)-α-methylhistamine pretreatment alone (e.g. after saline challenge) had no effect on hypothalamic histamine content in mice compared with saline pretreatment (Fischer's PLSD test, $P > 0.05$). ANOVA applied to the data represented in Fig. 5b identified a significant main effect of (*R*)-α-methylhistamine pretreatment ($F(1,42) = 8.049$, $P < 0.01$), and a significant (*R*)-α-methylhistamine pretreatment x METH challenge interaction, on hypothalamic N^{τ}-methylhistamine content ($F(2,42) = 5.328$, $P < 0.01$), but no significant main effect of METH challenge

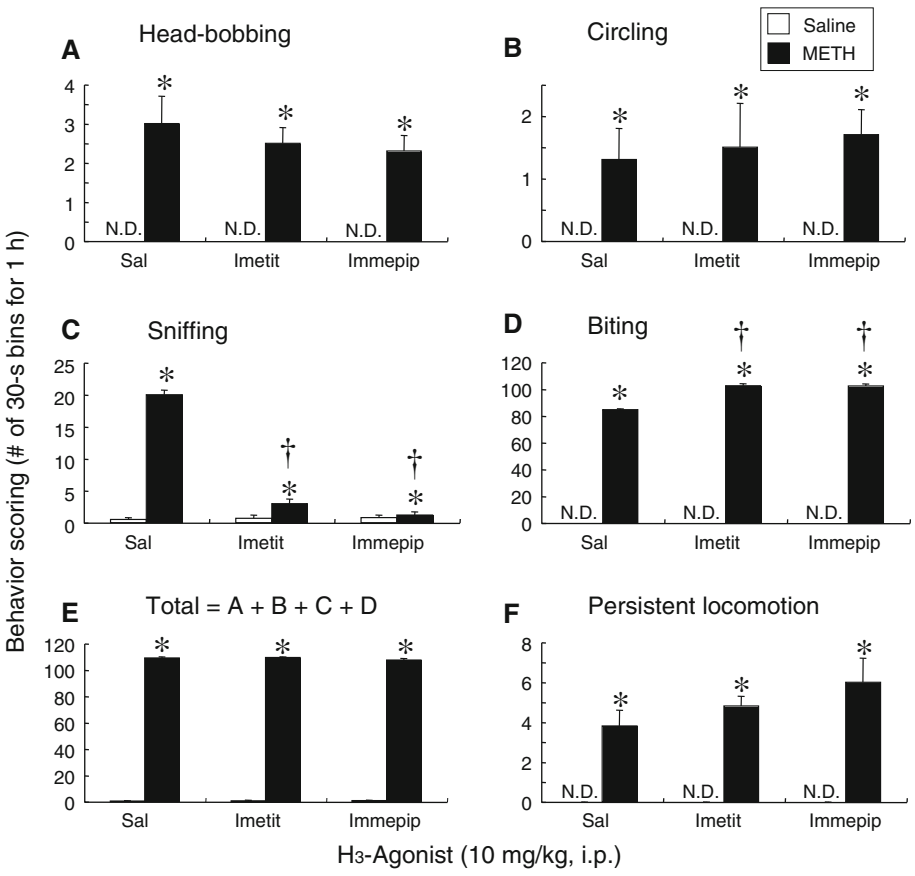

Fig. 4 Different types of stereotypical behavior in response to saline or methamphetamine in mice pretreated with 10 mg/kg imetit, 10 mg/kg immepip, or vehicle. Values are shown as the mean ± SEM ($n = 6$). N.D., not detected. METH: methamphetamine (10 mg/kg, i.p.). *$P < 0.05$, compared with the Saline challenge group (open column; Bonferroni/Dunn test). †$P < 0.05$, compared with the METH challenge group pretreated with saline (Bonferroni/Dunn test)

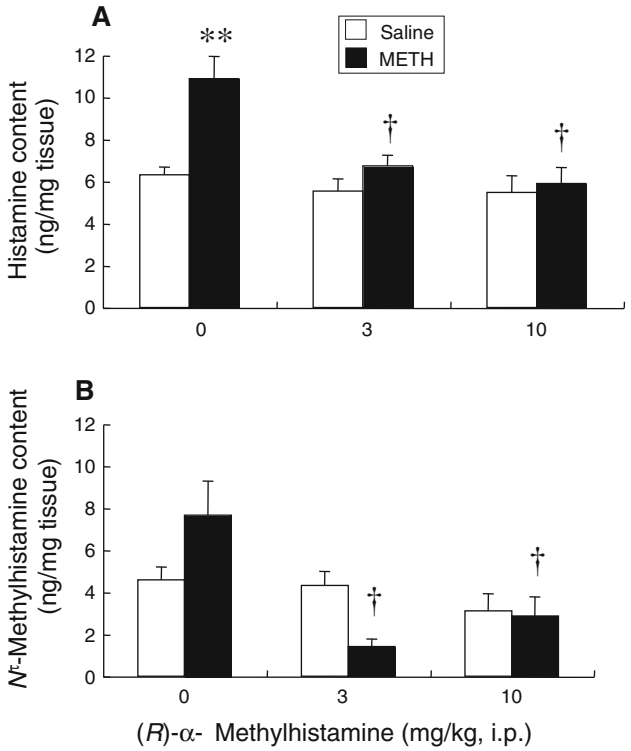

Fig. 5 Hypothalamic histamine **a** and N^{τ}-methylhistamine content **b** in mice pretreated with 3 and 10 mg/kg (*R*)-α-methylhistamine. Values are shown as the mean ± SEM ($n = 8$). METH: methamphetamine (10 mg/kg, i.p.). $^{**}P < 0.01$, compared with corresponding saline-challenged mice (Fischer's PLSD test). $^{\dagger}P < 0.05$, compared with saline-pretreated subjects (Fischer's PLSD test)

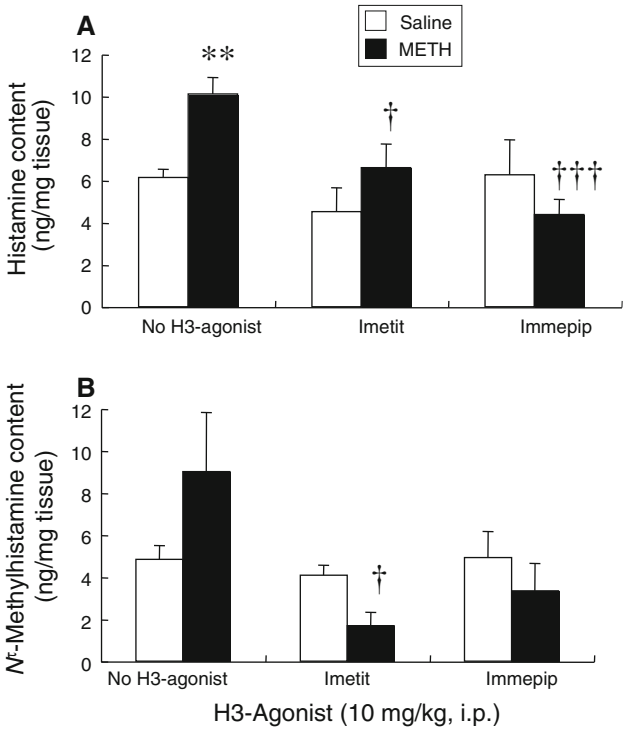

Fig. 6 Hypothalamic histamine **a** and N^{τ}-methylhistamine content **b** in mice pretreated with 10 mg/kg imetit and immepip. Values are shown as the mean ± SEM ($n = 6$). METH: methamphetamine (10 mg/kg, i.p.). $^{**}P < 0.01$, compared with corresponding saline-challenged mice (Fischer's PLSD test). $^{\dagger}P < 0.05$, $^{\dagger\dagger\dagger}P < 0.001$, compared with saline-pretreated subjects (Fischer's PLSD test)

($F(2,42) = 0.001$, $P = 0.9710$). *Post-hoc* comparisons showed that 3 and 10 mg/kg (*R*)-α-methylhistamine pretreatment significantly decreased hypothalamic N^{τ}-methylhistamine content compared to saline pretreatment after a challenge dose of METH (Fischer's PLSD test, $P < 0.05$). However, (*R*)-α-methylhistamine pretreatment alone had no effect on the hypothalamic N^{τ}-methylhistamine content compared with saline pretreatment.

The effects of imetit and immepip on the tissue contents of histamine and N^{τ}-methylhistamine were also analyzed. The tissue contents of histamine and N^{τ}-methylhistamine, shown in Fig. 6, were determined in the hypothalamus of mice for which the behavioral data represented in Figs. 3 and 4.

ANOVA applied to the imetit pretreatment data represented in Fig. 6a showed significant main effects of imetit pretreatment ($F(1,20) = 7.997$, $P < 0.05$) and METH challenge for histamine content ($F(1,20) = 11.156$, $P < 0.01$), but no significant imetit pretreatment x METH challenge interaction for histamine content ($F(1,20) = 1.082$, $P = 0.3108$). *Post-hoc* comparisons showed that METH challenge significantly increased histamine content compared with saline-challenged subjects (Fischer's PLSD test, $P < 0.01$) and that imetit pretreatment significantly decreased the hypothalamic histamine content of mice challenged with METH compared with saline pretreatment (Fischer's PLSD test, $P < 0.05$). However, imetit pretreatment alone had no effect on the hypothalamic histamine content of mice challenged with saline.

ANOVA applied to the immepip pretreatment data represented in Fig. 6A showed a significant main effect of immepip pretreatment ($F(1,20) = 7.795$, $P < 0.05$) and a significant immepip pretreatment x METH challenge interaction for histamine content ($F(1,20) = 8.573$, $P < 0.01$), but no significant overall effect of METH challenge on histamine content ($F(1,20) = 1.076$, $P = 0.3119$). *Post-hoc* comparisons showed that immepip pretreatment significantly decreased the hypothalamic histamine content of mice challenged with METH compared with saline pretreatment (Fischer's PLSD test, $P < 0.001$). However, immepip pretreatment alone had no effect on the hypothalamic histamine content of mice challenged with saline.

ANOVA applied to the imetit pretreatment data represented in Fig. 6b showed a significant main effect of imetit pretreatment ($F(1,20) = 7.310$, $P < 0.05$) and a significant imetit pretreatment × METH interaction for N^{τ}-methylhistamine content ($F(1,20) = 9.064$, $P < 0.01$), but no

significant main effect of METH challenge for N^{τ}-methylhistamine content ($F(1,20) = 0.356, P = 0.5574$). *Post-hoc* comparisons showed that imetit pretreatment significantly decreased hypothalamic N^{τ}-methylhistamine content in METH-challenged mice compared with saline-pretreated subjects (Fischer's PLSD test, $P < 0.05$), but not in the saline-challenged mice.

ANOVA applied to the immepip pretreatment data in Fig. 6b showed no significant main effects of immepip pretreatment ($F(1,20) = 2.707$, $P = 0.1155$) or METH challenge for N^{τ}-methylhistamine content ($F(1,20) = 0.579$, $P = 0.4555$) and no significant immepip pretreatment × METH interaction for N^{τ}-methylhistamine content ($F(1,20) = 2.865$, $P = 0.1060$).

Discussion

The main findings of the present study are that (1) pretreatment of mice with the histamine H_3 receptor agonists (*R*)-α-methylhistamine, imetit, and immepip significantly decreased stereotypical sniffing and increased stereotypical biting induced by METH, and (2) that pretreatment with histamine H_3 receptor agonists significantly decreased hypothalamic histamine levels, but only in mice challenged with METH. The actions of the histamine H_3 receptor agonists may be mediated by presynaptic histamine H_3 receptors. (*R*)-α-methylhistamine dose-dependently decreased METH-induced stereotypical sniffing and hypothalamic histamine levels (Figs. 2c, 5a) while increasing METH-induced stereotypical biting (Fig. 2d). The selective histamine H_3 receptor agonists imetit and immepip, that have quite different chemical structures from (*R*)-α-methylhistamine, had quite similar behavioral (Fig. 4) and neurochemical (Fig. 6) effects on METH actions. Present findings are parallel to previous results showing that METH-induced stereotypical sniffing was significantly increased in mice pretreated with the selective histamine H_3 receptor antagonists thioperamide and clobenpropit [20].

Although there is evidence supporting the previous analysis, that these effects are mediated by actions at presynaptic autoreceptors, there are other potential mechanisms that might be postulated to mediate these effects. Histamine H_3 receptors have been suggested to form heterodimers with dopamine D_2 receptors [18], and to antagonize D_2 receptor function, which may provide an alternative mechanism for some of the effects observed in the present study. Histamine H_3 receptors have also been suggested to have less direct interactions with dopamine D_1 receptors [22, 23]. However, because there is no broad effect upon the overall amount of stereotypy in the current study, it appears unlikely that these histaminergic affects universally affect dopaminergic stimulation produced by METH, and, as discussed below, may only affect dopamine D_2 receptors in particular striatal subregions. This may, somewhat paradoxically, be consistent with another study that failed to observe histamine H_3-dopamine D_2 interactions in the striatum [24] if different striatal regions were sampled in that study compared to this one. In further support of this argument the distribution of histamine H_3 receptors has been suggested to vary across the striatum with a strong dorsal–ventral gradient from areas of lower to higher expression [25, 26]. Thus, if histamine H_3 receptor agonists, based on the differential distribution of histamine H_3 receptors, produce a greater antagonism of ventral striatal dopamine D_2 receptors than dorsal striatal dopamine D_2 receptors, then it might be expected to produce a shift in the stereotypical effects of METH from more ventrally mediated dopaminergic behaviors to more dorsally mediated behaviors. Although interesting, a more detailed understanding of the regional variations in striatal histamine H_3 receptor distribution, particularly in the anterior-posterior directions, as well as regional variations in striatal histamine H_3-dopamine D_2 interactions, is needed to better evaluate this hypothesis.

Effects on histamine synthesis were also observed which, in addition to being consistent with effects on presynaptic histamine H_3 receptors, might indicate that other postsynaptic histaminergic receptor mechanisms, affected by changes in histamine release, may also interact with METH. Histamine-immunoreactive neurons are found solely in the tuberomammillary nucleus of the posterior hypothalamus in the rat brain, from which histamine-immunoreactive fiber bundles project to almost all major brain regions [27]. Consistent with this pattern of projections the brain histaminergic system is considered to widely modulate other neurotransmitter systems [11]. Measurement of hypothalamic levels of histamine and the metabolite N^{τ}-methylhistamine provides an estimate of the overall activity of the histaminergic system and its potential modulation of other brain systems that could mediate histaminergic modulation of METH actions. To our knowledge, this is the first report that acute administration with METH significantly increased tissue histamine levels in the hypothalamus of mice (Figs. 5a, 6a). In rats, METH challenge (3, but not 1, mg/kg, i.p.) significantly increased histamine release in the hypothalamic region measured by in vivo brain microdialysis [28]. The mechanism underlying this effect is unknown, but could include increased histamine synthesis and/or attenuation of metabolism by histamine *N*-methyltransferase. It seems more likely that tissue histamine levels would be more influenced by activation of histamine synthesis than by histamine release, to produce the increase in tissue histamine levels, but this

speculation would need to be clarified in further studies. With regard to this hypothesis, no alterations of histidine decarboxylase activity were observed in the diencephalon of rats challenged with METH (1–10 mg/kg) [29], but it should be noted that acute administration of METH (3 and 10 mg/kg, i.p.) significantly decreased tissue levels of histamine in the diencephalon of rats after 1 h [29]. This is inconsistent with our results (Figs. 5a, 6a) and may therefore represent regional brain variation in histamine responses to METH evaluated by these HPLC analyses (i.e. diencephalon consisting of thalamus, hypothalamus, subthalamus, and epithalamus vs. hypothalamus) or species differences (rat vs. mouse).

A mechanism that may mediate the inhibition of both histamine synthesis and release may be the activation of presynaptic histamine H_3 receptors [14, 15]. Selective histamine H_3 receptor agonists significantly attenuated the increased hypothalamic histamine levels observed after METH challenge (Figs. 5a, 6a). By contrast, pretreatment of mice with histamine H_3 receptor agonists had no effects on histamine levels after saline challenge (Figs. 5a, 6a), which is consistent with the report that treatment of mice with (*R*)-α-methylhistamine for 2 h had no effects on brain levels of histamine or N^{τ}-methylhistamine [16].

The histamine H_3 receptor antagonists thioperamide and clobenpropit potentiated METH-induced dopamine release in the shell of the nucleus accumbens [30], suggesting that activation of histamine H_3 receptors might attenuate METH-induced dopamine release and thereby attenuate some of the aberrant behavioral consequences of METH. However, in the present study, histamine H_3 receptor agonists potentiated METH-induced stereotypical biting and reduced METH-induced sniffing. A previous study found that histamine H_3 receptor antagonists had no effect on the METH-induced stereotypical biting, but enhanced METH-induced sniffing [20]. Based on this, and previous analyses [20] that have shown no overall effect of histaminergic drugs on stereotypical behavior, but rather on the type of behavior that is exhibited after METH, it would seem likely that histaminergic interactions with METH may differ in striatal subregions underlying different types of stereotypy. Decreases in hypothalamic histamine levels, or rather reversal of METH-induced increases in histamine levels, consequent histamine H_3 agonism, are likely to be associated with alterations in METH-induced stereotypical biting in mice pretreated with histamine H_3 receptor agonists (Fig. 2d). Consistent with this idea, increases in hypothalamic histamine levels induced by histamine *N*-methyltransferase inhibitors (metoprine and SKF 91488) and L-histidine are associated with decreases in METH-induced stereotypical biting [13, 20]. In line with these observations, it is suggested that levels of histamine in the hypothalamus may be negatively correlated with the incidence of METH-induced stereotypical biting.

There was a tendency toward increased tissue levels of N^{τ}-methylhistamine in mice challenged with METH (Figs. 5b, 6b). Similarly, acute administration with METH in mice increased tissue levels of N^{τ}-methylhistamine in the hypothalamus 3 h post-injection with an ED_{50} value of 0.8 mg/kg [31]. The increase was of similar magnitude in the results reported [31] and the present study (77% vs. 67 and 86% in Figs. 5b and 6b, respectively). This suggests that METH stimulates the release of histamine which, in turn, is metabolized by histamine *N*-methyltransferase in the hypothalamus. Although the physiological relevance of enhanced histamine levels in the hypothalamus after acute METH administration is still uncertain, it appears to be associated with a shift from the incidence of stereotypical biting to the incidence of stereotypical sniffing.

The total frequency of stereotypical behavior was slightly affected in mice pretreated with immepip 10 min after METH (or saline) challenge; there was a significant reduction and increase of the total frequency after drug and saline challenge, respectively, compared with mice pretreated with saline or imetit (Fig. 3) or (*R*)-α-methylhistamine (Fig. 1), suggesting that these phenomena may not be mediated by histamine H_3 receptors. This difference was only observed during the onset of stereotypy, which may indicate that at lower doses of METH, there may be broader affects of immepip on the overall incidence of stereotypy or greater differential effects on particular components of stereotypical behavior. Indeed, mice pretreated with immepip had a trend toward increases in the incidence of sniffing and persistent locomotion during the period between 5 and 10 min after saline and METH challenge, respectively (data not shown).

The incidence of METH-induced stereotypical biting appears to be associated with tissue levels of histamine in the hypothalamus of mice. The findings of the present study as well as our previous reports [13, 20] suggest that agents altering hypothalamic levels of histamine and probably affecting activity of brain histaminergic systems may alter the particular pattern, but not severity (or frequency), of stereotypical behavior induced by METH. The pattern of METH-induced stereotypical behavior appears to result from the activation of specific subregions of the striatum, since individual behavioral components of METH-induced stereotypies can be dissociated anatomically [32]. Therefore, it is likely that levels of hypothalamic histamine may either directly, or indirectly via modulation of other neurotransmitter system(s), affect dopaminergic activity in the striatum, but do so in a regionally selective manner that primarily affects the type of stereotypy observed rather than the overall amount of stereotypy.

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