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Effects of the H_3-receptor inverse agonist thioperamide on the psychomotor effects induced by acutely and repeatedly given cocaine in C57BL/6J mice

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Abstract

Previous studies have shown that histamine H_3 blockers potentiate the psychomotor and rewarding effects of cocaine. The present study examined the influence of thioperamide, an inverse H_3 receptor agonist, on the development of psychomotor sensitization and stereotyped activity induced by acute or intermittent cocaine in C57BL/6J mice. In the first experiment, mice were injected i.p. with saline, 10 or 20 mg/kg thioperamide and saline or 8 mg/kg cocaine, 10 min apart, before being tested for their locomotor activity (providing data on the acute effects of thioperamide on cocaine-induced activity). Subsequently, mice were treated in the same manner every other day over six additional sessions. Sensitization was assessed by the responsiveness to a cocaine challenge (8 mg/kg, i.p.) given 2 and 14 days following the intermittent treatment. In experiments 2 and 3, we tested the effects of thioperamide (10 or 20 mg/kg, i.p.) on gnawing and sniffing induced or affected by relatively high doses of cocaine (24 or 32 mg/kg, s.c.), the drugs being given 10 min apart. In the first experiment, both doses of thioperamide amplified cocaine-induced psychomotor hyperactivity almost on all experimental sessions. However, the histamine inverse agonist did not affect the induction of a psychomotor sensitization. All cocaine-treated mice showed similar levels of sensitized activity 2 and 14 days after the intermittent treatments, whether they received thioperamide or not. The second and the third experiments showed that thioperamide did not affect gnawing and sniffing induced by cocaine. Taken together, these results indicate that H_3 receptors clearly contribute to the neurobiological mechanisms of the locomotor component of cocaine-induced psychomotor activation, but less likely to those underlying the development of cocaine behavioral sensitization or the expression of cocaine-induced oro-facial stereotypies.

Keywords: Cocaine; C57BL/6J mice; H_3 receptors; Gnawing; Locomotion; Psychomotor sensitization; Stereotypy; Thioperamide

1. Introduction

Histamine neurons originate exclusively from the tuberomamillary nucleus (TMN) of the hypothalamus and project to the entire brain. To date, three types of histamine receptors have been identified in the brain: the H_1, H_2 and H_3 receptors. H_1 and H_2 receptors are mainly located postsynaptically and their activation leads to excitatory effects. The H_3 receptors were initially described as presynaptic autoreceptors located on histaminergic neurons, where they play a negative feedback role on histamine synthesis and release (Haas and Panula, 2003; Leurs et al., 2005). However, they are also widely distributed on non-histaminergic neurons. For example, H_3 receptors on dopaminergic and glutamatergic neurons operate as heteroreceptors inhibiting the release of dopamine and glutamate in the striatum (Schlicker et al., 1993; Molina-Hernandez et al., 2000; Molina-Hernandez et al., 2001). Histamine H_3 receptors are believed to be involved in many biological functions, such as circadian rhythms and sleep (Monti et al., 1991), antinociception (Malmberg-Aiello et al., 1994), water and food consumption (Clapham and Kilpatrick, 1993; Attoub et al., 2001) and learning and memory (Brown et al., 2001; Haas and Panula, 2003). Although the available results are conflicting, there is also growing evidence suggesting a role for histamine H_3 receptors in the behavioral effects of abused drugs. Consistently, a very high density of H_3 receptors has been found in the striatum and the nucleus accumbens, which are known to play a major role in the brain mechanisms of drug psychomotor and addictive effects (Pollard et al., 1993).

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Consistent with the high distribution of H_3 receptors in the striatum, there is evidence that the blockade of H_3 receptors can increase the neurochemical and behavioral effects of several psychomotor stimulants. For example, in vivo microdialysis studies show that the H_3 receptor inverse agonist thioperamide increases dopamine release in the rat ventral striatum in the presence of methamphetamine or cocaine (Munzar et al., 2004; Hyytiä et al., 2003). Additionally, thioperamide amplifies the subjective effects of methamphetamine and cocaine in rats trained to discriminate the subjective effects of these psychostimulants from those of saline using a drug-discrimination procedure (Mori et al., 2002; Munzar et al., 1998, 2004). The reinforcing effects of methamphetamine and cocaine can also be increased by H_3 receptors inverse agonists/antagonists. For example, thioperamide and clobenpropit, another H_3 antagonist, readily promote the intravenous self-administration of a low dose of methamphetamine (0.03 mg/kg) that was not self-administrated alone in rats (Munzar et al., 2004). Comparable results are available with cocaine self-administration in rats (Hyytiä et al., 2003) or with cocaine inducing a conditioned place preference in mice (Brabant et al., 2005). In contrast to these rewarding effects, H_3 receptor inverse agonists seem to exert opposite effects on hyperactivity induced by cocaine and other psychostimulants. Whereas thioperamide has been reported to enhance cocaine-induced locomotion in rats (Hyytiä et al., 2003) and mice (Brabant et al., 2005), it decreases the stimulant effects of amphetamine in mice (Clapham and Kilpatrick, 1994). Moreover, other potent H_3 receptor inverse agonists, such as ciproxifan and ABT-239, have been found to attenuate methamphetamine-induced psychomotor hyperactivity (Morisset et al., 2002; Fox et al., 2005). The modulation of H_3 receptor activity was also shown to alter the oro-facial stereotypies induced by apomorphine (Farzin and Attarzadeh, 2000). Whereas thioperamide potentiated apomorphine-induced licking stereotypies, the H_3 receptor agonist imetit induced the opposite effect. However, it remains unknown whether H_3 receptors are involved in the stereotypies induced by other stimulants and in particular by cocaine. Although the studies reported above indicated that the H_3 receptor activity modulates a number of acute effects of psychostimulants, such as methamphetamine and cocaine, no studies have investigated the role of these receptors in the sensitization of the psychostimulant effects after repeated administration.

The aim of the present study was to investigate the involvement of H_3 receptors in the behavioral sensitization and the stereotypies induced by cocaine in C57BL/6J mice. In a first experiment, we tested the effects of thioperamide on the development of the sensitization to the locomotor effects of cocaine after repeated administrations. Results from the first session provided information on the interactive effects between thioperamide and cocaine given acutely. Several experimental data suggest distinguishing short-term sensitization observed less than 10 days post-treatment from long-term sensitization that is observed later. These studies demonstrate that distinct neuronal mechanisms are involved in the induction of short- and long-term sensitization (Vanderschuren and Kalivas, 2000 for a review). Therefore, cocaine challenges were performed 2 and 14 days after the development phase of sensitization. A second experiment was conducted to study the effect of thioperamide on cocaine-induced gnawing activity directed onto corrugated paper, a form of oro-facial stereotypy typically caused by a relatively high dose of psychomotor stimulant. A third experiment investigated the potential effect of thioperamide on (acute) cocaine-induced sniffing and grooming considered as stereotypies in environmental conditions that were inadequate for gnawing to be expressed (no appropriate target).

2. Materials and methods

2.1. Subjects

A total of 226 male C57BL/6J mice aged 8–9 weeks at the beginning of the experiments (Central Animal Facility of the University of Liège, Belgium) were housed individually in transparent polycarbonate cages (L 26 × W 40.5 × H 20 cm) with pine sawdust bedding. Food (standard pellets, CARFIL QUALITY BVDA, Oud-Turnhout, Belgium) and water were available ad libitum for the whole experiment. The animal room was maintained on a 12:12-h light–dark cycle (lights on at 0800 h) and an ambient temperature of 19–22 °C. All experimental procedures were carried out in accordance with the standards of care and use of laboratory animals laid down by the European Communities Council (Directive No. 86/609/EEC, 24 November 1986). All experimental procedures were carried out during the light period of the light–dark cycle, between 0900 and 1300 h.

2.2. Pharmacological treatments

(−)-Cocaine hydrochloride (BELGOPIA, Louvain-La-Neuve, Belgium) and thioperamide maleate (SIGMA-ALDRICH, Bornem, Belgium) were dissolved in an isotonic saline solution (0.9% NaCl), before being administered at a volume of 0.01 ml/g body weight via the peritoneal or the subcutaneous (nape of the neck) route. The control treatment consisted of an equal volume of saline solution.

2.3. Behavioral apparatuses and experimental procedures

2.3.1. Effect of thioperamide on cocaine-induced psychomotor activation and sensitization

Locomotor activity was scored with a series of 10 individual test chambers, each one comprising a square enclosure made from 0.5 cm clear acrylglas panels without base (20.5 cm × 20.5 cm × 20.5 cm). An enclosure was placed on a square plate of 0.5 cm grey acrylglas, which served as a floor, and a removable, perforated, clear acrylglas plate served as a lid. Ambulatory activity was measured by a pair of infrared light-beam sensors located on each side of the enclosure at heights of 2 cm. Sensors were spaced 6.5 cm from each end of a side, so that the light-beams formed a matrix of 3 × 3 squares over the surface. A mouse had to traverse the full distance (at least 6.5 cm) between the beams for each activity count. Ambulatory counts were recorded by a single personal computer to which all

the testing chambers were connected. Interruptions of a single beam were not counted in the data analysis; similarly interruptions of the intersection between perpendicularly positioned beams were not recorded. Each chamber was encased in a sound-attenuating shell (100 cm × 90 cm × 150 cm height), artificially ventilated, illuminated by an "energy server" non-heating 60-W white light (625 lm), and maintained at an ambient temperature of 21–23 °C. A one-way window on each shell door allowed direct visual surveillance.

Two days prior to the intermittent treatment of 8 mg/kg cocaine (sensitization), mice were habituated to the injection procedure and to the test apparatus, receiving two saline injections 10 min apart and being confined in the testing chamber for 20 min during which their locomotor activity was measured. Mice were then randomly allocated to the six possible pharmacological conditions ($n=7$) of a design combining three treatments of thioperamide (saline, 10 or 20 mg/kg) with two treatments of cocaine (saline or 8 mg/kg). The procedure involved seven injections of either saline or cocaine (8 mg/kg, i.p.) given every other day and preceded 10 min earlier by one of the three thioperamide treatments (saline, 10 or 20 mg/kg, i.p.). Immediately after the second injection, mice were individually confined in the testing chamber for 20 min. Two and fourteen days after the last of the seven sessions, animals were submitted to sensitization tests (in the absence of thioperamide). All mice were firstly injected with saline and 10 min later with cocaine (8 mg/kg, i.p.) and then immediately placed in the testing chamber for 20 min.

2.3.2. Effect of thioperamide on cocaine-induced gnawing activity

Gnawing measurements were conducted according to the procedure of Tirelli et al. (1998) and were based on the biting-caused piercing of a corrugated paper sheet. The floor of each testing clear acrylglas chamber (25 cm × 22.5 cm × 13.5 cm) was covered with one of such sheets, the corrugations facing upward. The corrugated paper sheets, whose corrugations were 5 mm wide, 2 mm high and separated from each other by 6 mm, were used once. Gnawing was quantified subsequently by placing a metallic wire-mesh grid over the corrugated paper (6 × 6 mm meshes). A score of 1 was given for each grid mesh through which a biting-induced perforation of the paper was visible. A score of 2 was given for each grid mesh through which two unambiguous perforations of the paper were visible. When the whole surface covered by the grid mesh was completely pierced and torn out by the bites, a score of 3 was noted. The maximum possible score was 1008, but the highest value from this experiment was below 400.

A first sub-experiment was carried out to establish a partial dose–response curve for cocaine-induced gnawing in our C57BL/6J mice. The s.c. route of administration was chosen on the basis of previous studies, where gnawing was stronger when cocaine was given s.c. than when it was given i.p. (Tirelli and Witkin, 1995; Tirelli et al., 1998). Forty animals were randomly assigned to four groups ($n=10$) and were injected with saline, 16, 24 or 32 mg/kg cocaine. In a second sub-experiment, where the inability of thioperamide to induce gnawing was verified, 30 mice were randomly divided into three groups ($n=10$) and injected with 0, 10 or 20 mg/kg thioperamide (i.p.). In a third sub-experiment, which tested whether thioperamide is able to alter cocaine-induced gnawing, 60 mice were randomly assigned to six groups ($n=10$) and injected i.p. with saline, 10 or 20 mg/kg thioperamide 10 min before receiving either 24 or 32 mg/kg cocaine (s.c.). In all sub-experiments, mice were individually placed into the experimental chambers immediately after the second injection and tested for 75 min.

2.3.3. Effect of thioperamide on cocaine-modified sniffing and grooming

Sniffing and grooming were measured with video-recording and visual scoring. Mice were tested singly in clear acrylglas floorless chambers (height, 20 cm; area, 42 × 7 cm) obtained from the division of a larger rectangular arena into six of these chambers. That six-chamber set was placed on a square platform of clear glass held horizontally by a robust metallic frame. The glass platform was divided into six equally sized squares by black lines so that each chamber was similarly divided into six areas (6.5 × 6.5 cm). A removable, perforated, acrylglas plate served as a lid. A portable S-VHS video camera was positioned directly underneath in order to view the whole surface covered by the six-chamber set (forming a 42 × 42 cm squared surface), an arrangement that allowed filming six mice ventrally. A character generator was connected to the control monitor and was used to indicate elapsed time. Lighting was provided by four neon tubes fixed singly on each leg of the frame. Each apparatus was individually located in a small ventilated and heated (20–23 °C) room, the walls of which were painted white (height, 245 cm; area, 140 × 160 cm). Videotapes were marked and subsequently replayed for analysis on a high-quality videocassette recorder with slow motion and frame-by-frame control options.

Mice were randomly divided into six groups ($n=9$) and were injected i.p. with saline, 10 or 20 mg/kg thioperamide followed 10 min later by a s.c. injection of saline or 24 mg/kg cocaine, the six possible treatments forming a 2 × 3 factorial design. Immediately after the second injection, mice were placed into the experimental chambers and videotaped for 60 min. Sniffing, grooming, rearing and locomotion were subsequently scored using a time-sampling procedure (Michel and Tirelli, 2002). Each mouse was scored over five samples of 100 s, beginning 10 min after the injection of cocaine. The samples were spaced by 12 min (i.e., 10, 22, 34, 46 and 58 min after the cocaine injection). Samples from each animal were then summed. Videotapes were analyzed one time per behavior pattern, the duration of the occurrence of each behavior being quantified in seconds. The behavior patterns were defined as follows. (1) Sniffing consisted of episodes of rapid flaring and contracting of the nostrils associated with movements of the whiskers, the nose making no contact with the floor, while standing still; (2) grooming involved episodes of face, head or body stroking with the forelimbs, or licking of any part of the body; (3) rearing was defined as body vertical or nearly vertical with the forelimbs in the air or in

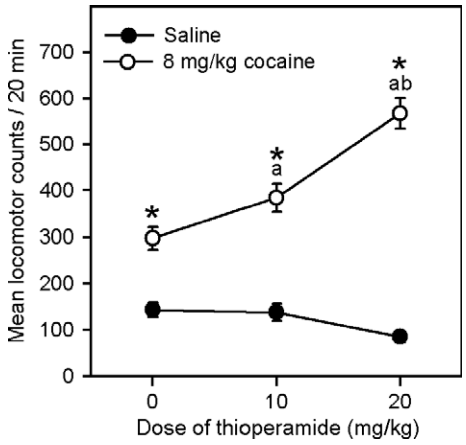

Fig. 1. Effect of thioperamide on cocaine-induced locomotor hyperactivity in C57BL/6J mice. Thioperamide (10 and 20 mg/kg, i.p.) or saline was given 10 min before either saline or 8 mg/kg cocaine (i.p.) and the mice were tested immediately thereafter for 20 min. (*) Value significantly different from that of the respective group treated without cocaine, (a) value significantly different from that of the saline-plus-8 m/kg cocaine group treated, (b) value significantly different from that of the 10 mg/kg thioperamide-plus-8 mg/kg cocaine group, as yielded by Holm–Sidak tests taken at least at $P<0.05$. The vertical brackets represent ±S.E.M.

contact with the wall, with or without obvious sniffing; (4) a locomotion activity count was recorded each time the mouse crossed one of the five lines (forming six lined squares) on the surface arena with both the head and forelegs at any time during the sampling periods.

2.4. Data analysis

The inter-observer agreement was estimated on a series of sampled cardboards (gnawing) and tapes (sniffing, grooming, rearing and locomotion), and was never lower than $r=0.88$. Data related to the potential interactive locomotor effects between thioperamide and cocaine on the first test session (acute interactive effects) were analyzed with a 2×3 fixed-model ANOVA involving thioperamide (saline, 10 or 20 mg/kg, 3 levels) and cocaine (saline or 8 mg/kg cocaine, 2 levels) as between-subject factors. The potential effects of thioperamide on the development curve of cocaine sensitization were analyzed with a 3×7 mixed-model ANOVA that compared the values derived from the three cocaine-injected groups having received one of the thioperamide treatments (saline, 10 or 20 mg/kg), the seven test sessions (7 levels) constituting a within-subject factor. Data from the groups receiving saline as a second treatment were analyzed similarly. Scores from the cocaine challenge test were analyzed using a 2×3 fixed-model ANOVA, thioperamide (saline, 10 or 20 mg/kg, 3 levels) and cocaine (saline or cocaine, 2 levels) being defined as between-subject factors. Data obtained with the video cameras related to sniffing, grooming, rearing and locomotion were analyzed with the same tools as those used for the acute cocaine plus thioperamide experiment. Where necessary, square-root transformations normalized raw data prior to ANOVA in order to more nearly meet the assumption of homoscedasticity (following a significant Levene's test; Zar, 1999); for the sake of clarity, means of the raw values are presented in the graphs. Relevant between-mean differences were assessed via the Holm–Sidak test (Glantz, 1997). Because the scores of gnawing massively violated the assumptions of normality, they were analyzed with the distribution-free Kruskal–Wallis H-test (H_2) that treated the pharmacological treatments as a between-subject factor. Relevant between-mean differences were assessed via the Student–Newman–Keuls (SNK) test adapted for nonparametric tests (Glantz, 1997). Complementary analyses conducted for specific reasons (i.e., absence of significant overall interaction) are mentioned in the text. Statistical significance was always set at $P<0.05$.

3. Results

3.1. Effect of thioperamide on cocaine-induced psychomotor activation and sensitization

Fig. 1 shows the interactive effects of thioperamide and cocaine as recorded on the first session of the experiment. Thioperamide dose-dependently enhanced the locomotor effects of 8 mg/kg cocaine but did not significantly increase locomotion on its own. That profile of effects was supported by a significant interaction between cocaine and thioperamide ($F_{2,36}=25.773$, $P<0.001$). Holm–Sidak tests revealed that 20 mg/kg thioperamide combined with cocaine induced higher levels of activity than cocaine alone or 10 mg/kg thioperamide plus cocaine ($P<0.001$). Moreover, animals treated with 10 mg/kg thioperamide plus cocaine were more active than those

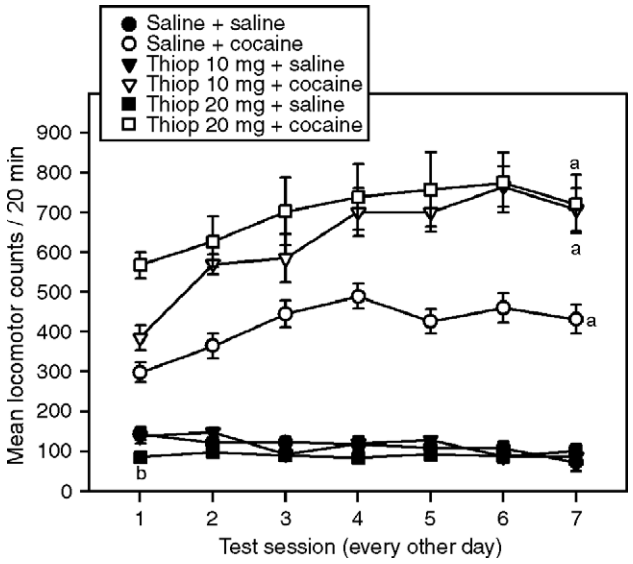

Fig. 2. Effect of thioperamide on cocaine-induced hyperactivity over seven successive test sessions performed every other day in C57BL/6J mice. Prior to each test session, which lasted 20 min, mice received thioperamide (10 and 20 mg/kg, i.p.) or saline and 10 min later either saline or 8 mg/kg cocaine (i.p.). (a) Value significantly different from that of the first session within a given group, (b) value significantly different from those of the two other groups treated without cocaine within a session, as yielded by Holm–Sidak tests taken at least at $P<0.05$ (note that no significant thioperamide-by-cocaine-by-session interaction was detected by an initial three-way 2×3×7 ANOVA). The vertical brackets represent ±S.E.M.

receiving only cocaine ($P<0.05$). Mice treated with 20 mg/kg thioperamide plus saline displayed a slightly reduced locomotor relative to the control group, although this effect did not reach statistical significance (Holm–Sidak, $P=0.089$).

Fig. 2 presents the seven-injection cocaine sensitization generated in presence of thioperamide. The two-way ANOVA (thioperamide × test sessions) performed on the scores from cocaine-treated animals yielded a significant effect of thioperamide ($F_{2,18}=11.35$, $P<0.001$) and a significant effect of test sessions ($F_{6,108}=18.05$, $P<0.001$) but no interaction between these factors ($F_{12,108}=1.46$, $P=0.15$). The absence of a significant interaction indicates that the three cocaine-treated groups (with or without thioperamide) exhibited similar rates of sensitization. The dose of 20 mg/kg thioperamide potentiated the stimulant effects of cocaine on each session (Holm–Sidak at least at $P<0.05$). At 10 mg/kg, thioperamide also potentiated cocaine-induced hyperactivity but not on the first session (Holm–Sidak at least at $P<0.05$). As regards the effect of thioperamide alone (plus saline), two-way ANOVA (thioperamide × test sessions) yielded no significant main effect of thioperamide ($F_{2,18}=1.57$; $P=0.236$) but a significant interaction between that treatment and the test sessions ($F_{6,12}=2.16$; $P<0.02$). That interaction accounts for a significant hypolocomotor effect induced by 20 mg/kg thioperamide on the first session and a significant habituation-related decrement of activity over the sessions in the two other groups (Holm–Sidak at least at $P<0.05$).

Fig. 3 depicts the effects of the cocaine challenge given to all groups 2 days after the seven-session pharmacological treatments. Two-way ANOVA (cocaine × thioperamide) yielded a significant main effect of cocaine ($F_{1,36}=30.63$, $P<0.0001$), no thioperamide effect ($F_{2,36}=1.534$, $P=0.229$) and no significant interaction between cocaine and thioperamide ($F_{2,36}=0.032$, $P=0.968$). Mice from the two groups having intermittently received thioperamide prior to cocaine and those from the

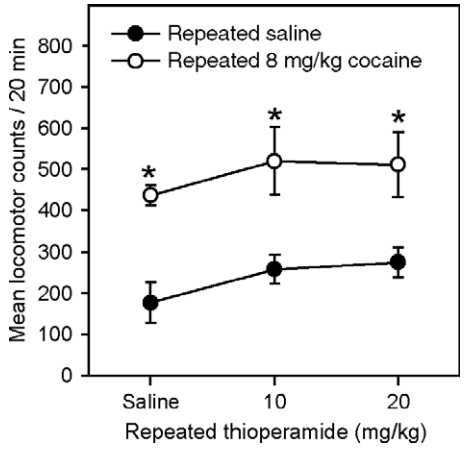

Fig. 3. Effect of a cocaine challenge on psychomotor responsiveness in C57BL/6J mice previously (2 days earlier) treated with intermittent injections of thioperamide plus cocaine over seven sessions separated by 2 days. Animals from all groups received 8 mg/kg cocaine i.p. and were tested during a unique 20-min session. (*) Value significantly different from that of the respective group treated without cocaine during the chronic treatment as yielded by Holm–Sidak tests taken at least at $P<0.05$. The vertical brackets represent ±S.E.M.

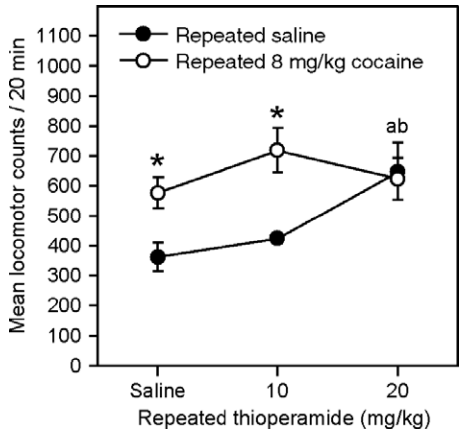

Fig. 4. Effect of a cocaine challenge on psychomotor responsiveness in C57BL/6J mice previously (14 days earlier) treated with intermittent injections of thioperamide plus cocaine over seven sessions separated by 2 days. Animals from all groups received 8 mg/kg cocaine i.p. and were tested during a unique 20-min session. (*) Value significantly different from that of the respective group treated without cocaine during the chronic treatment, (a) value significantly different from that of the group treated only with saline during the chronic treatment, (b) value significantly different from that of the group treated only with 10 mg/kg thioperamide during the chronic treatment, as yielded by Holm–Sidak tests taken at least at $P<0.05$. The vertical brackets represent ±S.E.M.

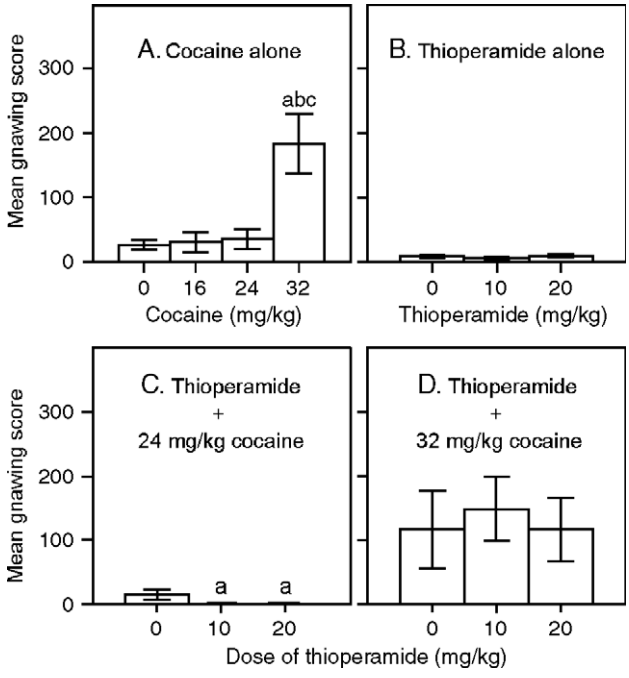

Fig. 5. Effect of thioperamide on cocaine-induced gnawing directed toward the corrugations of a cardboard sheet covering the floor of the test chamber in C57BL/6J mice. Panels A and B: effects of cocaine (16, 24 or 32 mg/kg, s.c.) and thioperamide (10 or 20 mg/kg, i.p.) given alone, respectively. Panels C and D: effects of thioperamide (10 or 20 mg/kg, i.p.) given 10 min before 24 or 32 mg/kg cocaine (s.c.), respectively. Testing begun immediately after the last injection and lasted 75 min. (a) Value significantly different from that of the saline group (panel A) or the saline-plus-cocaine group (panel C), (b) value significantly different from that of the 16 mg/kg cocaine group, (c) value significantly different from that of the 24 mg/kg cocaine group, as yielded by Neuman–Keuls tests taken at least at $P<0.05$ and based on distribution-free Kruskal–Wallis tests (that detected significant differences). The vertical brackets represent ±S.E.M.

saline-plus-cocaine group displayed equivalent levels of sensitized psychomotor activity, which were significantly greater than those of the respective control groups receiving no cocaine (supported by Holm–Sidak tests at least at $P<0.05$).

The sensitization test performed 14 days after the last drug treatment session is shown in Fig. 4. Two-way ANOVA (cocaine × thioperamide) yielded a significant main effect of cocaine ($F_{1,36}=9.453$, $P<0.01$), a thioperamide effect ($F_{2,36}=3.289$, $P<0.05$) and a significant interaction between cocaine and thioperamide ($F_{2,36}=3.287$, $P<0.05$). As indicated by the significant effect of cocaine, sensitization was still present in all cocaine-pretreated animals. Moreover, post-hoc tests revealed that all cocaine-pretreated animals showed similar levels of locomotor activity, whether they received thioperamide or not. However, the control animals treated with 20 mg/kg thioperamide and saline during the chronic treatment showed higher levels of activity than animals treated only with saline or 10 mg/kg thioperamide (Holm–Sidak at least at $P<0.05$), suggesting a cross-sensitization between the high dose of thioperamide and cocaine.

3.2. Effect of thioperamide on cocaine-induced gnawing

As depicted in Panel A of Fig. 5, 32 mg/kg cocaine induced gnawing levels at least six-fold significantly greater (despite a great variability) than those exhibited by the other groups, 16 and 24 mg/kg cocaine remaining practically ineffective ($H_3=8.701$, $P<0.05$ and subsequent SNK tests taken at $P<0.05$). Panel B of Fig. 5 shows that thioperamide alone (10 or 20 mg/kg, i.p.) has no effect on gnawing ($H_2=1.563$, $P=0.458$). In Panel C of Fig. 5, it can be seen that thioperamide significantly abolished gnawing activity in mice injected with 24 mg/kg cocaine ($H_2=7.714$ at $P<0.05$ and SNK tests taken at $P<0.05$). By contrast, as graphed in Panel D of Fig. 5, thioperamide did not affect gnawing activity induced with a great variability by 32 mg/kg cocaine ($H_2=0.677$, $P=0.713$).

3.3. Effect of thioperamide on cocaine-induced sniffing

Panel A of Fig. 6 shows that sniffing activity induced by 24 mg/kg cocaine was not significantly affected by thioperamide (significant main effect of cocaine: $F_{1,48}=45.758$, $P<0.001$; no significant interaction between thioperamide and cocaine: $F_{2,48}=2.308$, $P=0.110$). The significant effect of cocaine indicates that cocaine increased sniffing in all cocaine-treated groups (an effect also supported by Holm–Sidak tests at least at $P<0.05$). As depicted in Panel B of Fig. 6, although cocaine and thioperamide alone did not affect grooming activity, the combination of thioperamide with cocaine significantly decreased the sampled duration of grooming, as supported by a significant interaction between cocaine and thioperamide ($F_{2,48}=3.202$, $P<0.05$ and appropriate Holm–Sidak test at $P<0.05$). Panel C of Fig. 6 shows that cocaine did not affect rearing per se. Moreover, thioperamide had no

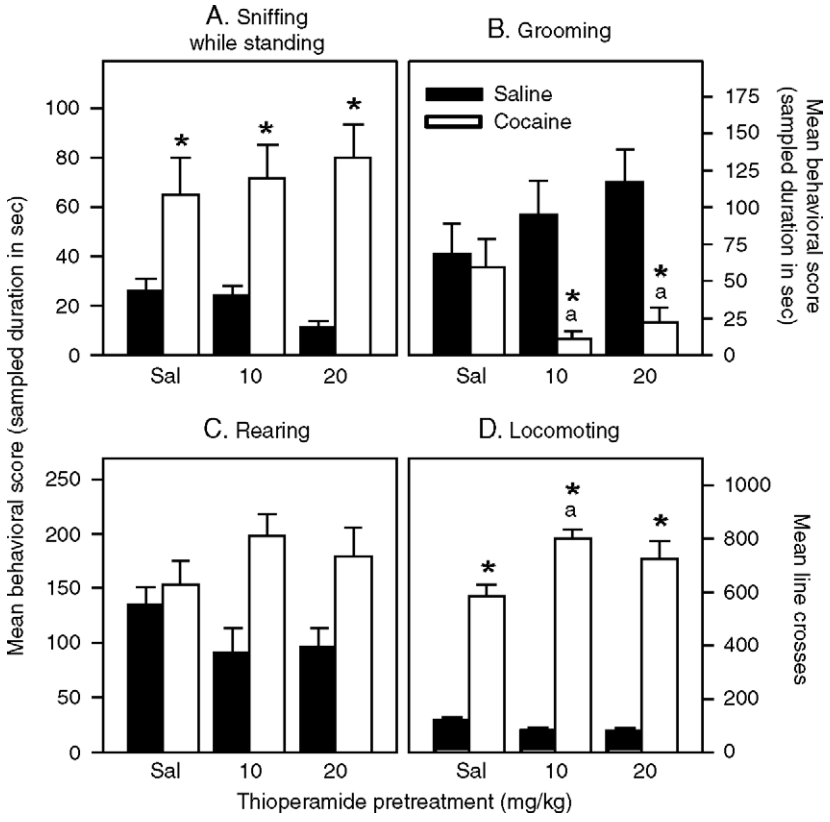

Fig. 6. Effect of thioperamide (0, 10 and 20 mg/kg, i.p.) on behavior patterns modified by a s.c. injection of 24 mg/kg cocaine. (*) Value significantly different from the respective control group treated without cocaine, (a) value significantly different from that of the group injected with cocaine alone, as yielded by Holm–Sidak tests taken at least at $P<0.05$. The vertical brackets represent ±S.E.M.

influence on rearing when administered alone or in combination with cocaine (no significant interaction between cocaine and thioperamide, $F_{2,48}=0.245$, $P=0.117$, and no significant effects of cocaine or thioperamide after additional one-way ANOVAs). Panel D of Fig. 6 shows that cocaine-induced locomotion was significantly enhanced by 10 mg/kg thioperamide (significant interaction between cocaine and thioperamide, $F_{2,48}=5.597$, $P<0.01$, and Holm–Sidak tests taken at least at $P<0.05$). The dose of 20 mg/kg thioperamide also enhanced the locomotor effects of cocaine but this effect did not reach statistical significance in the present experiment (Holm–Sidak test, $P=0.052$). The decrease induced by 20 mg/kg thioperamide in saline-injected mice was significant only after a less conservative one-way ANOVA ($F_{2,\ 24}=3.87$, $P<0.05$).

4. Discussion

The present study yielded the following findings. (1) Thioperamide dose-dependently (each mean being different from each other) increased the psychomotor stimulation induced by acutely given 8 mg/kg cocaine (Fig. 1), an amplifying effect that was more or less constantly induced on every session of the sensitization-inducing cocaine intermittent treatment (Fig. 2). (2) However, coadministration of thioperamide during that intermittent treatment did not affect subsequent acute cocaine sensitivity in cocaine-sensitized mice 2 or 14 days after the drug treatment (Figs. 3 and 4). (3) Unexpectedly, the intermittent treatment performed with 20 mg/kg cross-sensitized with cocaine 14 days but not 2 days after the drug treatment (Figs. 3 and 4). (4) Thioperamide given acutely did not affect stereotyped behavior patterns such as sniffing and gnawing intensified by relatively high doses of 24 or 32 mg/kg cocaine (Figs. 5 and 6). Although thioperamide alone had no effect on grooming, its combination with 24 mg/kg cocaine significantly reduced that behavior pattern, likely because of a facilitation of locomotor activity that did not allow the expression of behaviors while standing (due to a behavioral competition). The same principle of behavioral exclusion likely explains the almost total abolition of gnawing activity occurring in mice treated with 10 or 20 mg/kg thioperamide plus 24 mg/kg cocaine (Fig. 5).

In accordance with previous studies, thioperamide potentiated the locomotor effects induced by 8 or 24 mg/kg cocaine (Hyytiä et al., 2003; Brabant et al., 2005). Several studies indicated that the acute locomotor effects of cocaine are mediated by an increased dopaminergic activity in the ventral striatum. The ventral striatum is mainly formed by two major structures: the nucleus accumbens and the olfactory tubercle (Heimer et al., 1995). Although the locomotor stimulant effects of cocaine have long been assumed to result from an increased dopaminergic activity in the nucleus accumbens (Kelly and Iversen, 1976; Delfs et al., 1990), recent studies indicated that the olfactory tubercle might play the most important role. For example, Ikemoto (2002) have found higher levels of hyperactivity in rats when cocaine is directly infused in the olfactory tubercles relative to the nucleus accumbens. A detailed autoradiographic study demonstrated that the density of H_3 receptors is very high in the ventral striatum, and particularly in the olfactory tubercles (Pillot et al., 2002). Histamine H_3 heteroreceptors modulate the release of several neurotransmitters, including dopamine. It is therefore believed that the potentiating effects of cocaine-induced hyperactivity by thioperamide are mediated by an increased release of dopamine (Brabant et al., 2005). The high density of H_3 receptors in the olfactory tubercles, a brain area deeply involved in the locomotor effects of cocaine, could explain why thioperamide so importantly potentiated the cocaine-induced locomotion. The results of several studies support the idea that thioperamide potentiates the locomotor stimulant effects of cocaine through an increased dopamine release in the ventral striatum. In vitro studies showed that histamine inhibits the release of dopamine from striatal terminals through its action on H_3 heteroreceptors and that this effect is blocked by the administration of thioperamide (Schlicker et al., 1993). In vivo microdialysis studies also demonstrated that thioperamide increases the release of dopamine produced by cocaine and methamphetamine in the nucleus accumbens of rats (Hyytiä et al., 2003 ; Munzar et al., 2004). Taken together, these findings suggest that H_3 receptors exert an inhibitory action on dopamine release. Thioperamide probably suppresses this inhibitory control on dopamine release thereby potentiating the locomotor effects of cocaine.

Although thioperamide potentiates the locomotor effects of cocaine (Brabant et al., 2005, Hyytiä et al., 2003, present results), it was shown to decrease amphetamine-induced hyperactivity (Clapham and Kilpatrick, 1994). Other H_3 inverse agonists (ciproxifan and ABT-239) also reduce the stimulant effects of methamphetamine (Morisset et al., 2002; Fox et al., 2005). This discrepancy is difficult to explain according to our present level of understanding about the role of histamine in the locomotor effects of these two drugs. Some authors have suggested that H_3 inverse agonists decrease amphetamine- and methamphetamine-induced hyperactivity because it increases histamine release by blocking H_3 autoreceptors located on histaminergic terminals (Clapham and Kilpatrick, 1994; Morisset et al., 2002). In agreement with that hypothesis, it was also shown that the administration of histidine, leading to an increased concentration of histamine within the brain, reduced the locomotor stimulant effects of methamphetamine in mice (Itoh et al., 1984). In contrast, the thioperamide-induced enhancement of the locomotor stimulant effects of cocaine is probably due to an increased release of dopamine after the blockade of H_3 heteroreceptors located on mesolimbic dopaminergic fibers (Hyytiä et al., 2003; Brabant et al., 2005). Taken together, these results suggest that the role of the brain histaminergic system in the locomotor effects of cocaine is different from that of amphetamine and methamphetamine. A differential involvement of histamine in the behavioral effects of cocaine and methamphetamine was already reported by Masukawa et al. (1993). In that study, the blockade of the histaminergic system with chlorpheniramine, an H_1 antagonist, potentiated methamphetamine-, but not cocaine-induced conditioned place preference.

In the present study, whereas 10 mg/kg thioperamide did not affect locomotor activity, the dose of 20 mg/kg slightly reduced

locomotion, an effect that was already observed in our laboratory with C57BL/6J mice (Brabant et al., 2005). Moreover, thioperamide did not induce sensitization alone. Our results are in agreement with those from Komater et al. (2003) that also demonstrated that thioperamide alone produced no sensitization. Unexpectedly, we found in the present study that a chronic thioperamide pretreatment cross-sensitized with the stimulant effects of cocaine 14 but not 2 days after the last thioperamide injection. These results can be interpreted as a long-term, but not a short-term, cross-sensitization between a thioperamide pretreatment and a cocaine challenge. However, this interpretation should be considered very cautiously for two reasons. Firstly, cross-sensitization was observed only for the highest dose of thioperamide. This compound shows a high affinity for the serotonin 5-HT_3 receptor (Leurs et al., 1995; Leurs et al., 2005) that is known to play an important role in the neurochemical and behavioral effects of cocaine (Reith, 1990; Kankaanpaa et al., 2002). Accordingly, chronic administration of 5-HT_3 blockers has been found to alter subsequent sensitivity to the locomotor effects of cocaine (King et al., 2002; Szumlinski et al., 2003). Secondly, Komater et al. (2003) reported an absence of cross-sensitization between a cocaine pretreatment and a thioperamide challenge given 7 days after the last cocaine injection. Unfortunately, that study did not test the effects of a thioperamide challenge after a longer time interval following the last cocaine injection. Further studies are needed to confirm and better characterize this possible long-term cross-sensitization between an H_3 inverse agonist and cocaine that was revealed by the present experiment.

Our study did not demonstrate any convincing role of H_3 receptors in the development of sensitization to the stimulant effects of cocaine. Thioperamide potentiated cocaine-induced hyperactivity on every experimental sessions of the chronic intermittent treatment (see Fig. 2). However, 2 and 14 days after the induction of cocaine sensitization, animals administered with thioperamide and cocaine showed similar levels of sensitivity to the locomotor effects of cocaine. Therefore, our results suggest that H_3 receptors are involved in the acute locomotor effects of cocaine but not in the induction of short-term or long-term sensitization. Previous experiments demonstrated that glutamate transmission in the ventral tegmental area plays a critical role in induction of cocaine sensitization (Vanderschuren and Kalivas, 2000; Dunn et al., 2005). For example, a recent study demonstrated that the repeated stimulation of AMPA or metabotropic glutamate receptors in the ventral tegmental area mimics the initiation of behavioral sensitization to cocaine (Dunn et al., 2005). Since autoradiographic studies indicate very low densities of H_3 receptors in this cerebral region (Pollard et al., 1993; Pillot et al., 2002), one can understand why H_3 receptors are probably not involved in the induction of behavioral sensitization.

The present study did not show a potentiating effect of thioperamide on cocaine-induced stereotypies (sniffing and gnawing). Behavioral stereotypies produced by psychostimulants are mainly mediated by an increased dopaminergic activity in the dorsal striatum (Kelly et al., 1975; Kelly and Iversen, 1976). However, relative to amphetamine, cocaine induces a much lower dopamine release in the dorsal striatum together with lower levels of stereotypies in rats and mice (Kuczenski and Segal, 1992; Kankaanpaa et al., 1996; Tanda et al., 2005; Tolliver and Carney, 1994; Antoniou et al., 1998; Tirelli et al., 1998). Although H_3 receptors are also expressed in the dorsal striatum, a detailed autoradiographic study demonstrated that the density of these receptors is lower in this region relative to the ventral striatum (Pillot et al., 2002). Together with the lower dopaminergic effect of cocaine in the dorsal striatum relative to the ventral striatum, these latter results might explain why thioperamide potentiates the locomotor stimulant effects of cocaine that are mediated by the ventral striatum, but not cocaine-induced behavioral stereotypies that are mediated by the dorsal striatum.

In summary, the present results confirm that thioperamide strongly enhanced the locomotor effects of cocaine, likely through H_3 receptors located in the ventral striatum. In contrast, the blockade of H_3 receptors by thioperamide increased neither sensitization to cocaine nor cocaine-induced stereotyped behaviors (in particular gnawing and exacerbated sniffing).

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