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Characterization of serotonin-toxicity syndrome (toxidrome) elicited by 5-hydroxy-L-tryptophan in clorgyline-pretreated rats

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Abstract

Patients are at high risk of developing serotonin-toxicity syndrome (toxidrome) when they take multiple serotonergic drugs, particularly co-administered with monoamine oxidase inhibitors or 5-hydroxytryptamine (5-HT) reuptake blockers. The toxidrome can vary from mild to severe. The primary goal of the present study was to understand the relationship between behavioral signs and degrees of toxidrome induced by 5-hydroxy-L-tryptophan (5-HTP) in clorgylinized rats. The severity was obtained by scoring behavioral signs including head shakes, penile erection, forepaw treading, hind limb abduction, Straub tail and tremor. It was found that 5-HTP produced a dose-dependent increase in degrees of the toxidrome. Furthermore, correlation between the toxidrome and changes in body-core temperature ($\text{claudqc}T_{\text{cor}}$) was determined. There was hypothermia in the mild toxidrome ($\text{claudqc}T_{\text{cor}} < -1^\circ\text{C}$), high hyperthermia in the severe toxidrome ($\text{claudqc}T_{\text{cor}} > +2^\circ\text{C}$) and a small change in T_{cor} in the moderate toxidrome ($-1^\circ\text{C} < \text{claudqc}T_{\text{cor}} < +2^\circ\text{C}$). Thus, $\text{claudqc}T_{\text{cor}}$ in response to drugs can be used to estimate the severity of the toxidrome. The second attempt was to identify the receptors mediating those changes. 5-HT_{1A} receptors were involved in the hypothermic response while 5-HT_{2A} and NMDA receptors mediated head shakes, hyperthermia, forepaw treading and Straub tail. Lastly, antidotal effect of cyproheptadine and (+)-MK-801 was examined. Both drugs blocked hyperthermia and death. However, the effects on mortality became poor when the antidotes were injected 60 min after high hyperthermia had been induced. These findings demonstrate the importance of the time frame using antidotes in the treatment of the 5-HT toxidrome.

Keywords

Serotonin-toxicity syndrome; head shakes; hyperthermia; hypothermia; 5-HT_{1A} receptor; 5-HT_{2A} receptor

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1. Introduction

The first case of serotonin (5-HT) poisoning was reported half a century ago when a patient was co-administered the monoamine oxidase (MAO) inhibitor iproniazid with the synthetic narcotic compound meperidine (Mitchell, 1955). The incident did not receive much attention for decades because of the limited availability of serotonergic drugs. To date, about 100,000 patients each year in the United States alone suffer from antidepressant poisoning (The American Association of Poison Control Centers; <http://www.aapcc.org/annual.htm>). Toxic incident is also not uncommon in other western countries (Birmes et al., 2003; Dunkley et al., 2003). In patients, symptoms consisting of autonomic dysfunction, neuromuscular disorders and mental state alteration collectively are called serotonin-toxicity syndrome or toxidrome (Boyer and Shannon, 2005; Gillman, 2006).

Under laboratory conditions, a 5-HT toxidrome was usually induced in rats by injection of monoamine oxidase (MAO) inhibitors combined with 5-HT precursors (Grahame-Smith, 1971a; Modigh and Svensson, 1972; Jacobs and Klemfuss, 1975; Abdel-Fattah et al., 1995; Nisijima et al., 2000). The behavioral signs of the toxidrome are many, commonly including somatic (forepaw treading, flat body, hind limb abduction, head shakes, tremor, Straub tail) and autonomic responses (cyanosis, diarrhea, salivation, hyperthermia). Using *in vivo* microdialysis in rats, hypothalamic 5-HT was elevated 140-fold over the pre-drug level (Shioda et al., 2004). Thus, excess 5-HT in the brain is implicated for most, if not all, of the behavioral signs. Activation of 5-HT_{1A} receptors produced forepaw treading, hind limb abduction and Straub tail (Darmani and Zhao, 1998) while 5-HT_{2A} receptors were responsible for head shakes and hyperthermia (Peroutka et al., 1981; Abdel-Fattah et al., 1995; Van Oekelen et al., 2002). Recent investigations revealed a crucial role of the glutamatergic system in mediating the 5-HT toxidrome (Nisijima et al., 2004; Shioda et al., 2004).

However, the toxidrome is not homogenous. After a scrutiny of 100 cases, Mills (1995) reported that each symptom had only a 5 – 50% chance of being exhibited in an individual patient and that most signs were absent completely. Although the causes and neural pathways are not fully understood, appearance of those signs are largely dependent on the degrees of toxicity (Boyer and Shannon, 2005; Isbister and Buckley, 2005). Thus behavioral signs and symptoms can be mild, moderate or severe (Boyer and Shannon, 2005). Changes in body-core temperature (claudq T_{cor}), attributable to 5-HT-mediated thermoregulation in the central nervous system (Lin et al., 1998), are a most obvious indication for the toxidrome (Isbister and Buckley, 2005). Several studies conducted in animals have revealed both hyperthermia and hypothermia in the toxidrome (Abdel-Fattah et al., 1995, 1997; Nisijima et al., 2001, 2003). However, little is known about changes in body-core temperature in the context of different degrees of toxicity although in the severe state their relationship has been examined by others using a single dose protocol (Grahame-Smith, 1971b; Jacobs and Klemfuss, 1975; Nisijima et al., 2001). Here, we tested a hypothesis that hypothermia was an indication of a mild toxidrome, and hyperthermia was associated with a severe or lethal response to the drug administration. To test dose-dependent toxicity, a series of experiments were carried out in rats pretreated with clorgyline (2 mg/kg, i.p.), 12 h before the experiments. The degrees of the toxidrome and its behavioral signs were quantified in

response to 5-hydroxy-L-tryptophan (5-HTP) administration intraperitoneally at doses of 5, 10, 15, 20 or 25 mg/kg. Parametric measurements were used to quantify the frequency of head shakes and changes in body-core temperature, and non-parametric approaches were employed to score the penile erection, forepaw treading, hind limb abduction, Straub tail and tremor. The relationship between toxic intensity (mild, moderate and severe) and changes in body-core temperature was determined. In both humans and rats, ligands for 5-HT_{1A}, 5-HT_{2A} and N-methyl-D-aspartate (NMDA) receptors showed a potential therapeutic value in treatment of the toxidrome (Nisijima et al., 2001; Boyer and Shannon, 2005; Isbister and Buckley, 2005). Thus, the second goal of the current study was to examine the involvement of 5-HT_{1A}, 5-HT_{2A} and NMDA receptors in the toxidrome. Additionally, a selective 5HT₇ receptor antagonist SB-269970 (Faure et al., 2006) was used to test a possible role of 5-HT₇ receptors in hypothermia in the mild toxidrome. The third goal of this study was to reveal a critical time frame for antidotes to block toxicity induced by a lethal dose of 5-HTP (25 mg/kg, i.p.) in the clorgylinized animals. Cyproheptadine was examined because it had been considered the most effective antidotal agent for patients (Nisijima et al., 2004, Boyer and Shannon, 2005, Isbister and Buckley, 2005). For comparison, (+)-MK-801 was also tested for its antidotal efficacy.

2. Methods and materials

2.1 Animal preparation

Male Sprague-Dawley rats (Charles River Laboratories at Raleigh, NC, USA) were kept on a normal light-dark cycle (lights-on: 8:00 – 20:00) and housed in pairs with food and water available *ad libitum*. Animal use procedures were in strict accordance with the *NIH Guide for the Care and Use of Laboratory Animals* and approved by the local animal study committees. All efforts were made to minimize the number of animals used and their suffering.

2.2 Chemicals and schedule for drug injections

Clorgyline (N-Methyl-N-propargyl-3-(2,4-dichlorophenoxy)propylamine hydrochloride), 5-HTP (5-hydroxy-L-tryptophan), SB-269970 ((*R*)-3-[2-[2-(4-Methylpiperidin-1-yl)ethyl]pyrrolidine-1-sulfonyl]phenol hydrochloride) and WAY-100,635 (N-[2-[4-(2-Methoxyphenyl)-1-piperazinyl]ethyl]-N-2-pyridinylcyclohexanecarboxamide maleate salt) were purchased from Sigma (St Louis MO, USA). Ketanserin, (+)-MK-801 ((5*S*,10*R*)-(+)-5-Methyl-10,11-dihydro-5H-dibenzo[*a,d*]cyclohepten-5,10-imine maleate) and (-)-pindolol (1-(1*H*-Indol-4-yl)-3-[(1-methylethyl)amino]-2-propanol) were obtained from Tocris (Ellisville, MO, USA), cyproheptadine and methysergide maleate from USP (Rockville, MD, USA). Doses, vehicles and routes for drug administration were prepared as illustrated in Table 1. With the exception of 5-HTP, drugs were injected at a constant volume of 1 ml/kg body weight. 5-HTP was dissolved in the vehicle (0.9% NaCl) and administered at a volume of 3 ml/kg body weight.

2.3. Experimental procedures

2.3.1. Experiment 1: Head shakes—Clorgyline (2 mg/kg, i.p.) was administered to rats 12 h before an experiment. On the experimental day, the clorgylinized animals were

individually habituated in a transparent Plexiglas test chamber (40 cm × 20 cm × 20 cm) for at least 2 h prior to the test. The ambient temperature was set at 22.0 °C (± 0.5 °C) with humidity control (40 – 70%). Experiments were carried out between 10:00 and 16:00. The numbers of head shakes were counted at 15-min intervals throughout the 90-min observation period (6 time blocks), starting immediately after 5-HTP or vehicle (0.9% NaCl) injection. To test a possible involvement of 5-HT_{2A} and NMDA receptors, animals were injected with ketanserin and (+)-MK-801, respectively, 15 min before 5-HTP administration.

2.3.2. Experiment 2: Severity scores—Severity in response to 5-HTP injection was assessed at 4 levels ranging from 0 (no effect) to 1 (mild), 2 (moderate) and 3 (severe). The definition for each level was based on the following criteria: if an animal appeared tolerable to 5-HTP injection (e.g. a few head shakes and grooming) and also if there was no death, it was rated as mild (score = 1); if the injection caused intolerable behavioral changes (e.g., irritation, locomotion) but a low mortality rate (~10%), it was considered to be moderate (score = 2); a severe response (score = 3) was associated with injections at a nearly 50% death rate (LD₅₀). In this study, we focused on five behavioral signs: penile response, forepaw treading, hind-limb abduction, Straub tail and tremor. Toxic intensity was calculated as a mean rating scored by two experienced observers blind to drug injections. The rating was performed during the maximum response period between 45 – 75 min after 5-HTP administration. For caution, each rat was rated twice at time blocks of 45 – 60 min and 60 – 75 min. The final score was the mean response averaged from the two time blocks from two observers.

2.3.2.1. Penis: Intensity was scaled by erection and ejaculate spills. For a clear-cut observation, the animals were examined on a wire grid floor with a clean paper sheet underneath. Level 3 (severe) was scored for a full-length (~1 cm) erection during a 15-min block and/or a large quantity of spills (> 0.5 ml volume); level 2 (moderate) for a persistent erection but with no or a negligible amount of spills; level 1 (mild) for constant exploring or licking the penis, but with no erection; level 0 (no effect) absence of signs mentioned above.

2.3.2.2. Forepaw treading test: Frequency (times/min) was counted on either the left or right paw. Level 3 (severe) if the counts were over 120; level 2 (moderate) for treading from 60 to 120 times/min; level 1 (mild) if treading occurred occasionally or less than 60 times/min; level 0 (no effect) if the signs of treading were not obvious during a 15-min block.

2.3.2.3. Hind limb abduction: Intensity was estimated by the position of the distal limbs relative to the body midsagittal plane and by the angles between the proximal and distal limbs. During the wake-quiet condition, distal limbs are normally positioned underneath the body and parallel to the midsagittal plane. The angles between proximal and distal limbs are less than 90 degrees and the toes point rostrally. Level 3 (severe) was assigned if the toes and distal limbs swung backwards to the caudal/tail side and the angles between the two parts of the limbs became straight (180 degrees); level 2 (moderate) if the limbs were completely abducted by showing that the distal limbs were perpendicular to the midsagittal plane and the body was sustained by the belly on the floor; level 1 (mild) if the distal but not proximal limbs were dragged out, there was no visible change in toe direction and the body

was still sustained by the limbs; the level was rated as 0 (no effect) in absence of the signs mentioned above,

2.3.2.4. Straub tail test: Level 3 (severe) was assigned when the vertical tail persisted over 10 s each time and occurred at least twice during a 15-min block; level 2 (moderate) if the tail was erected by the observers and also if the erection stayed over 2 s; level 1 (mild) if the observers felt the tail was stiff even though they were not able to erect it; level 0 (no effect) if the tail was wagging on the floor.

2.3.2.5. Body trunk for estimating tremor: Level 3 (severe) was rated if tremors occurred throughout the whole trunk and also if the animal became immobilized; level 2 (moderate) if tremors occurred mainly at the anterior trunk and also if the animal was able to crawl around the floor; level 1 (mild) if there were occasional shivers; level 0 (no effect) if the animal showed no shivers during a 15-min block.

2.3.3. Experiment 3: Changes in body-core temperature (T_{cor})—Animals were pretreated with clorgyline (2 mg/kg, i.p.) 12 h before the experiments. The experiments were performed during lights-on of the light-dark cycle (between 10:00 and 16:00) after 2 h in the test chambers. The body-core temperature (T_{cor}) was measured at intervals of 15 min using a vinyl-jacketed thermoprobe (402 model; YSI Inc, Dayton, Ohio, USA) by inserting a 4.6 cm probe via the rat rectum into the colon and displaying on a digital meter (Traceable®, Fisher Scientific, Pittsburgh, USA). 5-HTP injections were made immediately at time zero after two consecutive T_{cor} were taken. The receptor antagonists (methysergide, ketanserin, WAY-100,635, SB-269970, cyproheptadine and (+)-MK-801) were given 15 min before 5-HTP administration. To test antidotal effects of cyproheptadine and (+)-MK-801, the antidotes were injected in 15 min or 60 min after a lethal injection of 5-HTP (25 mg/kg, i.p.).

2.4. Data calculation and statistics

Unless otherwise specified, data samplings were collected at intervals of 15 min and expressed as the mean ($\pm$ S.E.M.). A one-way repeated measures ANOVA was used to determine an overall effect of drugs including respective control groups. If significant ($P < 0.05$), the effect was further examined by a *post-hoc* Scheffe test for head shakes and T_{cor} . However, toxic intensity for penile erection, forepaw treading, hind limb abduction, Straub tail and tremor was examined by a Mann-Whitney U test. If appropriate, a Student's *t*-test was also used.

3. Results

3.1. Experiment 1: Head shakes

Head shakes were evoked within 5 min after injection of 5-HTP to the clorgylinized rats. Fig 1 shows a dose-dependent change in frequency across 6 blocks at 15 min intervals (totally 90 min). Note that there were no obvious head shakes following 1 mg/kg ($n = 2$; data not shown) or vehicle injections. Dose-dependent increases were observed following 5, 10 and 15 mg/kg (Fig 1A; $F_{(2,22)} = 15.759$; $P < 0.0001$). Maximum increase occurred between 30 –

45 min and then declined gradually. As the doses were increased to 20 and 25 mg/kg, the frequency of shakes was high during the first 30 min and declined rapidly (Fig 1B). Statistical analysis shows that frequency induced by the two higher doses was not significantly different from the vehicle control ($F_{(2,13)} = 3.618$, $P = 0.0563$).

The next experiments were to determine types of neural receptors mediating head shakes. Since the submaximum effect was at 10 mg/kg, this dose was thus chosen for the further test. As shown in Fig 1C, the shaking behavior was examined by the 5-HT_{2A} receptor antagonist ketanserin and NMDA receptor antagonist (+)-MK-801. A one-way repeated measures ANOVA revealed that the effect was blocked by ketanserin (5 mg/kg, i.p.; $F_{(1,11)} = 7.519$, $P = 0.0192$) but not significantly attenuated by (+)-MK-801 (0.5 mg/kg, s.c.; $F_{(1,11)} = 3.995$, $P = 0.079$).

3.2. Experiment 2: Intensity of the toxidrome measured by non-parametric approaches

Fig 2A shows a dose-dependent change in penile erection ($F_{(4,32)} = 12.307$, $P < 0.0001$), forepaw treading ($F_{(4,32)} = 24.211$, $P < 0.0001$), hind limb abduction ($F_{(4,32)} = 20.562$, $P < 0.0001$), Straub tail ($F_{(4,32)} = 12.992$, $P < 0.0001$) and tremor ($F_{(4,32)} = 22.005$, $P < 0.0001$). There was no detectable effect on these symptoms following 5 mg/kg of 5-HTP as compared to the vehicle treatment. However, the response was gradually intensified as the dose was increased to over 10 mg/kg. Fig 2B shows the effect of the 5-HT_{2A} receptor antagonist ketanserin and NMDA receptor antagonist (+)-MK-801 on the toxic signs following administration of 25 mg/kg of 5-HTP to the clorgylinized rats. The intensity of these toxic signs was not significantly altered by ketanserin pretreatment (5 mg/kg, i.p.). In contrast, (+)-MK-801 (0.5 mg/kg, s.c.) reduced the intensity level of forepaw treading (Mann-Whitney test; $U = 20$, $P < 0.05$) and Straub tail ($U = 19$, $P < 0.05$) although the response of penile erection, hind limb abduction and tremor was not altered. (+)-MK-801 plus ketanserin produced the same effect as that of (+)-MK-801 alone.

In a separate test, the relationship between severity score, $\text{claudqc}T_{\text{cor}}$ and mortality rate were examined as shown in Table 2. Note that the overall toxicity response was calculated as means averaged by the individual score for penile erection, forepaw treading, hind limb abduction, Straub tail and tremor. 5-HTP at 10 mg/kg produced a mild change in these signs with the overall score at ~0.8. The toxidrome was intensified and became moderate (overall score, 1.8) following 15 mg/kg and became severe by injection of 20 mg/kg or 25 mg/kg. Changes in body-core temperature ($\text{claudqc}T_{\text{cor}}$) were measured 60 min after 5-HTP injection. 5-HTP at doses of 5 mg/kg and 10 mg/kg produced a decrease in T_{cor} (or hypothermia). In contrast, 20 mg/kg and 25 mg/kg evoked an increase (hyperthermia). The effect of 15 mg/kg was inconsistent (2 out of 6 rats decreased vs. 4 animals showing increased T_{cor}). Of those with hyperthermia, most animals were killed when $\text{claudqc}T_{\text{cor}}$ were increased by over 3 degrees Celsius (10 of 13 rats; mortality rate 70%; Fig 7).

3.3. Experiment 3: Changes in body-core temperature ($\text{claudqc}T_{\text{cor}}$)

3.3.1 Dose-dependent effects—At low doses of 5-HTP (10 mg/kg; Fig 3A), there were small but significant decreases in body temperature compared to the pre-drug level ($F_{(2,13)} = 5.141$, $P = 0.0226$). Again, the effect of 15 mg/kg was not consistent. In the five

rats examined, there was a small decrease in two animals, an increase in two and no change in one. Overall, 15 mg/kg had no significant effect on the body-core temperature as shown in Fig 3B ($F_{(1,8)} = 0.18$, $P = 0.6828$). Injection of the high doses (20 and 25 mg/kg) of 5-HTP elevated the body-core temperature significantly ($F_{(2,14)} = 5.713$, $P = 0.0153$; Fig 3C). In summary, 5-HTP produced either hypothermia (10 mg/kg) or homeothermia (15 mg/kg) or hyperthermia (20 mg/kg) in the clorgylinized animals (Fig 3D).

3.3.2. Receptors mediating hypothermic response—The 5-HTP dose of 10 mg/kg was chosen for identifying what types of 5-HT receptors mediated hypothermia. Collectively, the baseline T_{cor} was 36.8°C (± 0.1 , $n = 55$). Methysergide, a broad-spectrum antagonist for 5-HT receptors, was injected 15 min before 5-HTP. As shown in Fig 4A, methysergide alone (10 mg/kg, i.p.; methy) had no significant effect on T_{cor} , but reduced 5-HTP-induced hypothermia ($F_{(1,7)} = 6.53$, $P = 0.0378$). The blocking effect was apparent in the late phase of hypothermia. Ketanserin was used to test whether the hypothermia was mediated through 5-HT_{2A} receptors. As shown in Fig 4B, ketanserin failed to have any effect on hypothermia induced by 10 mg/kg of 5-HTP ($F_{(1,11)} = 0.334$, $P = 0.5750$). The next experiment was to examine an involvement of the 5-HT_{1A} receptors by using selective antagonist WAY-100,635.

Injection of WAY-100,635 alone (0.1 mg/kg, i.p.) slightly but consistently elevated T_{cor} in the control animals ($0.8 \pm 0.3^{\circ}\text{C}$, $n = 5$). The increase was statistically significant ($P = 0.0458$; paired Student's *t*-test). Compared to the vehicle+5-HTP group, WAY-100,635 also attenuated the hypothermia induced by 5-HTP (Fig 4C). A one-way ANOVA revealed that the attenuation was statistically significant ($F_{(1,8)} = 8.47$, $P = 0.0196$). Note that WAY-100,635 (0.1 mg/kg, s.c.) blocked only a 40% reduction in hypothermia (from -2.6°C to $-1.5^{\circ}\text{C} \pm 0.5$). The same level of attenuation was obtained as the dose increased from 0.1 mg/kg to 0.5 mg/kg of WAY-100,635 ($n = 3$; data not shown). Lastly, SB-269970 was used to test an involvement of 5-HT₇ receptors in hypothermia. The drug alone induced a small increase in T_{cor} ($0.8 \pm 0.2^{\circ}\text{C}$ above the baseline; $P = 0.0074$, paired Student's *t*-test). In this study, SB-269970 at either 1 mg/kg or 3 mg/kg failed to alter hypothermia (Fig 4D; 1 mg/kg, $F_{(1,8)} = 0.282$, $P = 0.6097$; 3 mg/kg, $F_{(1,8)} = 0.611$, $P = 0.457$).

3.3.3. Receptors mediating hyperthermic response—The 5-HTP dose of 25 mg/kg was used for subsequent hyperthermic experiments. The baseline T_{cor} was 37.1°C (± 0.1 , $n = 47$). In this study, cyproheptadine and ketanserin were used to examine the effect mediated by 5-HT_{2A} receptors, (+)-MK-801 for NMDA receptors and (-)-pindolol for 5-HT_{1A} receptors. As shown in Fig 5A, cyproheptadine alone (cypro; 10 mg/kg, i.p.) elicited slight decreases in T_{cor} . Compared to the pre-drug level, the mean reduction was -0.7°C (± 0.3 , $n = 6$; $P < 0.05$; paired Student's *t*-test). Cyproheptadine injected 15 min before 5-HTP completely blocked the hyperthermic effect ($F_{(1,11)} = 36.801$, $P = 0.0001$; * $P < 0.05$). Furthermore, the hyperthermia was reversed to hypothermia within 30 min. The mean reduction was -1.7°C (± 0.5), with significantly lower temperatures being observed as compared to cyproheptadine alone ($F_{(1,10)} = 5.324$, $P = 0.0434$; # $P < 0.05$). The same results were obtained in rats pretreated with ketanserin as shown in Fig 5B. Similarly, ketanserin

blocked not only the hyperthermic effect ($F_{(1,11)} = 49.105$, $P = 0.0001$; $*P < 0.05$), but also reversed it to hypothermia for 90 min ($\#P < 0.05$).

(+)-MK-801 (0.5 mg/kg, s.c.) alone had no significant effect on T_{cor} . As shown in Fig 5C, T_{cor} was significantly reduced after (+)-MK-801 pretreatment followed by 5-HTP. A one-way repeated measures ANOVA confirmed that the difference from vehicle+5-HTP group was significant ($F_{(1,12)} = 20.319$, $P = 0.0002$; $*P < 0.05$). Moreover, the body temperature fell below the pre-drug level and became hypothermia for at least 90 min. The reduction was also significant compared to (+)-MK-801 alone ($F_{(1,17)} = 9.93$, $P = 0.0058$; $\#P < 0.05$). In contrast, the effect of 5-HTP was unaltered by (-)-pindolol pretreatment (Fig 5D; $F_{(1,9)} = 1.070$, $P = 0.3279$; $P > 0.05$).

3.4. Experiment 4: Antidotal effect of cyproheptadine and (+)-MK-801

Cyproheptadine has been recommended in clinics for treating the 5-HT toxidrome (Boyer and Shannon, 2005; Isbister and Buckley, 2005). Thus, cyproheptadine (10 mg/kg, i.p.) was injected post 15 min to test whether the response was prevented before hyperthermia was elicited or post 60 min to test whether the hyperthermia was able to be reversed after being developed. Fig 6A shows that in the animals treated with cyproheptadine post 15 min, $\text{claudqc}T_{\text{cor}}$ were significantly prevented ($F_{(1,9)} = 40.167$, $P = 0.0001$) but still showed a small increase, $\sim +1$ °C above the pre-5-HTP level. Interestingly, the post-treatment failed to reverse the hyperthermia during the 75-min observation. In the second set of the experiment as shown in Fig 6B, animals had hyperthermia (2.1 ± 0.7 °C over the pre-5-HTP level) after 5-HTP injection. Post injection of cyproheptadine (60 min after 5-HTP) gradually decreased T_{cor} to the control level in 90 min, but did not reverse it to the hypothermic state.

In the case of (+)-MK-801, injection at post-15 min prevented development of hyperthermia (Fig 6C; $F_{(1,9)} = 34.976$, $P = 0.0002$) and at post-60 min reduced the hyperthermic T_{cor} to the control level within 90 min (Fig 6D).

4. Discussion

In this study, there were three major findings: *a*) the signs for the toxidrome (penile response, forepaw treading, hind limb abduction, Straub tail and tremor) were progressively severe with increasing doses of 5-HTP. However, the dose-response relationship for head shakes and $\text{claudqc}T_{\text{cor}}$ varied remarkably. For instance, head shaking behaviors were obvious at small (10 mg/kg) and moderate doses (15 mg/kg) but were inhibited by the high doses (20 mg/kg). Most strikingly, hypothermia was evoked by low doses of 5-HTP in contrast to hyperthermia by high doses. Thus, caution is needed in interpreting the relationship between a response and toxic intensity; *b*) it is likely that changes in body-core temperature are strongly related to toxic intensity. It appeared that a mild toxidrome was always associated with hypothermia, and severe toxicity was concurrent with high hyperthermia ($> +2$ °C from baseline). Regarding a moderate toxidrome, there was a tendency toward normothermia or a small change in core temperature (-1 °C to $+2$ °C). We also observed that 70% of the animals were killed when $\text{claudqc}T_{\text{cor}}$ were over $+3$ °C. Thus, it appears that controlling T_{cor} is important for the toxidrome treatment; *c*) the 5-HT_{2A} and NMDA receptor antagonists blocked hyperthermia and death. The results corroborate the

earlier assumption that blocking hyperthermia or reversing the hyperthermia to hypothermia was crucial for reducing the high death rates associated with the toxidrome (Isbister and Buckley, 2005; Nelson et al., 2007). However, penile erection, hind limb abduction and tremor were still in a severe state after the blockers. Thus, the receptors responsible for those behavioral signs remain to be identified.

In experiment 1, head shakes were elicited after injection of 5-HTP in the clorgylinized animals. The same observation was reported in previous investigations (Nisijima et al., 2004; Shioda et al., 2004; Izumi et al., 2006). In this study, however, the numbers of head shakes were counted in response to the doses ranging from the non-toxic to toxic and to lethal injections. The behavioral expression of shaking activity was very similar to that of ($\pm$)-1-(2,5-Dimethoxy-4-iodophenyl)-2-aminopropane hydrochloride (DOI), a hallucinogenic drug (Dursun and Handley, 1996; Vickers et al., 2001). To date the relationship between hallucination and DOI-induced shaking behavior is not fully understood. A recent molecular study revealed a possible link of head shakes to hallucination (Garcia et al., 2007). If this link is confirmed to be the case, it suggests that head shaking behavior may reflect not only physical but also mental changes. Indeed, there were mental state changes in patients with the toxidrome (Boyer and Shannon, 2005). The similarity of the shaking behavior induced by DOI or 5-HTP suggests that both are acting at the 5-HT_{2A} receptor site. Interestingly, the shaking response induced by 5-HTP in the clorgylinized animals was not only blocked by ketanserin but also attenuated by (+)-MK-801, suggesting that both 5-HT_{2A} and NMDA receptors were involved. We noted that the first shake in response to high doses was observed as early as 5 min after an injection, occurring before a pronounced elevation of brain 5-HT (Shioda et al., 2004). In spite of early occurrence, the response was dramatically inhibited as other symptoms became severe at high doses. It is conceivable that intensified neuromuscular activity, which was evoked much later, restrained the head shaking response. Clearly, our data support the hypothesis that behavioral signs can be altered dependent on the degrees of the toxidrome.

In experiment 2, signs of the toxidrome were characterized using non-parametric approaches. It should be kept in mind that, in addition to the five signs investigated in this study, other changes were also observed including locomotion, hunched back, flat body, diarrhea and cyanosis (Grahame-Smith, 1971b; Jacobs and Klemfuss, 1975). However, there was no consensus available in the literature regarding the definition of the symptoms and relative severities although several attempts had been made (Jacobs and Klemfuss, 1975; Darmani and Zhao, 1998; Izumi et al., 2006). In this study, a severity score on a given sign was assigned and optimized following a series of pilot observations. To minimize possible biases and errors from observers, double-blind tests were used throughout the experiments. The most interesting findings were that there were strong correlations between non-parametric measurements, $\text{claudqc}T_{\text{cor}}$ and the death rate. Regardless of dose injections, a severe toxidrome was always shown to be correlated with high T_{cor} as shown in Fig 7. Together, we concluded that a life-threatening incident likely occurred with $\text{claudqc}T_{\text{cor}}$ over +3 °C.

We demonstrated that the NMDA receptor antagonist (+)-MK-801 reduced the severity scores of behavioral signs induced by 5-HTP in the clorgylinized animals. Interestingly, the

effects were selective only for forepaw treading and Straub tail, but not penile erection, hind limb abduction or tremor. We also observed that the 5-HT_{2A} receptor antagonist ketanserin but not (+)-MK-801 improved cyanosis, however both effectively blocked hyperthermia and death. Our data suggest that neural circuits mediating those changes were different although the primary cause was the same, presumably initiated by an excess of 5-HT (Boyer and Shannon, 2005). In addition to 5-HT, other neurotransmitters such as dopamine, noradrenalin and glutamate were also elevated during the 5-HT toxidrome (Nisijima et al., 2001; Shioda et al., 2004). This is expected since the large increase in 5-HT from serotonergic terminals, which are widely distributed in the brain, would interact with other neuronal systems resulting in a massive and indiscriminate release of other neurotransmitters as well. Our results were corroborated with findings that 5-HT_{2A} receptors had an excitatory effect on postsynaptic neurons (Aghajanian and Marek, 1999) and activated a neural circuit between dorsal raphe serotonergic and prefrontal cortical glutamatergic neurons, regions crucial in maintaining mood stability and mental health (Martin-Ruiz et al., 2001; Puig et al., 2003). Although the raphe-cortical circuit was proposed to be implicated in schizophrenia and hallucination (Aghajanian and Marek, 2000; Martin-Ruiz et al., 2001), it is most likely that the same pathway was responsible for mental disorientation in patients during the 5-HT toxidrome (Mills, 1995; Terao and Hikichi, 2007). Moreover, our studies suggest that head shakes and hyperthermic responses might be also integrated with the pathway since the NMDA receptor blocker (+)-MK-801 had the same selective effects on the symptoms as the 5-HT_{2A} receptor antagonist ketanserin. However, networks and neurotransmissions responsible for hind limb abduction and tremor remain to be determined. Regardless of this, all animals survived the lethal dose of injection, supporting the therapeutic value of 5-HT_{2A} and NMDA receptor antagonists in the treatment of toxicity (Isbister and Buckley, 2005).

Our studies provide evidence that body temperature was decreased (hypothermia) in a mild toxidrome. Hypothermia was mediated by many types of receptors including serotonergic 5-HT_{1A} (Hillegaart, 1991; Millan et al., 1993; Lin et al., 1998), 5-HT₇ (Guscott et al., 2003; Faure et al., 2006), dopaminergic D₂ (Oerther, 2000) and adrenergic α_2 receptors (Feleder et al., 2004). It appears that both 5-HT_{1A} and 5-HT₇ receptors exerted a small tonic inhibition on T_{cor} , consistent with the role of those binding sites in thermoregulation (Lin et al., 1998). Using the selective 5-HT₇ receptor antagonist SB-639970 we ruled out a possible involvement of 5-HT₇ receptors in hypothermia under our experimental condition. Consistent with the earlier findings (Abdel-Fattah et al., 1995), activation of 5-HT_{1A} receptors is responsible for hypothermia. Interestingly, the 5-HT_{1A} receptor antagonist WAY-100,635 (0.1 – 0.5 mg/kg, s.c.) blocked only 40% of the hypothermia associated with a mild syndrome, clearly suggesting that other receptors were also involved. Likewise, methysergide had an incomplete effect on the hypothermia (~60%), further supporting the involvement of other binding sites as well. We also noticed the difference of the time frame for the effect of WAY-100,635 and methysergide. It appears that WAY-100,635 blocked the early reduction, and methysergide antagonized the late response. The discrepancy between two antagonists is not fully understood and might be due to the broad-spectrum effect of methysergide. Nevertheless, further studies are needed to examine the potential role of D₂ and α_2 receptors in hypothermia induced by 5-HTP in the clorgylinized animals.

In contrast to the mild toxidrome, the severe toxidrome correlated with a high increase in core temperature ($> +2\text{ }^{\circ}\text{C}$) was life-threatening. It was most likely that animals were killed as the core temperature was increased by over $+3\text{ }^{\circ}\text{C}$. Since the death and severity were highly correlated with $\text{claudq}T_{\text{cor}}$ (Fig 7), we therefore decided to examine further the effect on the core temperature induced by 5-HTP in the clorgylinized animals. Hyperthermia was completely blocked and even reversed to hypothermia by either ketanserin, cyproheptadine or (+)-MK-801 but not by (-)-pindolol, suggesting that 5-HT_{2A} and NMDA receptors were involved in the mediation of such a hyperthermic response to excessive 5-HT in the toxidrome. It has been evident that there is a neural circuit between raphe serotonergic and prefrontal glutamatergic neurons consisting of NMDA and 5-HT_{2A} receptors, respectively, in mediating behavioral processes relevant to psychotic symptoms (Martin-Ruiz et al., 2001). Although it has not yet been examined, it is most likely that such a neural circuit may also participate in the development of the severe toxidrome evoked by excessive 5-HT. After blocking hyperthermia, other symptoms such as tremor and hind limb abduction were still severe, suggesting that additional neural mechanisms, for instance, 5-HT_{1A} receptors, might be involved (Darmani and Zhao, 1998; Fox et al., 2007).

Cyproheptadine and (+)-MK-801 were thoroughly examined as antidotes via the animal model of the toxidrome in this study. Cyproheptadine was selected since it has been commonly recommended for patients (Boyer and Shannon, 2005). Although (+)-MK-801 is not in clinical use because of its side effects, the current study provides useful information for future exploration of possible therapeutic drugs regarding NMDA and glutamatergic receptors. To simulate the clinical situations, antidotes were given only after a toxidrome had been induced, as indicated by the signs including head shaking, flat body, hunched back, forepaw treading and tremor. It appears that both drugs were efficient at either time frame (post 15 min or post 60 min) in preventing or blocking hyperthermia induced by 5-HTP. However, whether the animals eventually survived from the lethal injection was dependent on the $\text{claudq}T_{\text{cor}}$ before the antidotes. We observed that death was prevented when the antidotes were injected in post-15 min before hyperthermia was elicited. After hyperthermia was developed, antidotes were able to prolong the lives (over 5 h vs. ~2 h without antidotes) but failed to prevent deaths. The findings are of particular interest, suggesting that the timing of antidotes is a matter of life or death. Among many possibilities, one could argue that the damage to some vital organs, which were injured by hyperthermia (Grahame-Smith, 1971b), was not reversible by the antidotes. It is worthwhile noting that a moderate toxidrome, manifested by a small increase in T_{cor} ($> +2\text{ }^{\circ}\text{C}$), was usually self-resolved without antidotal interferences.

In summary, we have characterized the 5-HT toxidrome induced by 5-HTP in the clorgylinized rats. All behavioral signs obtained from animal studies were found to be comparable to symptoms described in patients with 5-HT poisoning (Mills, 1995; Boyer and Shannon, 2005). Thus the results of the present studies provide valid procedures and 5-HTP doses crucial for investigating the emerged mental illness in humans. Since high hyperthermia is associated with an increased mortality rate, further studies are needed to decipher the neural circuit consisting of 5-HT_{2A} and glutamatergic NMDA receptors in regulating T_{cor} during serotonergic polytherapy.

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References

- Abdel-Fattah AF, Matsumoto K, el-Hady KA, Watanabe H. 5-HT_{1A} and 5-HT₂ receptors mediate hypo- and hyperthermic effects of tryptophan in pargyline-pretreated rats. *Pharmacol Biochem Behav.* 1995; 52:379–384. [PubMed: 8577805]
- Abdel-Fattah AF, Matsumoto K, Murakami Y, Adel-Khalek Gammaz H, Mohamed MF, Watanabe H. Central serotonin level-dependent changes in body temperature following administration of tryptophan to pargyline- and harmaline-pretreated rats. *Gen Pharmacol.* 1997; 28:405–409. [PubMed: 9068981]
- Aghajanian GK, Marek GJ. Serotonin, via 5-HT_{2A} receptors, increases EPSCs in layer V pyramidal cells of prefrontal cortex by an asynchronous mode of glutamate release. *Brain Res.* 1999; 825:161–171. [PubMed: 10216183]
- Aghajanian GK, Marek GJ. Serotonin model of schizophrenia: Emerging role of glutamate mechanisms. *Brain Res Rev.* 2000; 31:302–312. [PubMed: 10719157]
- Birmes P, Coppin D, Schmitt L, Lauque D. Serotonin syndrome: A brief review. *CMAJ.* 2003; 168:1439–1442. [PubMed: 12771076]
- Boyer EW, Shannon M. The serotonin syndrome. *N Engl J Med.* 2005; 352:1112–1120. [PubMed: 15784664]
- Darmani NA, Zhao W. Production of serotonin syndrome by 8-OH-DPAT in *Cryptotis parva*. *Physiol Behav.* 1998; 65:327–331. [PubMed: 9855483]
- Dunkley EJ, Isbister GK, Sibbritt D, Dawson AH, Whyte IM. The Hunter Serotonin Toxicity Criteria: Simple and accurate diagnostic decision rules for serotonin toxicity. *QJM.* 2003; 96:635–642. [PubMed: 12925718]
- Dursun SM, Handley SL. Similarities in the pharmacology of spontaneous and DOI-induced head-shakes suggest 5HT_{2A} receptors are active under physiological conditions. *Psychopharmacology (Berl).* 1996; 128:198–205. [PubMed: 8956381]
- Faure C, Mnie-Filali O, Scarna H, Debonnel G, Haddjeri N. Effects of the 5-HT₇ receptor antagonist SB-269970 on rat hormonal and temperature responses to the 5-HT_{1A/7} receptor agonist 8-OH-DPAT. *Neurosci Lett.* 2006; 404:122–126. [PubMed: 16759802]
- Feleder C, Perlik V, Blatteis CM. Preoptic α 1- and α 2-noradrenergic agonists induce, respectively, PGE₂-independent and PGE₂-dependent hyperthermic responses in guinea pigs. *Am J Physiol Regul Integr Comp Physiol.* 2004; 286:R1156–1166. [PubMed: 14962823]
- Fox MA, Jensen CL, Gallagher PS, Murphy DL. Receptor mediation of exaggerated responses to serotonin-enhancing drugs in serotonin transporter (SERT)-deficient mice. *Neuropharmacol.* 2007; 53:643–656.
- Garcia EE, Smith RL, Sanders-Bush E. Role of G_q protein in behavioral effects of the hallucinogenic drug 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane. *Neuropharmacol.* 2007; 52:1671–1677.
- Gillman PK. A Review of Serotonin Toxicity Data: Implications for the Mechanisms of Antidepressant Drug Action. *Biol Psychiatry.* 2006; 59:1046–1051. [PubMed: 16460699]
- Grahame-Smith DG. Inhibitory effect of chlorpromazine on the syndrome of hyperactivity produced by L-tryptophan or 5-methoxy-N,N-dimethyltryptamine in rats treated with a monoamine oxidase inhibitor. *Br J Pharmacol.* 1971a; 43:856–864. [PubMed: 4261561]
- Grahame-Smith DG. Studies in vivo on the relationship between brain tryptophan, brain 5-HT synthesis and hyperactivity in rats treated with a monoamine oxidase inhibitor and L-tryptophan. *J Neurochem.* 1971b; 18:1053–1066. [PubMed: 4254940]
- Guscott MR, Egan E, Cook GP, Stanton JA, Beer MS, Rosahl TW, Hartmann S, Kulagowski J, McAllister G, Fone KC, Hutson PH. The hypothermic effect of 5-CT in mice is mediated through the 5-HT₇ receptor. *Neuropharmacol.* 2003; 44:1031–1037.

- Hillegaart V. Effects of local application of 5-HT and 8-OH-DPAT into the dorsal and median raphe nuclei on core temperature in the rat. *Psychopharmacology (Berl)*. 1991; 103:291–296. [PubMed: 1829235]
- Isbister GK, Buckley NA. The pathophysiology of serotonin toxicity in animals and humans: Implications for diagnosis and treatment. *Clin Neuropharmacol*. 2005; 28:205–214. [PubMed: 16239759]
- Izumi T, Iwamoto N, Kitaichi Y, Kato A, Inoue T, Koyama T. Effects of co-administration of a selective serotonin reuptake inhibitor and monoamine oxidase inhibitors on 5-HT-related behavior in rats. *Eur J Pharmacol*. 2006; 532:258–264. [PubMed: 16488409]
- Jacobs BL, Klemfuss H. Brain stem and spinal cord mediation of a serotonergic behavioral syndrome. *Brain Res*. 1975; 100:450–457. [PubMed: 1192191]
- Lin MT, Tsay HJ, Su WH, Chueh FY. Changes in extracellular serotonin in rat hypothalamus affect thermoregulatory function. *Am J Physiol*. 1998; 274:R1260–1267. [PubMed: 9644038]
- Martin-Ruiz R, Puig MV, Celada P, Shapiro DA, Roth BL, Mengod G, Artigas F. Control of serotonergic function in medial prefrontal cortex by serotonin_{2A} receptors through a glutamate-dependent mechanism. *J Neurosci*. 2001; 21:9856–9866. [PubMed: 11739593]
- Millan MJ, Rivet JM, Canton H, Le Marouille-Girardon S, Gobert A. Induction of hypothermia as a model of 5-hydroxytryptamine_{1A} receptor-mediated activity in the rat: A pharmacological characterization of the actions of novel agonists and antagonists. *J Pharmacol Exp Ther*. 1993; 264:1364–1376. [PubMed: 8450471]
- Mills KC. Serotonin syndrome. *Am Fam Physician*. 1995; 52:1475–1482. [PubMed: 7572570]
- Mitchell R. Fatal toxic encephalitis occurring during iproniazid therapy in pulmonary tuberculosis. *Ann Intern Med*. 1955; 42:417–424. [PubMed: 14350468]
- Modigh K, Svensson TH. On the role of central nervous system catecholamines and 5-hydroxytryptamine in the nialamide-induced behavioural syndrome. *Br J Pharmacol*. 1972; 46:32–45. [PubMed: 4263796]
- Nelson LS, Erdman AR, Booze LL, Cobaugh DJ, Chyka PA, Woolf AD, Scharman EJ, Wax PM, Manoguerra AS, Christianson G, Caravati EM, Troutman WG. Selective serotonin reuptake inhibitor poisoning: An evidence-based consensus guideline for out-of-hospital management. *Clin Toxicol (Phila)*. 2007; 45:315–332. [PubMed: 17486478]
- Nisijima K, Shioda K, Yoshino T, Takano K, Kato S. Diazepam and chlormethiazole attenuate the development of hyperthermia in an animal model of the serotonin syndrome. *Neurochem Int*. 2003; 43:155–164. [PubMed: 12620284]
- Nisijima K, Shioda K, Yoshino T, Takano K, Kato S. Memantine, an NMDA antagonist, prevents the development of hyperthermia in an animal model for serotonin syndrome. *Pharmacopsychiatry*. 2004; 37:57–62. [PubMed: 15048612]
- Nisijima K, Yoshino T, Ishiguro T. Risperidone counteracts lethality in an animal model of the serotonin syndrome. *Psychopharmacology (Berl)*. 2000; 150:9–14. [PubMed: 10867971]
- Nisijima K, Yoshino T, Yui K, Katoh S. Potent serotonin (5-HT_{2A}) receptor antagonists completely prevent the development of hyperthermia in an animal model of the 5-HT syndrome. *Brain Res*. 2001; 890:23–31. [PubMed: 11164765]
- Oerther S. Temperature set-point changes induced by DA D_{2/3} and 5-HT_{1A} receptor agonists in the rat. *Neuroreport*. 2000; 11:3949–3951. [PubMed: 11192607]
- Peroutka SJ, Lebovitz RM, Snyder SH. Two distinct central serotonin receptors with different physiological functions. *Science*. 1981; 212:827–829. [PubMed: 7221567]
- Puig MV, Celada P, Diaz-Mataix L, Artigas F. In vivo modulation of the activity of pyramidal neurons in the rat medial prefrontal cortex by 5-HT_{2A} receptors: relationship to thalamocortical afferents. *Cereb Cortex*. 2003; 13:870–882. [PubMed: 12853374]
- Shioda K, Nisijima K, Yoshino T, Kato S. Extracellular serotonin, dopamine and glutamate levels are elevated in the hypothalamus in a serotonin syndrome animal model induced by tranylcypromine and fluoxetine. *Prog Neuropsychopharmacol Biol Psychiatry*. 2004; 28:633–640. [PubMed: 15276688]

- Terao T, Hikichi T. Serotonin syndrome in a case of depression with various somatic symptoms: the difficulty in differential diagnosis. *Prog Neuropsychopharmacol Biol Psychiatry*. 2007; 31:295–296. [PubMed: 16916568]
- Van Oekelen D, Megens A, Meert T, Luyten WH, Leysen JE. Role of 5-HT₂ receptors in the tryptamine-induced 5-HT syndrome in rats. *Behav Pharmacol*. 2002; 13:313–318. [PubMed: 12218512]
- Vickers SP, Easton N, Malcolm CS, Allen NH, Porter RH, Bickerdike MJ, Kennett GA. Modulation of 5-HT_{2A} receptor-mediated head-twitch behaviour in the rat by 5-HT_{2C} receptor agonists. *Pharmacol Biochem Behav*. 2001; 69:643–652. [PubMed: 11509227]

Fig 1.

Effect of 5-HTP on head shakes in the rats pretreated with clorgyline (2 mg/kg, i.p.). 5-HTP was injected at time zero, indicated by solid arrows. The frequency was counted every 15 min. **A**, compared to vehicle control, injection of 5 mg/kg, 10 mg/kg and 15 mg/kg produced a dose-dependent increase. * $P < 0.05$ and ** $P < 0.01$ vs. vehicle control, examined by a repeated measures ANOVA followed by a *post-hoc* Scheffe test. **B**, compared to vehicle, injection of 20 mg/kg and 25 mg/kg had no significant effect. **C**, the 5-HT_{2A} receptor antagonist ketanserin (5 mg/kg, i.p.) or the NMDA receptor antagonist (+)-MK-801 (0.5 mg/kg, s.c.) was administered 15 min before 5-HTP, as shown by an open arrow. The effect

was blocked by ketanserin but not (+)-MK-801. $*P < 0.05$ compared to 5-HTP alone (one-way ANOVA followed by *post-hoc* Scheffe test).

Fig 2.

Toxic intensity during the time period between 45 – 75 min was expressed as mean $\pm$ S.E.M. (0, no effect; 1, mild; 2, moderate or 3, severe). **A**, the behavioral signs (penile erection, forepaw treading, hind limb abduction, Straub tail and tremor) were dose-dependently intensified following 5-HTP administration in the clorgylinized rats. Statistical significance was compared to saline control as indicated by * for $P < 0.05$ and ** for $P < 0.01$, examined by a nonparametric Mann-Whitney U test. **B**, the antagonistic effect of the 5-HT_{2A} receptor antagonist ketanserin (5 mg/kg, i.p.) or/and NMDA receptor blocker (+)MK-801 on toxic signs induced by 5-HTP (25 mg/kg, i.p.) in the clorgylinized rats. Statistical significance was compared to 5-HTP alone as indicated by * for $P < 0.05$ and ** for $P < 0.01$, examined by a nonparametric Mann-Whitney U test.

Fig 3.

Effect of 5-HTP on body-core temperature (T_{cor}) in the clorgylinized animals (2 mg/kg, i.p.). Data are expressed as the mean ($\pm$ S.E.M.). Arrows indicate the time of 5-HTP injection. **A**, small doses of 5-HTP induced a decrease in T_{cor} * $P < 0.05$ vs. vehicle control, examined by a one-way repeated measures ANOVA followed by a *post-hoc* Scheffe test. **B**, no effect was found at an intermediate dose of 5-HTP. **C**, high doses of 5-HTP evoked an increase in T_{cor} . # $P < 0.05$ at 20 mg/kg vs. vehicle; * $P < 0.05$ and ** $P < 0.01$ at 25 mg/kg vs. vehicle. **D**, the bar graph compares changes in T_{cor} in response to different doses of 5-HTP. The values summarize the mean T_{cor} between 60–90 min from Fig 3A–C.

Fig 4.

Effect of methysergide (**A**), ketanserin (**B**), WAY-100,635 (**C**) and SB-269970 (**D**) on hypothermia induced by 5-HTP (10 mg/kg, i.p.) in the clorgylinized rats. Data are expressed as changes in T_{cor} (claudqc T_{cor}) from the mean baseline before 5-HTP injection. The open arrows indicate the time for an antagonist injection and solid arrows for 5-HTP injection. Injection of 5-HTP evoked hypothermia, -2.6°C (± 0.6 ; mean decreases averaged from 60 min to 120 min). **A**, claudqc T_{cor} in rats pretreated with methysergide (methy; 10 mg/kg, i.p.) 15 min prior to 5-HTP. Compared to vehicle+5-HTP, methysergide blocked ~60% of the hypothermia (from -2.6°C to $-1.0 \pm 0.1^{\circ}\text{C}$). **B**, claudqc T_{cor} in rats pretreated with ketanserin (5 mg/kg, i.p.) 15 min prior to 5-HTP. The 5-HT_{2A} receptor antagonist ketanserin had no effect on hypothermia induced by 5-HTP. **C**, claudqc T_{cor} in rats pretreated with WAY-100,635 (WAY; 0.1 mg/kg, i.p.) 15 min prior to 5-HTP. The 5-HT_{1A} receptor antagonist WAY-100,635 blocked 40% of the hypothermia (from -2.6°C to $-1.5 \pm 0.2^{\circ}\text{C}$). **D**, claudqc T_{cor} in rats pretreated with SB-269970 (SB 1, 1 mg/kg, i.p.; SB 3, 3 mg/kg, i.p.) 15 min prior to 5-HTP. There was no difference in T_{cor} between SB-269970 and vehicle pretreated animals. Differences compared to vehicle+5-HTP are indicated by $*P < 0.05$ and $**P < 0.01$, examined by a one-way repeated measures ANOVA followed by a *post-hoc* Scheffe test.

Fig 5.

Effect of cyproheptadine (**A**), ketanserin (**B**), (+)-MK-801 (**C**) and (-)-pindolol (**D**) on the hyperthermia induced by 5-HTP (25 mg/kg, i.p.) in the clorgylinized rats. Data are expressed as changes in body-core temperature (ΔT_{cor}) from the mean baseline before 5-HTP injection. The open arrows indicate the time for an antagonist injection and solid arrows for 5-HTP injection. **A**, ΔT_{cor} in rats pretreated with cyproheptadine (cypro; 10 mg/kg, i.p.) 15 min prior to 5-HTP. Cyproheptadine blocked the hyperthermia, and further reversed T_{cor} to the hypothermic state. **B**, ΔT_{cor} in rats pretreated with ketanserin (5 mg/kg, i.p.) 15 min prior to 5-HTP. Similarly, the 5-HT_{2A} receptor antagonist ketanserin not only blocked the hyperthermia but also reversed T_{cor} to the hypothermic state. **C**, ΔT_{cor} in rats pretreated with (+)-MK-801 (0.5 mg/kg, s.c.) 15 min prior to 5-HTP. The NMDA receptor antagonist (+)-MK-801 blocked the hyperthermia and further reversed T_{cor} to the hypothermic state. **D**, ΔT_{cor} in rats pretreated with (-)-pindolol (8 mg/kg, i.p.) 15 min prior to 5-HTP. There was no difference in T_{cor} between (-)-pindolol and vehicle pretreated animals. Differences compared to vehicle+5-HTP are indicated by * $P < 0.05$ and ** $P < 0.01$, compared to the respective antagonist+vehicle by # $P < 0.05$, examined by a one-way repeated measures ANOVA followed by a *post-hoc* Scheffe test.

Fig 6.

Antidotal effect of cyproheptadine (A–B) and (+)-MK-801 (C–D) on hyperthermia induced by 5-HTP in the clorgylinized rats. Data are expressed as changes in body-core temperature (ΔT_{cor}) from the mean baseline before 5-HTP injection. The open arrows indicate the time for an antagonist injection and solid arrows for 5-HTP injection. **A**, hyperthermic effect was blocked by cyproheptadine (cypro; 10 mg/kg, i.p.) 15 min after 5-HTP injection. **B**, injection of cyproheptadine gradually decreased hyperthermia to homeothermic T_{cor} in 60 min. **C**, (+)-MK-801 injected 15 min after 5-HTP blocked development of hyperthermia. **D**, (+)-MK-801 administered 60 min after 5-HTP reduced hyperthermia to baseline level in 60 min. * $P < 0.05$ and ** $P < 0.01$ indicate a significant difference compared to the vehicle+5-HTP group, examined by a one-way repeated measures ANOVA followed by a Scheffe test. # $P < 0.05$ and ## $P < 0.01$ show a difference compared to the maximum T_{cor} , examined by an unpaired Student t-test.

Table 1

Drug doses and vehicles used in this study

Drug	Dose (mg/kg)	Vehicle	Route
clorgyline	2	0.9% saline	i.p.
5-HTP	1; 5; 10; 15; 20; 25	0.9% saline	i.p.
(-)-pindolol	8	H ₂ O	i.p.
ketanserin	5	H ₂ O	i.p.
cypheptadine	10	10% ethanol in H ₂ O	i.p.
(+)-MK-801	0.1; 0.5	0.9% saline	s.c.
methysergide	10	0.9% saline	i.p.
WAY-100,635	0.1; 0.5	0.9% saline	i.p.
SB-269970	1; 3	0.9% saline	i.p.

Table 2

Correlation of changes in body-core temperature (T_{cor}) and the severity of toxidrome induced by 5-HTP (mg/kg, i.p.) in the clorgylinized rats

Dose	n	T_{cor} ($\pm$ S.E.M)	Severity score	Deaths/total test (%)
vehicle	6	+0.40 ($\pm$ 0.21)	0	0/6 (0%)
5	4	-1.57 ($\pm$ 0.38)	0.1 $\pm$ 0.1	0/4 (0%)
10	7	-1.19 ($\pm$ 0.65)	0.8 $\pm$ 0.1	0/7 (0%)
15	6	+0.87 ($\pm$ 1.09)	1.8 $\pm$ 0.3	1/6 (~17%)
20	6	+2.88 ($\pm$ 0.51)	2.4 $\pm$ 0.2	3/8 (~38%)
25	10	+3.55 ($\pm$ 0.31)	2.8 $\pm$ 0.03	9/10 (90%)

claudqc T_{cor} were obtained by calculating the difference in core temperature between the pre-drug baseline and the level 60 min after 5-HTP injection. Severity is expressed as means ($\pm$ S.E.M) in the average of the toxic scores for penile erection, forepaw treading, hind limb abduction, Straub tail and tremor as described in the **Methods and materials**.