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The effect of serotonin and serotonin receptor antagonists on motion sickness in *Suncus murinus*

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Abstract

In the present study, we investigated the effect of 5-hydroxytryptamine (5-HT) and 5-HT receptor agonists and antagonists on motion sickness in *Suncus murinus*, and the possibility that the emetic stimulus of 5-HT can alter the sensitivity of the animals to the different emetic stimulus of motion sickness. 5-HT (1.0, 2.0, 4.0 and 8.0 mg/kg ip) induced emesis and that was antagonised by methysergide (1.0 mg/kg ip), the 5-HT_4 receptor antagonist sulphamate[1-[2-[(methylsulphonyl)amino]ethyl]-4-piperidinyl]methyl-5-fluoro-2-methoxy-1*H*-indole-3-carboxylate (GR125487D; 1.0 mg/kg ip) and granisetron (0.5 mg/kg ip). Pretreatment with 5-HT caused a dose-related attenuation of the emetic response induced by a subsequent motion stimulus, which was not significantly modified by methysergide, granisetron or GR125487D pretreatment. (+)-1-(2,5-Dimethoxy-4-iodophenyl)-2-amino-propane (DOI; 0.5 and 1.0 mg/kg ip), 8-hydroxy-2(di-*n*-propylamino)tetralin (8-OH-DPAT; 0.1 mg/kg ip) but not methysergide, GR125487D or granisetron, attenuated motion-induced emesis, and that was not affected by pretreatment with ketanserin (2.0 mg/kg, ip) or *N*-{2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl}-*N*-(2-pyridinyl)cyclohexanecarboxamide trihydrocholoride (WAY-100635; 1.0 mg/kg ip), respectively. Indeed, ketanserin alone (0.1, 0.3, 1.0 and 2.0 mg/kg ip) attenuated motion sickness. These data indicate that 5-$HT_{1/2}$, 5-HT_3 and 5-HT_4 receptors are involved in the induction of 5-HT-induced emesis. However, agonist action at the 5-$HT_{1A/7}$ and 5-HT_2 receptors, and antagonist action at the 5-HT_{2A} receptors can attenuate motion sickness in *S. murinus*.

Keywords: Motion sickness; 5-Hydroxytrptamine; *Suncus murinus*

1. Introduction

Motion sickness occurs as a result of a mismatch or conflict between the information arising from the vestibular system, the visual and proprioceptive inputs (Reason and Brand, 1975). Many different circumstances can cause motion sickness including travelling on land, sea, air or space (see reviews by Money, 1970; Money et al., 1996). The most unpleasant sensations are described as vertigo, dizziness and nausea with the frequent appearance of vomiting and changes in gastrointestinal motility and autonomic function. Differences in susceptibility to the disorientating stimuli in animals or man appear to be related to a genetic factor (Lentz and Collins, 1977; Abe et al., 1970), levels of hormones (Kucharczyk et al., 1991) and age (Reason and Brand, 1975; Chinn, 1956).

Although the exact mechanisms or neuronal circuitry involved in motion sickness are not fully understood, the vestibular system has been shown to provide a most important input for the generation of motion sickness as deaf mutes are immune to motion sickness (Graybiel, 1964; Kennedy et al., 1965). Furthermore, it has been shown that individuals with bilateral vestibular dysfunction are not susceptible to motion sickness (Cheung et al., 1991; Reason, 1978).

Receptors that predominately mediate stimulatory actions in the vestibular nuclei include muscarinic acetylcholine receptors, D_2 dopaminergic receptors, 5-HT_2 serotonergic receptors and the H_1 and H_2 histamine receptors; inhibitory receptors include μ and δ opioid receptors and the 5-HT_{1A} receptor (see Smith and Darlington, 1996). Thus, drugs acting at many different types of receptors could affect the neuronal mechanisms within the vestibular system. However, since the underlying neuronal mechanisms for motion

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sickness are not known, the effect of drug binding to particular receptor types in the vestibular system to motion sickness is not understood.

The currently used antihistaminergic and anticholinergic agents are the most effective drugs in alleviating the symptoms of motion sickness (see review by Yates et al., 1998). It has been shown that motion sickness in inhibited both in the cat and *Suncus murinus* by depleting histamine with the synthesis inhibitor, α-fluromethylhistidine (Kaji et al., 1991; Wood et al., 1990). The cholinoceptor antagonist, scopolamine which blocks all five subtypes of muscarinic receptors is antiemetic in man, the squirrel monkey and *S. murinus* (Stott et al., 1989; Cheung et al., 1992; Ueno et al., 1988). Although, these drugs do not completely block emesis and their antiemetic site of action is poorly understood.

The neurokinin NK_1 receptor antagonist (+)-(2*S*,3*S*)-3-(2-methoxybenzylamino)-2-phenylperidine (CP-99,999) has also been shown to reduce or prevent motion sickness in the cat, ferret and the dog (Lucot et al., 1997; Watson et al., 1995). Preliminary clinical studies in cancer patients have shown that an NK_1 receptor antagonist in combination with other drugs reduces emesis and possibly nausea after administration of cisplatin (Hesketh et al., 1998). Further clinical studies are required to determine the effectiveness of NK_1 receptor antagonists in treating motion sickness.

The available 5-HT_3 receptor antagonists are effective against the acute emetic response induced by radiation and cytotoxic drugs, but they do not block the emetic response to most centrally acting emetic stimuli such as opioid and dopaminergic agonists, and certain peripherally acting emetic stimuli such as copper sulphate and also motion sickness (see Andrews, 1994). However, this does not negate a role for the serotonergic system in the mechanism of motion sickness since previous studies have shown the antiemetic effects of 5-HT_{1A} and 5-HT_2 receptor agonists on motion sickness (Lucot, 1995; Okada et al., 1995).

In the present study, attempts were made to further investigate the role of the serotonergic system in motion sickness in *S. murinus.*

In addition, experiments were designed to investigate the potential interaction between a chemically induced emetogenic stimulus 5-hydroxytryptamine (5-HT) and the stimulus of motion and to characterise the receptor mechanisms involved. Furthermore, the muscarinic receptor antagonist, scopolamine and the NK_1 receptor antagonist, CP-99,994 were employed to confirm the value of *S. murinus* as a model of motion-induced emesis.

2. Methods

2.1. Animals and housing conditions

The experiments were carried out using both adult female (38.6 ± 1.0 g) and adult male (71.2 ± 1.3 g) Japanese House Musk shrew, *S. murinus* (Bradford University strain); the animals were age-matched. Animals were housed in groups of not more than six in each cage and were allowed food (AQUATIC 3, trout pellets) and water 'ad libitum.' Animals were also fed with cat food three times per week. The floor of the cages were covered with sawdust and cleaned twice a week. The animal room was maintained at a humidity between 45% and 50% at 24 °C and illuminated between 21:00 and 07:00 h on a normal light–dark cycle.

2.2. Behavioural observations

Immediately after the administration of a drug or vehicle, each animal was placed individually in a transparent cage

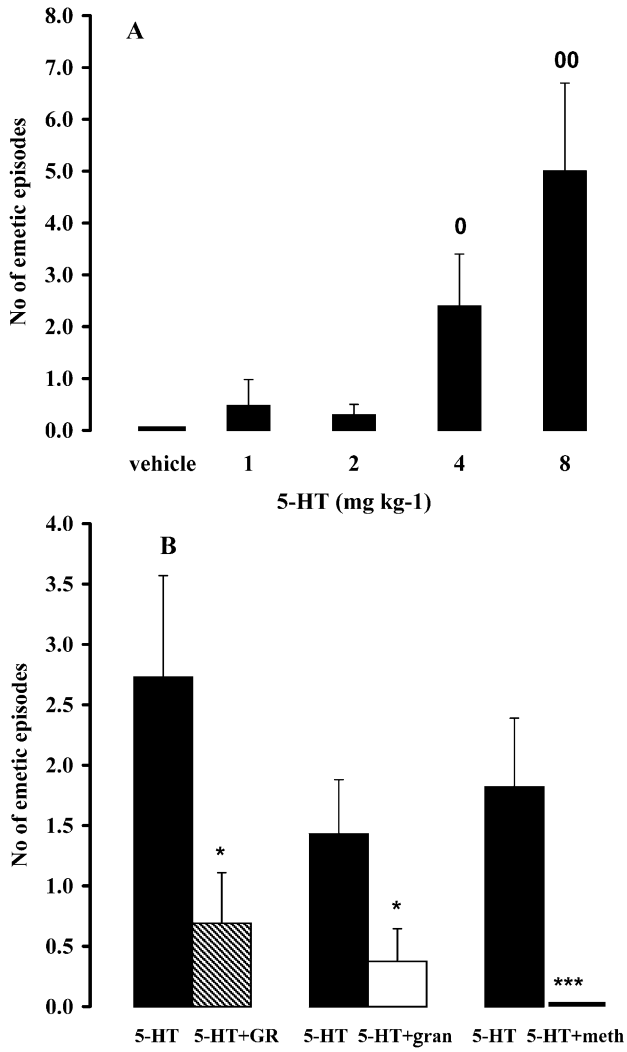

Fig. 1. (A) The effect of 5-HT (1.0, 2.0, 4.0 or 8.0 mg/kg ip) to induce emesis in *S. murinus*. (B) The effect of a 35-min pretreatment with methysergide (meth, 1.0 mg/kg ip), granisetron (gran, 0.5 mg/kg ip) or GR125487D (GR, 1.0 mg/kg ip) to antagonise emesis induced by 5-HT (4.0 mg/kg ip). Each histogram represents the mean ± S.E.M. of the number of emetic episodes recorded over a 10-min period, $n = 6$; * $P < .05$ and * * * $P < .001$ compared to the control 5-HT treatment; $^{o}P < .05$ and $^{oo}P < .01$ compared to the vehicle-treated animals.

(100 W × 150 L × 150 H mm) of six linked units and observed for any behavioural change. After a described time, a horizontal motion stimulus of 1 Hz and a 40-mm amplitude of shaking was commenced for 10 min. Preliminary experiments showed that these parameters were suitable to induce a reliable and reproducible emetic response (Javid and Naylor, 1999). In all experiments, the number of the emetic episodes (vomiting/retching) and the latency of onset to the first emetic episode were recorded. The emetic episodes were easily observed as a highly characteristic behavioural change: marked abdominal contractions, ventroflection of the head and a wide gaping mouth with protrusion of the tongue and licking, with vomiting occurring as the passage of solid material following the burst of sustained abdominal contractions. In many cases, the gastric material was stained yellow with bile. It should be noted that the animals were kept and tested in exactly the same environment to obviate confounding differences of olfactory, visual and other cues.

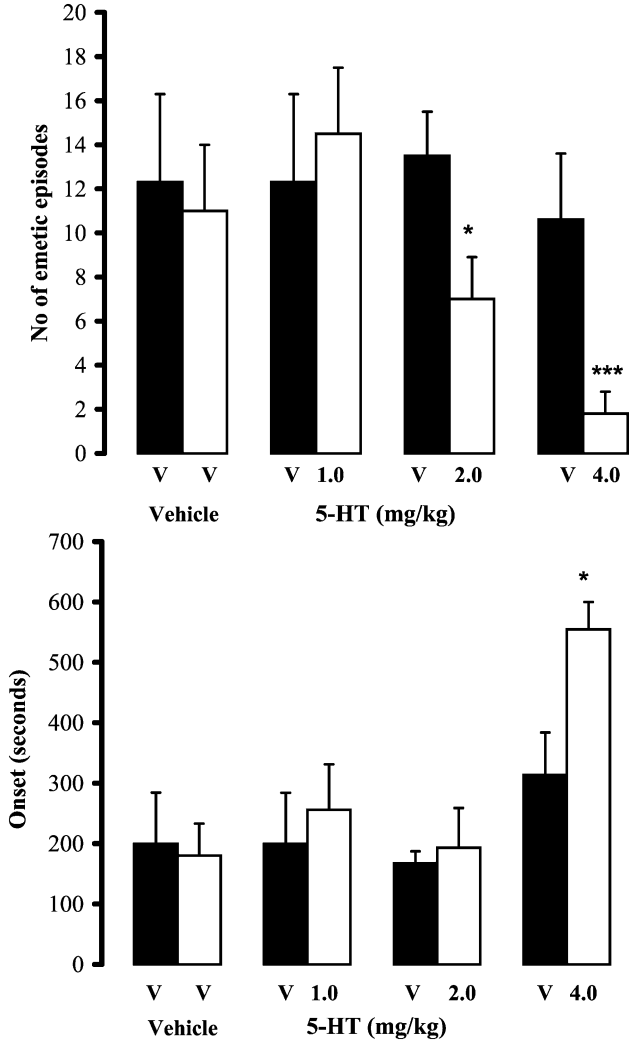

Fig. 2. The effect of 5-HT on motion induced emesis. In the first test, animals were challenged with saline prior to a motion stimulus (vehicle, v); in the second test, animals were challenged with saline or 5-HT 1.0, 2.0 or 4.0 mg/kg ip 10 min prior to a motion stimulus. The number of emetic episodes and the latency of onset to the first emetic episode to motion stimuli were measured during a 10-min shaking period at a frequency of 1 Hz with an amplitude of 40-mm movement. If an animal did not develop emesis within 10 min, the latency was considered to be 600 s. Each histogram represents the mean ± S.E.M., $n = 12$; * $P < .05$ and * * * $P < .001$ compared to the saline treatment.

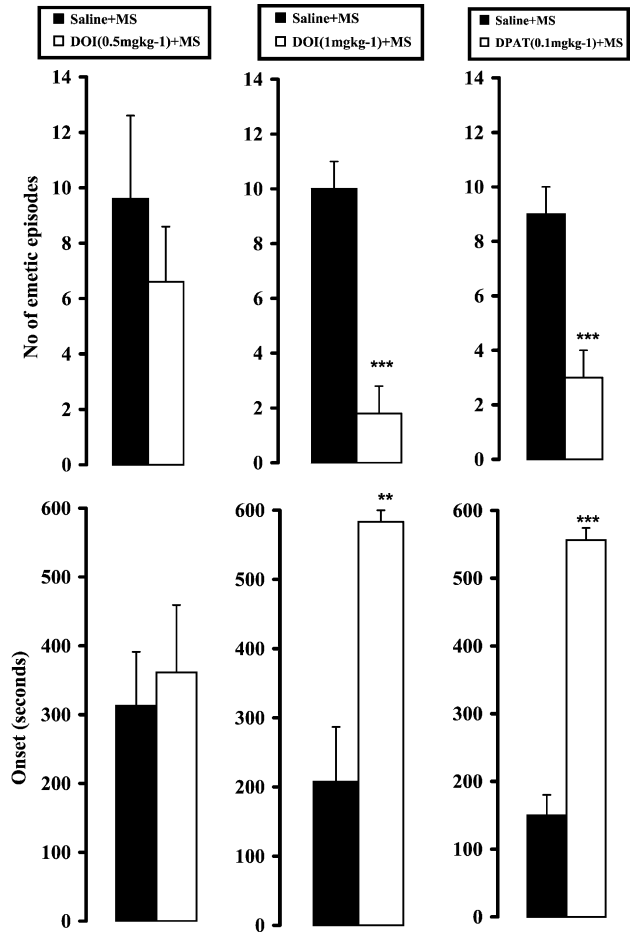

Fig. 3. The effect of DOI (0.5 and 1.0 mg/kg ip) and 8-OH-DPAT (DPAT, 0.1 mg/kg ip) on motion induced emesis. In the first test, animals were challenged with saline and motion stimulus and, in the second test, animals were challenged with DOI or 8-OH-DPAT 30 min prior to a motion stimulus (MS). The number of emetic episodes and the latency of onset to the first emetic episode were measured during a 10-min shaking period at a frequency of 1 Hz with an amplitude of 40-mm movement. If an animal did not develop emesis within 10 min, the latency was considered to be 600 s. Each histogram represents the mean ± S.E.M., $n = 6$; * * $P < .01$ and * * * $P < .001$ compared to the saline treatment.

2.3. Experimental design

Animals received 5-HT at 1.0, 2.0, 4.0 or 8.0 mg/kg ip or vehicle and were observed for 1 h for any behavioural change. In another set of experiments, animals were injected with either vehicle, granisetron (0.5 mg/kg ip), sulphamate[1-[2-[(methylsulphonyl)amino]ethyl]-4-piperidinyl]methyl-5-fluoro-2-methoxy-1*H*-indole-3-carboxylate (GR125487D;

1.0 mg/kg ip) or methysergide (1.0 mg/kg ip) 35 min prior to the administration of 5-HT (4.0 mg/kg ip).

In other experiments, animals received saline (intraperitoneally), 5-HT (1.0, 2.0 or 4.0 mg/kg ip), (+)-1-(2,5-dimethoxy-4-iodophenyl)-2-amino-propane (DOI; 0.5 or 1.0 mg/kg ip), 8-hydroxy-2(di-*n*-propylamino)tetralin (8-OH-DPAT; 0.1 mg/kg ip), ketanserin (0.1, 0.3, 1.0 and 2.0 mg/kg ip), *N*-{2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl}-*N*-(2-pyridinyl)cyclohexanecarboxamide trihydrocholoride (WAY-1006 35; 1.0 mg/kg ip), a combination of ketanserin (2.0 mg/kg ip) plus DOI (1.0 mg/kg ip), a combination of WAY-100635 (1.0 mg/kg ip) plus 8-OH-DPAT (0.1 mg/kg ip), granisetron (0.5 mg/kg ip), GR125487D (1.0 mg/kg ip), methysergide (1.0 mg/kg ip), CP-99,994 (5.0 mg/kg ip), scopolamine (2.0 mg/kg ip) 10–60 min (as detailed in Section 3) prior to a motion stimulus of 10 min duration.

Experiments were also carried out on animals that were injected intraperitoneally with either saline or 5-HT (4.0 mg/kg ip) 10 min prior to a motion stimulus (10 min duration); the latter experiments were carried out in the absence and presence of granisetron (0.5 mg/kg ip), GR1254 8D (1.0 mg/kg, ip) or methysergide (1.0 mg/kg ip) as 35-min pretreatments prior to the administration of 5-HT (4.0 mg/kg ip).

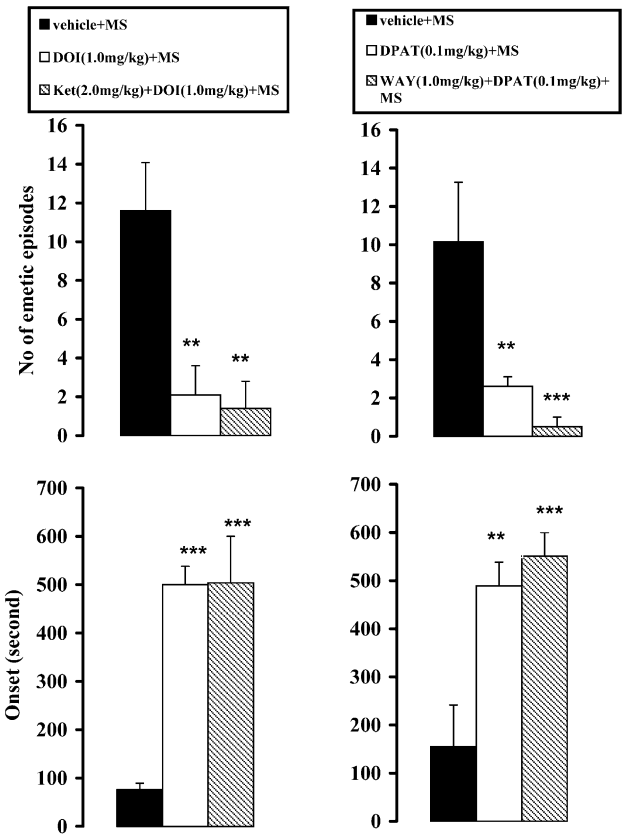

Fig. 4. The effect of DOI (1.0 mg/kg ip, 30-min pretreatment), 8-OH-DPAT (DPAT, 0.1 mg/kg ip, 30-min pretreatment), a combination of ketanserin (ket, 2.0 mg/kg ip, 45-min pretreatment) plus DOI (1.0 mg/kg ip, 30-min pretreatment), a combination of WAY 100635 (WAY, 1.0 mg/kg ip, 60-min pretreatment) plus 8-OH-DPAT (DPAT, 0.1 mg/kg ip, 30-min pretreatment) or vehicle on motion induced emesis. The number of emetic episodes and the latency of onset to the first emetic episode were measured during 10-min shaking period at a frequency of 1 Hz with an amplitude of 40-mm movement are shown. If an animal did not develop emesis within 10 min, the latency was considered to be 600 s. Each histogram represents the mean ± S.E.M., $n=6$; $^{**}P<.01$ and $^{***}P<.001$ compared to the vehicle treatment.

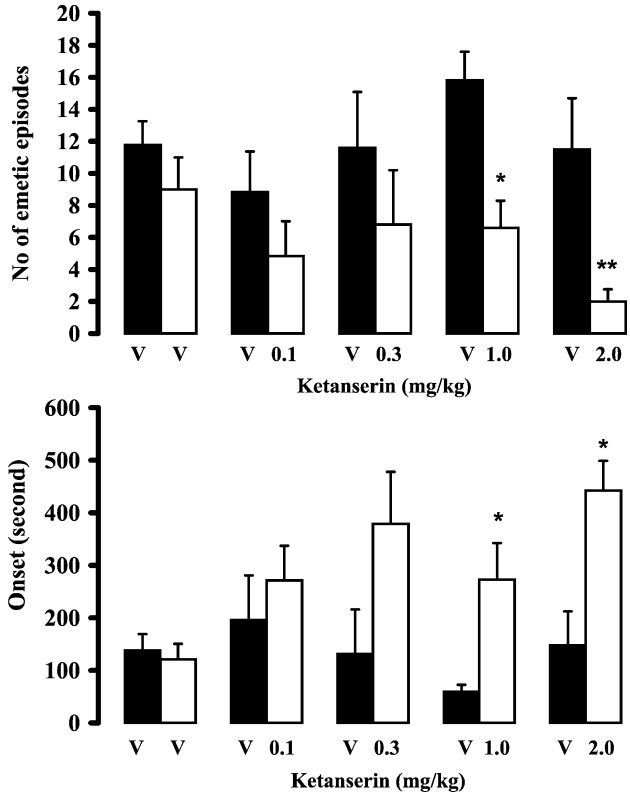

Fig. 5. The effect of ketanserin on motion induced emesis. In the first test, animals were challenged with saline prior to a motion stimulus (vehicle, v); in the second test, animals were challenged with saline or ketanserin (ket, 0.1, 0.3, 1.0 and 2.0 mg/kg ip) 45 min prior to motion stimulus. The number of emetic episodes and the latency of onset to the first emetic episode were measured during a 10-min shaking period at a frequency of 1 Hz with an amplitude of 40-mm movement are shown. If an animal did not develop emesis within 10 min, the latency was considered to be 600 s. Each histogram represents the mean ± S.E.M., $n=6$; $^{*}P<.05$ and $^{**}P<.01$ compared to the vehicle treatment.

In all experiments, a motion stimulus of 1 Hz and 40-mm amplitude of shaking was used for a 10-min period. Emesis induced by motion did not occur outside the period for which the animals were exposed. The number of the emetic episodes and the latency of onset to the first emetic episode were recorded.

All the experimental procedures were in compliance with the UK Animals (Scientific Procedures) Act 1986.

2.4. Drugs

5-HT maleate (Sigma), DOI (Sigma), methysergide maleate (Sandoz), GR125487D (Glaxo-Welcome), granisetron HCl·2H_2O (Smithkline Beecham), CP-99,994 (Pfizer), scopolamine HBr (Sigma), 8-OH-DPAT (RBI), ketanserin tart-

rate H_2O (RBI) and WAY-100635 were dissolved in distilled water. All doses of the drugs used were calculated on the basis of the weight of drug base and administered in a volume of 1 ml/100 g body weight.

2.5. Statistical analysis

Data were expressed as the mean ± S.E.M. and analysed using a paired *t*-test or analysis of variance, which was followed by Bonferroni–Dunnett's *t*-test as appropriate, where $*P<.05$, $**P<.01$ and $***P<.001$ were taken as significant.

3. Results

3.1. The effects of 5-HT to induce emesis and to modify motion-induced emesis

The intraperitoneal administration of 5-HT at doses of 1.0 ($P>.05$), 2.0 ($P>.05$), 4.0 ($P<.05$) and 8.0 mg/kg ($P<.01$) induced emesis in a dose-dependent manner (Fig. 1A). 5-HT at 4.0 and 8.0 mg/kg also produced overt salivation in all animals. The latency of onset to the first emetic episode was 26.9 ± 11.9 and 36.8 + 19.9 s at 4.0 and 8.0 mg/kg, respectively. There were no other overt changes in behaviour.

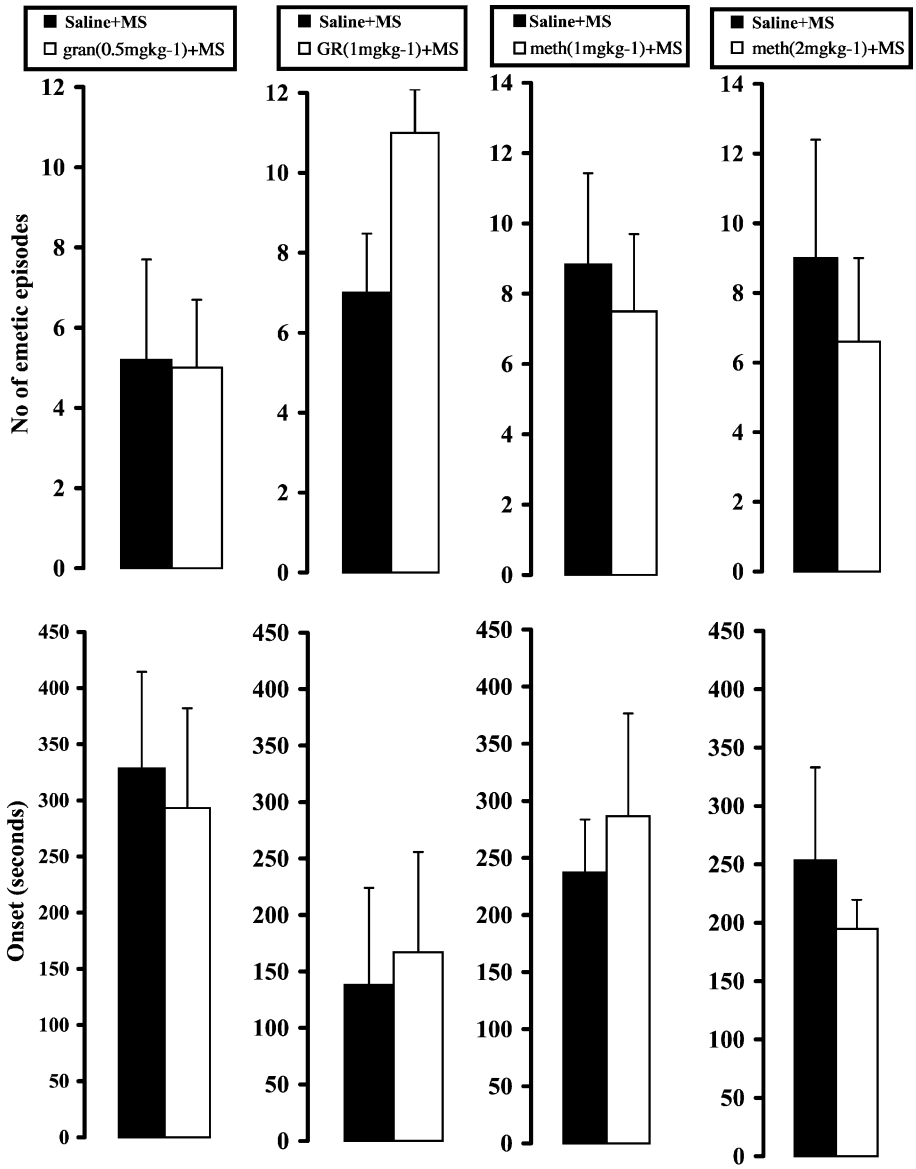

Fig. 6. The effect of methysergide (meth, 1.0 and 2.0 mg/kg ip), granisetron (gran, 0.5 mg/kg ip) or GR125487D (GR, 1.0 mg/kg ip) on motion induced emesis. In the first test, animals were challenged with saline and motion stimuli (MS); in the second test, animals were challenged with granisetron or GR125487D 45 min prior to a MS. The number of emetic episodes and the latency of onset to the first emetic episode were measured during a 10-min shaking period at a frequency of 1 Hz with an amplitude of 40-mm movement are shown. If an animal did not develop emesis within 10 min, the latency was considered to be 600 s. Each histogram represents the mean ± S.E.M., $n=6$.

The effect of a 10-min pretreatment with 5-HT was to attenuate dose-dependently the emetic response induced by a subsequent challenge to motion stimulus (Fig. 2). The attenuation of the emetic response to a motion stimulus achieved significance at 2.0 ($P<.05$) and 4.0 mg/kg ($P<.001$) of 5-HT (7.0 ± 2.0 and 1.8 ± 1.0 emetic episodes, respectively) as compared to the control values of 13.5 ± 2.0 and 10.6 ± 3.0 emetic episodes, respectively, obtained in the saline treated animals. Furthermore, pretreatment with 5-HT increased the latency to the first emetic episode induced by motion stimulus which achieved significance only at 4.0 mg/kg 5-HT ($P<.05$) (Fig. 2).

3.2. The effects of 5-HT receptor antagonists to modify 5-HT-induced emesis

The administration of granisetron (0.5 mg/kg ip) or GR125487D (1.0 mg/kg ip) 35 min prior to 5-HT (4.0 mg/kg ip) attenuated significantly ($P<.05$) the emesis induced by 5-HT (Fig. 1B). The emetic response to 5-HT

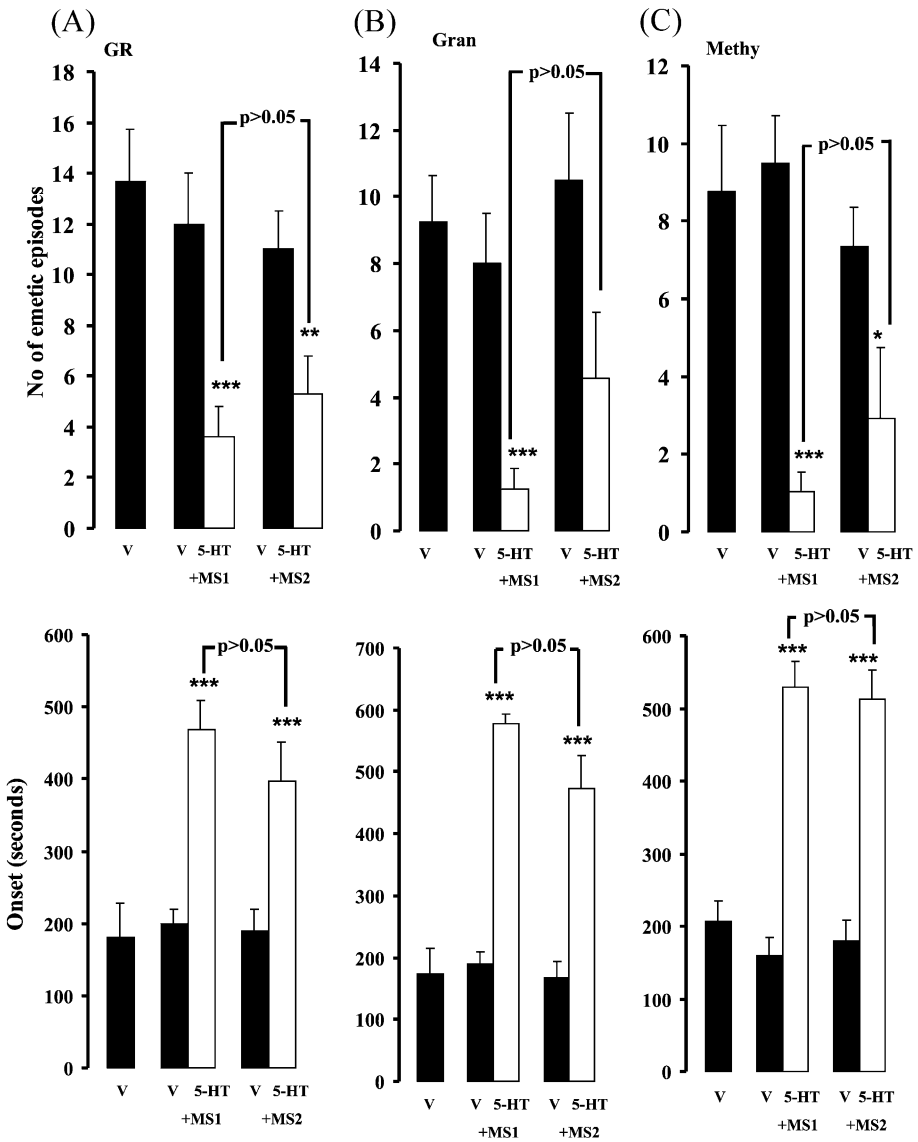

Fig. 7. The effect of preexposure to 5-HT (4.0 mg/kg ip) in the absence and presence of (A) GR125487D (GR, 1.0 mg/kg ip), (B) granisetron (gran, 0.5 mg/kg ip) and (C) methysergide (meth, 1.0 mg/kg ip) on the sensitivity of the animals to motion stimuli. In the first test ($n=18$), animals were challenged with saline (v) and motion stimuli; in the second test, animals were divided into two groups ($n=9$) and challenged with either saline or 5-HT 10 min prior to a motion stimulus (5-HT + MS1); in the third test, animals were challenged either with saline prior to a motion stimulus, or with the antagonist 35 min prior to a single injection of 5-HT and then (10 min later) with the motion stimuli (5-HT + MS2). The number of emetic episodes and the latency of onset to the first emetic episode were measured during a 10-min shaking period at a frequency of 1 Hz with an amplitude of 40-mm movement are shown. If an animal did not develop emesis within 10 min, the latency was considered to be 600 s. Each histogram represents the mean ± S.E.M.; * $P<.05$, ** $P<.01$ and *** $P<.001$ compared to the saline treatment.

was completely blocked by methysergide (1.0 mg/kg, $P<.001$).

3.3. Effects of 5-HT receptor agonists and antagonists on motion-induced emesis

DOI at 0.5 and 1 mg/kg, when administered 30 min prior to a motion stimulus, produced a dose related reduction in the emetic response induced by motion and this achieved significance ($P<.001$) at 1.0 mg/kg (Fig. 3). Also, pretreatment with DOI produced a significant increase in the latency to the first emetic episode ($P<.01$) at 1.0 mg/kg DOI (Fig. 3). DOI alone did not induce an emetic response. But unlike 5-HT, which failed to induce any overt behavioural changes in the *S. murinus*, DOI induced scratching and a 'stationary-like' behaviour at 0.5 and 1.0 mg/kg, respectively. The attenuation of motion-induced emesis by DOI (1.0 mg/kg, injected 30 min prior to motion stimulus) was not affected by pretreatment with ketanserin (2.0 mg/kg, injected 45 min prior to motion stimulus) (Fig. 4). Indeed, pretreatment with ketanserin alone (0.1, 0.3, 1.0 and 2.0 mg/kg) 45 min prior to the motion stimulus attenuated motion sickness and this achieved significance at 1.0 ($P<.05$) and 2.0 ($P<.01$) mg/kg (Fig. 5). This was followed by an increase in the latency of onset to the first emetic episode ($P<.05$) (Fig. 5). Although pretreatment with ketanserin did not reverse the inhibitory action of DOI on motion sickness, it reversed the stationary or freezing-like behaviour but not ear scratch like behaviour in the animals. Furthermore, ketanserin did not induce emesis in its own right.

The intraperitoneal administration of 8-OH-DPAT (0.1 mg/kg) 30 min prior to the motion stimuli reduced significantly ($P<.001$) the number of emetic episodes as compared to the saline treated animals from 9.0 ± 1.0 to 3.0 ± 1.0; this was associated with a significant ($P<.001$) increase in the latency of onset of emesis (Fig. 3). The inhibition of motion sickness induced by the administration of 8-OH-DPAT was not affected by pretreatment with WAY-100635 (1.0 mg/kg ip), which was injected 60 min prior to the motion stimuli (Fig. 4). Furthermore, the administration of WAY-100635 alone did not affect motion sickness.

However, the administration of the 5-HT_3 receptor antagonists granisetron (0.5 mg/kg), GR125487D (1.0 mg/kg) or methysergide (1.0 and 2.0 mg/kg), 45 min prior to the motion stimulus of 1 Hz and a 40-mm amplitude of movement, did not inhibit motion-induced emesis (Fig. 6). Also, the onset of the emetic episodes was not affected by these antagonists. None of the 5-HT receptor antagonists in their own right induced an overt behavioural change in the animals.

3.4. Effect of 5-HT receptor antagonists to modify the inhibitory effects of 5-HT on motion-induced emesis

In these experiments, three groups of animals were used to assess the interaction between 5-HT and GR125487D, granisetron and methysergide. All animals initially received a vehicle injection followed by a motion stimulus to ensure a comparable level of emesis between the groups. Animals then were injected with vehicle or 5-HT (4.0 mg/kg ip) in the absence or presence of a 35-min pretreatment with the antagonist. The intraperitoneal administration of 5-HT at 4.0 mg/kg significantly ($P<.01$ and $P<.001$) reduced the number of emetic episodes induced by a subsequent motion stimulus by 70–89% (Fig. 7). 5-HT also significantly increased the latency of onset of emesis by approximately 130–230% as compared to the control animals ($P<.001$). While pretreatment of the animals with GR125487D (1.0 mg/kg ip), granisetron (0.5 mg/kg ip) or methysergide (1.0 mg/kg ip) alone failed to modify motion-induced emesis (see Fig. 6), these antagonists also failed to significantly ($P>.05$) modify the inhibitory effect of 5-HT on motion-induced emesis (Fig. 7).

3.5. Effect of the neurokinin NK_1 receptor antagonist CP-99,994 and the cholinergic receptor antagonist scopolamine on motion-induced emesis

CP-99,994 when administered intraperitoneally at a dose of 5.0 mg/kg significantly ($P<.001$) reduced the number of

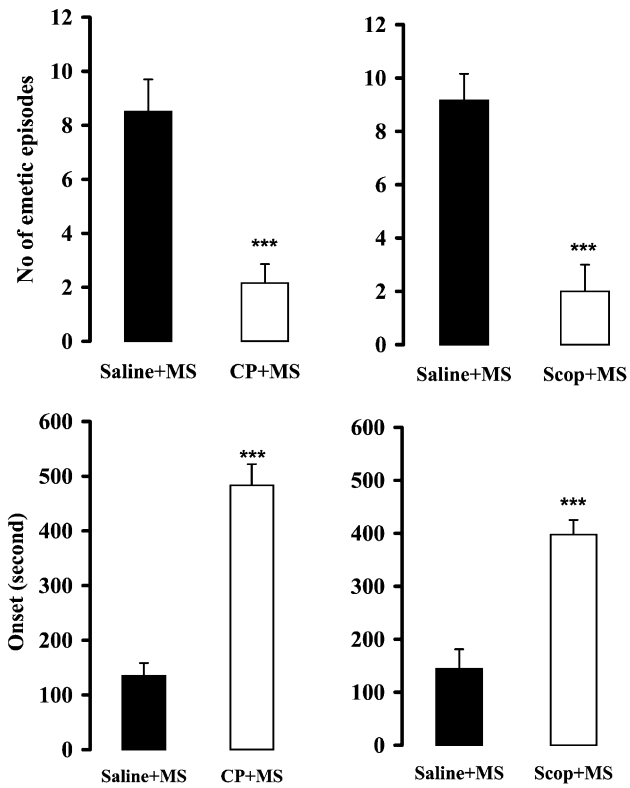

Fig. 8. The effect of CP-99 994 (CP, 5.0 mg/kg ip) or scopolamine (Sco, 2.0 mg/kg ip) administered as 45-min pretreatments on motion induced emesis. The number of emetic episodes and the latency of onset to the first emetic episode were measured during a 10-min shaking period at a frequency of 1 Hz with an amplitude of 40-mm movement are shown. If an animal did not develop emesis within 10 min, the latency was considered to be 600 s. Each histogram represents the mean ± S.E.M., $n=6$; * * * $P<.001$ compared to the saline treatment.

emetic episodes from 8.5 ± 1.2 to 2.2 ± 0.7 (Fig. 8). The onset of the emetic response was also significantly ($P < .001$) delayed from 135.0 ± 23.0 s in the saline treated animals to 483.0 ± 38.0 s (Fig. 8).

Scopolamine (2.0 mg/kg ip) when administered 45 min prior to the motion stimuli significantly ($P < .001$) reduced the number of emetic episodes from 9.2 ± 1.7 (obtained in the saline treated animals) to 2.0 ± 1.1. This was followed by a significant ($P < .001$) increase in the latency to the first emetic episode from 144.0 ± 36.0 to 397.0 ± 27.0 s (Fig. 8).

Neither CP-99,994 nor scopolamine produced any other overt behavioural change in the animals. In particular, the animals did not look sedated.

4. Discussion

The present study has confirmed that *S. murinus* affords a model of motion sickness susceptible to inhibition by the reference antiemetic agent scopolamine and also the NK_1 receptor antagonist CP-99,994. Indeed, the NK_1 receptor antagonists have been shown to have a breadth of action to inhibit emesis induced by drugs or motion in the cat (Lucot et al., 1997), dog (Watson et al., 1995), *S. murinus* (Gardner et al., 1995) and in the cancer patient (Navari et al., 1999). Therefore, the present paradigm used to induce motion sickness detects agents with proven antiemetic action in man and other species.

In an analysis of the mechanisms mediating motion sickness in *S. murinus*, the present study first attempted to investigate the importance of endogenous 5-HT using 5-HT receptor antagonists. The 5-$HT_{1/2}$ receptor antagonist methysergide has been shown to inhibit clonidine-induced emesis in cats (Japundzic-Zigon et al., 1995) and block the emesis elicited by apomorphine and orally administered copper sulphate in the dog (Lang and Marvig, 1989). However, in the present studies, the intraperitoneal administration of methysergide at pharmacologically effective doses did not antagonise motion-induced emesis in *S. murinus*. It has been shown that the 5-HT_3 receptor antagonists also fail to reduce motion sickness in man (Stott et al., 1989) and, in the present study, granisetron failed to reduce motion sickness in *S. murinus*. Similarly, in the present studies, the intraperitoneal administration of the potent 5-HT_4 receptor antagonist, GR125487D, at a pharmacologically effective dose (Twissell et al., 1995), did not protect the animals against motion-induced emesis.

Such data provides no support for a role of endogenous 5-HT to induce motion sickness in *S. murinus*, nor that a blockade of 5-$HT_{1/2/3/4}$ receptors is relevant to the control of motion sickness in this species. However, this does not exclude a role for 5-HT receptors in the pharmacological induction or control of emesis in *S. murinus*.

Thus, the intraperitoneal administration of 5-HT demonstrated a dose-dependent emetogenic effect with a very short onset and duration of action (generally less than 1 min) in *S. murinus*. The short onset of action suggests that the emetogenic effect of 5-HT is mediated peripherally and may involve an activation of 5-HT receptors located on the vagal afferent fibres. It has been shown in the ferret that the intravenous injection of 5-HT or the 5-HT_3 receptor agonist 2-methyl-5-HT produced a dose-dependent increase in abdominal afferent vagus nerve activity that was antagonised by a 5-HT_3 receptor antagonist (Hagihara et al., 1994). These authors also showed using different emetogens that cisplatin or copper sulphate-induced emesis is also associated with increases in afferent vagus nerve activity, which is antagonised by 5-HT_3 receptor antagonists. In addition, the intraperitoneal administration of 5-hydroxytryptophan and the intravenous infusion of 5-HT in cats and dogs (Bogdanski et al., 1958; Cahen, 1964) has produced retching and vomiting. Furthermore, the oral administration of the 5-HT_3 receptor agonists 2-methyl-5-HT and phenylbiguanide-induced emesis in the ferret that was blocked by a 5-HT_3 receptor antagonism (Sancilio et al., 1991). These findings demonstrate that 5-HT_3 receptors may activate a vagal afferent activity that may be relevant to 5-HT, cytotoxic drugs or copper sulphate-induced emesis.

The present study provided further evidence for the involvement of 5-HT_3 receptors in 5-HT-induced emesis since pretreatment with granisetron was able to attenuate the emetic effect of 5-HT in *S. murinus*. These results are similar to those reported by Torii et al. (1991) who showed that the emesis induced by the intraperitoneal administration of 5-HT in *S. murinus* was blocked by tropisetron. However, 5-HT receptors in addition to the 5-HT_3 receptors may also be involved in the emetic effects of exogenous 5-HT. Thus, pretreatment with methysergide also blocked the effects of 5-HT in *S. murinus*. In addition, the 5-HT_4 receptor antagonist GR125487D (Gidda et al., 1993) also attenuated 5-HT-induced emesis. The data in the present study indicate that in *S. murinus* exogenous 5-HT may have the potential to induce emesis via 5-HT_1, 5-HT_2, 5-HT_3 or 5-HT_4 receptors.

Irrespective of the brevity of action of 5-HT to induce emesis, and the possibility of its rapid metabolism following absorption, a 10-min pretreatment with 5-HT administered intraperitoneally caused a dose related *antagonism* to the subsequent stimulus of motion-induced emesis in *S. murinus*. The actions of 5-HT to inhibit motion sickness in *S. murinus* may involve a desensitisation of a common downstream pathway involved in emesis that follows the depolarisation of the 5-HT_3 receptors on the vagus nerve, which normally triggers the emetic reflex (Yakel and Jackson, 1988; Craig et al., 1990; Neijt et al., 1989).

However, electrical stimulation of the cervical vagus nerve has been shown to inhibit emesis induced by the stimulation of the abdominal vagus nerve in the dog, xylazin and motion-induced emesis in the cat and monkey, respectively (Zabara, 1992). Therefore, it is possible that 5-HT_3 receptors are also located on the cervical vagus nerve and

their stimulation by 5-HT could lead to the antagonism of emesis. However, in both cases, the hypothesis of an abdominal or cervical action clearly involves a peripheral site of action. But this does not exclude a central effect, although it should be noted that surgical ablation of the chemoreceptor trigger zone does not prevent motion-induced vomiting in cats and monkeys (Borison and Borison, 1986; Wilpizeski et al., 1986) and (b) vagotomy and sympathectomy does not change the incidence of motion sickness in dogs (Wang et al., 1957).

In a further study of the role of 5-HT receptors in motion sickness, the effect of different 5-HT receptor antagonists to modify the inhibitory effect of 5-HT on motion sickness was investigated. However, pretreatment with methysergide, granisetron or the 5-HT_4 receptor antagonist GR125487D failed to modify the inhibitory action of 5-HT on motion-induced emesis.

Notwithstanding an antiemetic effect mediated via the 5-HT_3 receptors, this does not exclude an involvement of 5-HT_1 and 5-HT_2 receptors in the control of emesis where, previously, the 5-$HT_{1A/7}$ receptor agonist 8-OH-DPAT and the 5-HT_2 receptor agonist DOI have been shown to antagonise cisplatin and motion-induced emesis in the cat, ferret, *S. murinus* and pigeon (Lucot and Crampton, 1989; Lucot, 1992; Okada et al., 1994; Rudd et al., 1992; Wolf and Leander, 1994; Baxter et al., 1995). Furthermore, in the present study, neither 8-OH-DPAT nor DOI induced emesis in their own right.

The action of 8-OH-DPAT on 5-HT_{1A} receptors may account for the inhibition of motion sickness. It has been shown that the vestibular nucleus in the brainstem receives 5-HT-containing projections from the dorsal raphe nucleus, and binding studies have demonstrated the presence of 5-HT_{1A}, 5-HT_{1B} and 5-HT_2 receptors on vestibular nucleus neurones (Zanni et al., 1995). The application of 5-HT to the mouse lateral vestibular nucleus in vivo using the iontophoretic technique has shown in the majority of cases an increase in the neuronal firing rate and in some cases a reduction followed by an increase. The increase in the firing rate is believed to be mediated by 5-HT_2 receptors due to its sensitivity to antagonism by methysergide and ketanserin (Licata et al., 1990). However, activation of 5-HT_{1A} receptors is believed to cause the reduction in the firing rate as this effect could be blocked by the 5-HT_{1A} receptor antagonist NAN-190 (Kishimoto et al., 1994). The decrease in the firing rate was mimicked by 8-OH-DPAT (Licata et al., 1993). Furthermore, in vitro electrophysiological studies have also reported similar results (Johnston et al., 1993). Whether 8-OH-DPAT administered peripherally acts on the dorsal raphe nuclei or directly on neurones in the vestibular nucleus remains to be investigated. If 8-OH-DPAT acts on the somatodendritic autoreceptors located on the raphe nuclei neurones, then activation of these may reduce synaptic 5-HT release within the vestibular nucleus. This would reduce the endogenous 5-HT tone at the excitatory 5-HT_2 receptor. Also, the action of a 5-HT_{1A} receptor agonist at the inhibitory receptors would also continue to reduce firing. In both cases, the final effect would be to decrease vestibular firing and the input to the 'vomiting centre'.

However, in the present study, the involvement of 5-HT_{1A} receptors in motion sickness seemed unlikely as we examined the effect of WAY-100635, a highly selective 5-HT_{1A} receptor antagonist (Fletcher et al., 1996) on 8-OH-DPAT-induced inhibition of motion sickness. The pretreatment with WAY-100635 failed to reverse the inhibitory action of 8-OH-DPAT on motion-induced emesis. Furthermore, pretreatment with WAY-100635 alone did not induce emesis in its own right nor did it modify the emetic response to motion. 8-OH-DPAT is a relatively selective 5-HT_{1A} receptor agonist with at least 10 times higher affinity for the 5-HT_{1A} receptor than for any other receptor type (Hoyer et al., 1994). However, recent results show that 8-OH-DPAT also possesses a relatively high affinity for the cloned rat and human 5-HT_7 receptors (Boess and Martin, 1994; Eglen et al., 1997). Therefore, an involvement of 5-HT_7 receptors in the inhibitory action of 8-OH-DPAT could be hypothesised. It should be noted that WAY-100635 has a negligible affinity for the 5-HT_7 receptors (Fletcher et al., 1996).

In the present studies using ketanserin, attempts were made to reverse the inhibitory action of DOI on motion sickness. Although ketanserin was able to inhibit the freezing-like behaviour (but not the ear scratching behaviour), it failed to reverse the inhibitory action of DOI on motion-induced emesis. But these results are inconclusive of a 5-HT_2 receptor involvement in the actions of DOI since ketanserin when administered alone was capable of attenuating motion-induced emesis in a dose-related manner. It is possible that the stimulus of motion causes a release of an endogenous substance, which probably acts on 5-HT_2 receptors to mediate an emetic response. Pretreatment with ketanserin blocks the 5-HT_2 receptors (particularly 5-HT_{2A} receptors) leading to an inhibition of emesis. However, it is not understood why ketanserin but not methysergide antagonised motion sickness, unless ketanserin was effective through a non-5-HT receptor system.

In conclusion, the precise role of 5-HT and its related receptors in mediating emesis are not clear. In terms of an agonist action, 5-HT and 5-HT_3 receptor agonists would be predicted to induce emesis. However, 5-HT may interact with other receptors to mediate an inhibitory response at the 5-$HT_{1A/7}$ and 5-HT_2 receptors. The failure of WAY-100635 to reverse the inhibition of motion sickness by 8-OH-DPAT suggests the unlikely involvement of 5-HT_{1A} receptors. The pretreatment with methysergide completely antagonised the emetic respone to exogenous 5-HT. This provides evidence that the actions of exogenous 5-HT to induce emesis are mediated via different systems to the activation of endogenous neurotransmitters involved in motion sickness. This may account for the prevention of 5-HT-induced emesis or motion sickness by 5-$HT_{1/2}$ receptor antagonism and 5-$HT_{1/2}$ receptor agonism, respectively.

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