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Effect of 5-HT_{1A} receptor agonist, 8-OH-DPAT, on cough responses in the conscious guinea pig

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Abstract

We have studied the role for 5-hydroxytryptamine (5-HT) in the modulation of the cough reflex by examining the effect of a selective 5-HT_{1A} receptor agonist, 8-hydroxy-2-(di-*n*-propylamino) tetralin (8-OH-DPAT) on cough and respiratory rate in conscious guinea pigs. Animals were placed in a box and exposed to the tussive agent citric acid (0.5 M) for 10 min, 3 min after terbutaline (0.05 mg/kg i.p.) was administered to prevent bronchoconstriction. 8-OH-DPAT inhibited at low doses (0.008 and 0.016 mg/kg) but potentiated at high doses (0.25 and 0.5 mg/kg) the citric acid-induced number of coughs, but dose-dependently increased respiratory rate. Methysergide (0.05–5 mg/kg), a $5HT_1$ and $5HT_2$ receptor antagonist, and ketanserin (0.05 mg/kg), a $5HT_2$ receptor antagonist, had no effects on cough or respiratory rate. Methysergide inhibited the increased cough responses and respiratory rate induced by 8-OH-DPAT at high doses, while ketanserin was without effect. These results suggest that 8-OH-DPAT may induce both an inhibition and activation of the cough reflex, the latter involving central $5HT_1$-receptor activation. © 1997 Elsevier Science B.V.

Keywords: Citric acid; Cough reflex; 5-HT (5-hydroxytryptamine, serotonin); Ketanserin; Methysergide

1. Introduction

5-hydroxytryptamine (5-HT) is an important modulator of brainstem processes such as vomiting and the control of breathing. In addition, recent work has supported a role for 5-HT in the control of the cough reflex in several species including man (Kamei et al., 1986, 1988, 1991; Stone et al., 1993). There are good grounds for suggesting that the 5-HT receptor subtype, 5-HT_{1A}, may modulate the sensitivity of the cough reflex. 5-HT_{1A} receptors are present on medullary brainstem neurons in regions thought to control respiration and cough and 5-HT has been found within visceral primary sensory neurons projecting to the nucleus tractus solitarius (Gaudin-Chazal et al., 1981, 1982), where cough and respiratory responses are thought to be integrated (Jordan and Spyer, 1986). 5-HT_{1A} receptors are known to exist on peripheral chemoreceptor endings (Nishi, 1975). Moreover, 5-HT_1 receptors display a very high affinity for 5-HT (Schmidt and Peroutka, 1989), which itself has been shown to diminish sensitivity of the cough reflex in man (Stone et al., 1993).

To examine whether 5-HT_{1A} receptor-mediated mechanisms are specifically involved in the modulation of cough and ventilation in the conscious state, we have studied the effects of the specific 5-HT_{1A} receptor agonist, 8-hydroxy-2-(di-*n*-propylamino)–tetralin (8-OH-DPAT), on these parameters in the conscious guinea pig. 8-OH-DPAT has a high affinity for 5-HT_{1A} receptors within the nanomolar range (Hjorth et al., 1982), and it is probable that most of its effects are mediated centrally, as the drug causes biochemical and behavioural phenomena characteristic of central serotoninergic stimulation (Arvidsson et al., 1981; Ahlenius et al., 1981; Tricklebank et al., 1985). The effects of 8-OH-DPAT may be mediated via a number of pathways because 5-HT_{1A} receptors are distributed widely throughout the brain and 8-OH-DPAT binds to presynaptic autoreceptors to reduce 5-HT release and also to post-synaptic 5-HT_{1A} receptors to increase the firing of serotoninergic neurons (Dickenson, 1989). Thus, contrasting effects of 8-OH-DPAT might be anticipated at different doses. In our studies in the conscious guinea pig, we show that 8-OH-DPAT has both inhibitory and excitatory effects on the cough reflex, but is a stimulant of ventilation.

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2. Materials and methods

2.1. Animals

Male Dunkin–Hartley guinea pigs (300–400 g) were caged individually in boxes with autoclaved sawdust to reduce the risk of mycoplasmal infection (Broderson et al., 1976) and were allowed free access to fluid and a standard rodent diet (B and K Universal, Hull, UK). Cages were housed in a constantly humidified environment (70%) at 23 ± 0.5°C with filtered air transported across the room in a laminar fashion.

2.2. Drugs

8-OH-DPAT (Sigma, Poole, UK), methysergide (Sandoz, Basel, Switzerland), and ketanserin (Sigma) were dissolved in 0.9% NaCl which was also used as the control agent in all studies. All drugs were given subcutaneously. 8-OH-DPAT was mixed with methysergide or ketanserin in studies investigating combinations of these drugs. The doses of methysergide and ketanserin used are known to act as receptor antagonists of the 5-HT_1 and 5-HT_2, and of the 5-HT_2 receptors, respectively (Vargaftig et al., 1982; Buckner et al., 1991). Control animals received an equivalent volume of 0.9% NaCl to those given 8-OH-DPAT and antagonist in the combination studies.

2.3. Studies

Changes in ventilation and sensitivity of the cough reflex were studied according to a rolling protocol described below, in which control and drug treatments were given 20 min before exposure of animals to citric acid (0.5 M). To minimise respiratory distress caused by citric acid, animals were pretreated with terbutaline sulphate (0.05 mg/kg, s.c.) 3 min before challenge.

Three groups of experiments were performed:

(1) Effects of 8-OH-DPAT (0.004–1 mg/kg) alone on resting respiratory rate and cough responses with doubling increments in doses used in separate groups of 6 animals for each dose

(2) Effects of the antagonists methysergide (0.05–5 mg/kg) and ketanserin (0.05–5 mg/kg) alone on respiratory rate and cough responses.

(3) Effects of each antagonist (5 mg/kg) on the influence of 8-OH-DPAT (0.004–0.016 mg/kg and 0.25–1 mg/kg) over respiratory rate and cough. The doses of 8-OH-DPAT (i.e., low and high) in this group of experiments were chosen on the basis of data obtained in the first studies.

2.4. Experimental set-up

A modification of the method of Forsberg and Karlsson (1986) was used to assess RR and cough frequency. After a period of acclimatisation to laboratory conditions, animals were placed one at a time into a perspex box ($10 \times 10 \times 25$ cm) that was shielded on all sides except that facing the observer. A constant airflow of 0.6 l/min was maintained through the box. A fixed resistance was placed on the exhaust port to allow the aerosol to permeate the box. The tussive agent (citric acid 0.5 M) was introduced into the box via a mini-ultrasonic nebuliser (Pulmosonic, Philadelphia, PA, USA). The particle size produced had an aerodynamic mass median diameter of 3.0 μm and the output of the nebuliser was 0.4 ml per minute.

To record resting respiratory rate and cough, a pneumotachograph (Fleisch 000) was inserted into the box above the air entry port. The other end of this device was placed into a tube that connected to an airtight chamber, which allowed damping of the system. Changes in airflow across the pneumotachograph were recorded by a transducer (Furness Controls, Bexhill) and recorded directly onto a moving pen recorder (Lectromed, UK). A tie-clip microphone (Sony, Japan) was placed in the roof of the box and was connected to a preamplifier and loudspeaker to monitor cough sounds. A regular sinusoidal trace was obtained (Fig. 1a) and respiratory rate was determined by examining the ten breaths. Resting respiratory rate was typically 100/min, and settled after an acclimatisation period in the box.

In order to induce cough, guinea pigs were exposed for 10 min to citric acid, and cough frequency was derived in cough/min over this time. Cough was usually preceded by sneeze (Fig. 1b). Coughs were defined as rapid increases in airflow accompanied by their characteristic sound.

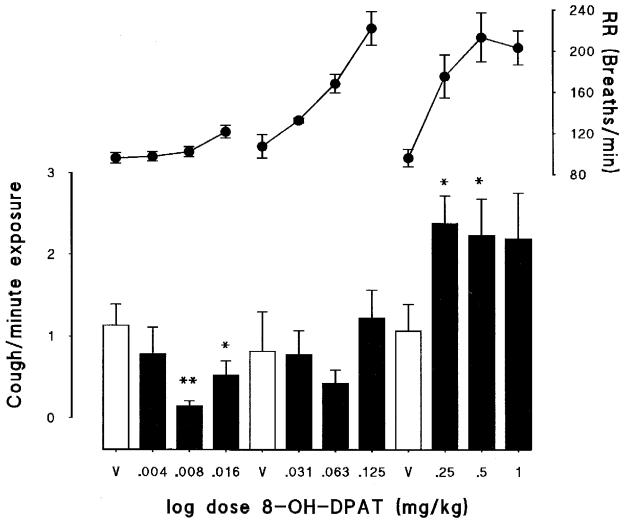

Fig. 1. Effect of increasing doses of 8-OH-DPAT on cough response and respiratory rate (RR) induced by exposure to citric acid for 10 min in conscious guinea pigs ($n = 6$ for each group). The study was performed in 3 batches of 24 guinea pigs, with vehicle (V) compared to 3 ascending doses. Low doses (0.004–0.016 mg/kg) inhibited, while high dose (0.25–1 mg/kg) augmented the cough response. Doses above 0.031 mg/kg increased respiratory rate. Data shown as mean ± SEM. * $P < 0.05$; ** $P < 0.01$ compared to respective vehicle.

Cough and sneeze could be differentiated on the basis of observation and sound (Forsberg and Karlsson, 1986). Citric acid also causes a sudden onset of tachypnoea followed by exaggerated abdominal movements in some animals, changes representing respiratory distress. Such movement is accompanied by a slowing of respiratory rate with a change in the trace pattern (Fig. 1c) and removal of animals from the box at this stage reveals an audible wheeze that can be reversed rapidly by the administration of terbutaline. To minimise respiratory distress, animals were pretreated with a low dose of terbutaline (0.05 mg/kg i.p.) 3 min prior to being placed in the box. Initial studies showed that the terbutaline pretreatment had no effect on the respiratory rate or cough responses.

2.5. Exposure protocol

Drug or control injections were given subcutaneously in a volume of 1 ml/kg, 20 min prior to citric acid exposure. After treatment, animals were placed in the box until 3 min prior to exposure, when they were given terbutaline (0.05 mg/kg, i.p.). Animals were challenged once only and were studied in parallel groups of 6 after repeatability studies showed a typical cough frequency of 1 per min. Single exposures were used because repeated challenges were accompanied by behavioural reduction in respiratory rate and cough.

2.6. Data analysis

Cough responses are reported as group medians. To allow for any changes in experimental conditions throughout study days, respiratory rate and cough frequency of drug-treated animals were compared against animals treated with control on the same day using a Mann–Whitney test. A P value less than 0.05 was taken as significant.

3. Results

3.1. Effects of 8-OH-DPAT on cough and respiratory rate

8-OH-DPAT had a dual effect on the sensitivity of the cough reflex. Cough responses were inhibited at the lower doses of 8-OH-DPAT (0.008 and 0.016 mg/kg) when compared against those of control treated animals. The group median change in frequency was from 0.91 to 0.15 min^{-1} for 0.004 mg/kg and 0.91 to 0.45 min^{-1} for 0.16 mg/kg (Fig. 2). By contrast, cough responses were potentiated at the higher doses of 8-OH-DPAT. The group median cough frequency rose from 1.15 to 2.7 min^{-1} at 0.25 mg/kg, and from 1.15 to 2.45 min^{-1} for 0.5 mg/kg (Fig. 2). Cough responses were also potentiated at the highest dose of 8-OH-DPAT (group median from 1.15 to 2.1 min^{-1}), but the increase did not achieve statistical significance. The cough frequency was not changed by exposure to 0.9% NaCl alone.

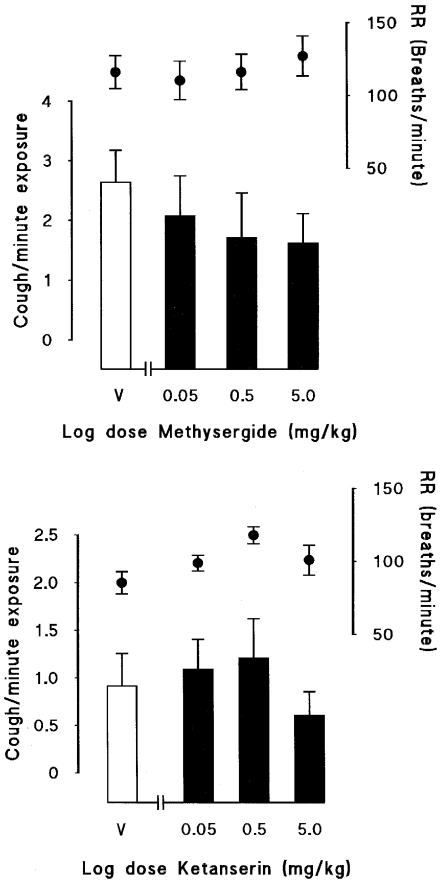

Fig. 2. Effect of increasing doses of methysergide (upper panel) and of ketanserin (lower panel) on cough response and respiratory rate induced by exposure to citric acid for 10 min in conscious guinea pigs ($n = 6$ for each group). There was no significant effect of either antagonist. Data shown as mean ± SEM.

8-OH-DPAT had a profound excitatory influence on ventilation (Fig. 2), causing a dose-dependent increase in respiratory rate which began at 0.016 mg/kg and plateaued between the 0.125 mg/kg and 0.25 mg/kg concentrations (Fig. 2), with a maximal increase in ventilation of 122%. The height of respiratory excursions in animals treated with the higher doses of 8-OH-DPAT was markedly reduced (Fig. 5). At the higher doses of 8-OH-DPAT, guinea pigs exhibited behavioural changes with increased activity and excitability, adopting a flat body posture with activities which included chewing, lip movement and forepaw treading.

Changes in respiratory rate and cough were not directly linked, because the respiratory rate had already begun to rise at a point when the sensitivity of the cough reflex was still reduced (Fig. 2). Moreover, cough responses had only begun to rise by the time that stimulation of respiratory rate was virtually maximal (Fig. 2). The respiratory rate of control-treated animals were not different between groups.

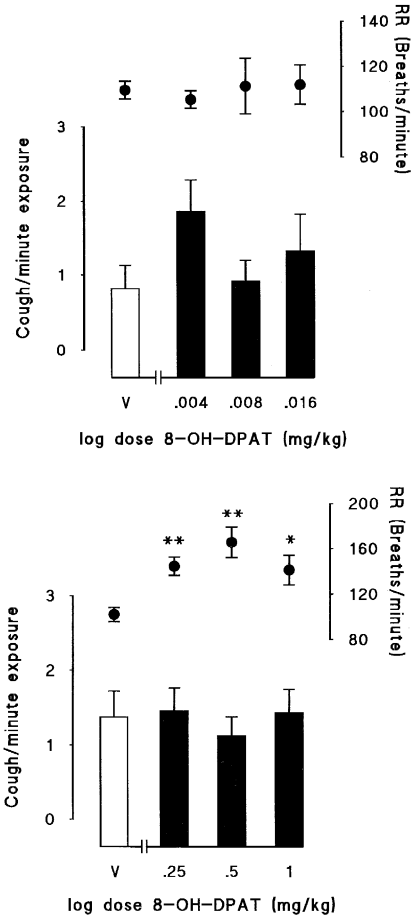

Fig. 3. Effect of methysergide (5.0 mg/kg) and 8-OH-DPAT at low doses (upper panel) and high doses (lower panel) on the cough response and respiratory rate (RR) of conscious guinea pigs exposed to citric acid for 10 min ($n = 6$ for each group). The inhibition of cough responses observed at low doses of 8-OH-DPAT and the enhancement of cough responses observed at high doses of 8-OH-DPAT in the absence of methysergide (see Fig. 2) were not observed in these experiments. At high doses of 8-OH-DPAT, the respiratory rate was still stimulated in the presence of methysergide but was attenuated when compared to responses shown in Fig. 2. Data shown as mean ± SEM. * $P < 0.05$; ** $P < 0.01$ compared to vehicle (V) for methysergide.

3.2. Effects of 5-HT antagonists on cough and respiratory rate

Neither methysergide nor ketanserin affected the sensitivity of the cough reflex or resting respiratory rate (Fig. 3). There were no behavioural effects observed following administration of methysergide or ketanserin. The batch of animals receiving methysergide and control coughed more than the animals receiving ketanserin or control (Fig. 3), demonstrating that sensitivity to citric acid may differ between batches but remains consistent within batches.

3.3. Effects of 8-OH-DPAT on cough and respiratory rate in the presence of antagonists

Because 8-OH-DPAT alone had inhibited cough responses at low doses and potentiated cough and ventilation at high doses, the influence of antagonists at either end of the 8-OH-DPAT dose spectrum was investigated. Methysergide had differential effects on both the sensitivity of the cough reflex and ventilatory responses of animals treated with low and high doses of 8-OH-DPAT (Fig. 4). No significant effect on cough responses occurred when low doses of 8-OH-DPAT were given in the presence of methysergide (Fig. 4). Group median cough frequencies with respect to control-treated animals were 0.75 to 2.15 min^{-1} (0.004 mg/kg), 0.75 to 0.78 min^{-1} (0.008 mg/kg) and 0.75 to 1.35 min^{-1} (0.016 mg/kg) but the changes did not reach statistical significance.

The effects of high dose 8-OH-DPAT on cough and ventilation were attenuated in the presence of methysergide (Fig. 4). 8-OH-DPAT did not potentiate cough responses: group median changes in cough frequency from

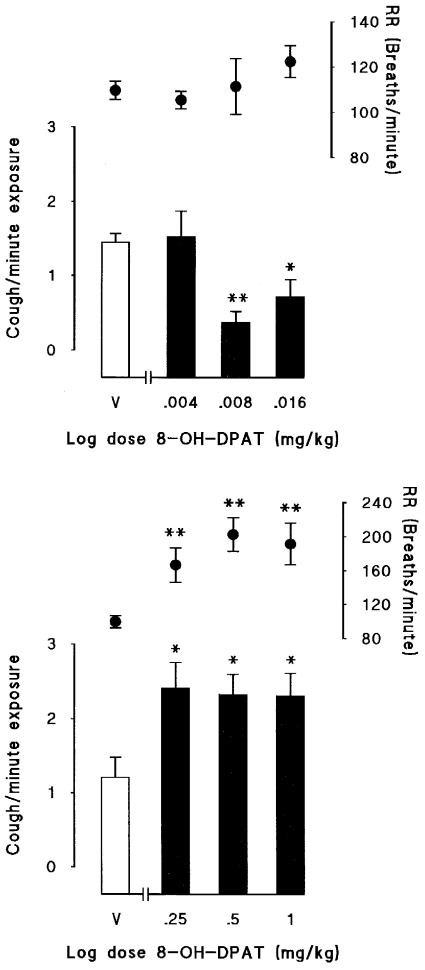

Fig. 4. Effect of ketanserin (5.0 mg/kg) and 8-OH-DPAT at low doses (upper panel) and high doses (lower panel) on the cough response and respiratory rate of conscious guinea pigs exposed to citric acid for 10 min ($n = 6$ for each group). Ketanserin had no effect on the inhibition of cough by low doses of 8-OH-DPAT or the stimulation of cough induced by high doses of 8-OH-DPAT observed in the absence of ketanserin (see Fig. 2). In addition, ketanserin had no effect on the increase in respiratory rate (RR) induced by high doses of 8-OH-DPAT. Data shown as mean ± SEM. * $P < 0.05$; ** $P < 0.01$ compared to vehicle for ketanserin.

control treated animals were 1.4 to 1.4 min^{-1} (0.25 mg/kg), 1.4 to 1.1 min^{-1} (0.5 mg/kg) and 1.4 to 1.6 min^{-1} (1 mg/kg). The respiratory rate rose significantly (Fig. 4), but the rise was less than when 8-OH-DPAT was given alone (62% vs. 122%). Behavioral effects from the higher doses of 8-OH-DPAT were blunted in the presence of methysergide. Animals receiving high doses of 8-OH-DPAT still exhibited behaviour characteristics of the serotonin syndrome, but seemed less agitated and certainly less active.

In contrast to methysergide, ketanserin had no effects on the cough reflex or ventilatory responses of animals to either low or high doses of 8-OH-DPAT (Fig. 5). The sensitivity of the cough reflex was again reduced at low doses of 8-OH-DPAT and potentiated at high doses. At the lower doses of 8-OH-DPAT, group median changes in cough frequency from control treated animals were 1.45 to 1.65 min^{-1} (0.004 mg/kg), 1.45 to 0.45 min^{-1} (0.008 mg/kg), and 1.45 to 0.95 min^{-1} (0.016 mg/kg). At the higher doses of 8-OH-DPAT the respective changes from control treated animals were 1.3 to 2.4 min^{-1} (0.25 mg/kg), 1.3 to 2.5 min^{-1} (0.5 mg/kg), and 1.3 to 2.2 min^{-1} (1 mg/kg). The ventilatory effects of 8-OH-DPAT were again marked in the presence of ketanserin. Ventilation rose at the 0.016 mg/kg dose of 8-OH-DPAT (Fig. 4a), peaking at the dose of 0.5 mg/kg (Fig. 5). The maximum rise in ventilation was 106% and was not significantly different from that observed with 8-OH-DPAT alone. Behavioural changes in animals treated with high doses of 8-OH-DPAT were obvious.

4. Discussion

Our studies show that an agonist at the 5-HT_{1A} receptor, 8-OH-DPAT, when administered subcutaneously, consistently had dual inhibitory and excitatory effects on the sensitivity of the cough reflex and an excitatory effect on ventilation in the conscious guinea pig. Changes in sensitivity of the cough reflex were not directly paralleled by changes in ventilation. The effects of 8-OH-DPAT on cough and ventilation were antagonised at each end of the dose-spectrum by methysergide, an antagonist of 5-HT_1 and 5-HT_2 receptors, but not by ketanserin, an antagonist of the 5-HT_2 receptor, thus supporting the effects of 8-OH-DPAT at the 5-HT_{1A} receptor. Higher doses of 8-OH-DPAT induced behavioural effects which were blunted by methysergide. Neither antagonist affected cough or ventilatory responses themselves. Our data extend those of others (Kamei et al., 1991) in that we now demonstrate stimulation of the serotoninergic system by a specific 5-HT_{1A} agonist modulating both cough and ventilation in the conscious guinea pig.

It is unlikely that a significant tonic serotoninergic influence over cough and ventilation is mediated via 5-HT_1 or 5-HT_2 receptors, as neither receptor antagonist attenuated these parameters when given alone. The site(s) at which 8-OH-DPAT exerts its effects cannot be determined from these studies, although the drug is likely to act via more than one mechanism. Low doses of 8-OH-DPAT could reduce the sensitivity of the cough reflex by slowing the activity of central serotoninergic neurons via presynaptic autoreceptors stimulation, with excitatory effects of high doses being mediated by a separate mechanism. Supporting this argument is evidence that other inhibitory effects of 5-HT_{1A} receptor agonists such as avoidance of conflict (Engel et al., 1984) and anxiolysis (Dickenson, 1989) are mediated by activation of presynaptic autoreceptors. However, the weight of evidence favours effects of the drug that are mediated primarily via the post-synaptic stimulation of central serotoninergic neurons situated in the rostral and caudal brainstem (Dahlström and Fuxe, 1965). Methysergide classically antagonises post-synaptic 5-HT receptors (Dickenson, 1989) and attenuated each effect of 8-OH-DPAT in these experiments. Moreover, Kamei et al. (1991) showed that 8-OH-DPAT inhibited cough-like responses in rats even at relatively high doses and argued that the effect was post-synaptic because it was not affected by pretreatment with *para*-chloro-phenylalanine which depletes 5-HT neurones.

The stimulation of ventilation and behaviour by high-dose 8-OH-DPAT also implies that its effects are mediated centrally. Although data reporting respiratory effects of 5-HT are conflicting, excitation of central respiratory drive by 8-OH-DPAT has been shown in the rabbit by Shepheard et al. (1990). Microinjection of 8-OH-DPAT into the dorsal motor nucleus and nucleus tractus solitarius complex of the rat increases phrenic nerve activity (Sporton et al., 1989), and intravenous administration of 8-OH-DPAT in the cat increases minute volume (Gillis et al., 1989). Thus, there is evidence for a centrally-mediated ventilatory influence of 8-OH-DPAT, with the antagonistic effect of methysergide in these studies supporting a mechanism that is post-synaptically mediated. The behavioural changes observed at high doses of 8-OH-DPAT were characteristic of the so-called serotonin syndrome which has been ascribed to an increase in central 5-HT function (Dickenson, 1989) and which is mediated primarily via 5-HT_{1A} receptors (Frazer et al., 1990). 8-OH-DPAT has been shown to induce the syndrome via selective activation of post-synaptic 5-HT_{1A} receptors (Tricklebank et al., 1985), occurring typically after higher doses of 8-OH-DPAT (Hjorth et al., 1987). These behavioural changes are blocked by methysergide (Dickenson, 1989). The occurrence of the syndrome in the current studies and its apparent reduction by methysergide is further evidence for the observed effects of 8-OH-DPAT being mediated centrally via post-synaptic stimulation of serotoninergic neurones.

While our studies and others suggest that 8-OH-DPAT may modulate cough and ventilatory responses via central 5-HT_{1A} receptors, other workers have also implied that peripherally sited 5-HT_{1A} receptors may modulate nocicep-

tive stimuli (Millan et al., 1991) and serotonin-associated hyperalgesia (Millan et al., 1989). If higher doses of 8-OH-DPAT induced pain, the changes in the sensitivity of the cough reflex, ventilation or behaviour could have resulted from this mechanism. Co-existent or additional peripheral influences of 8-OH-DPAT could also have contributed to the current findings as the drug was given subcutaneously. In preliminary studies, we have found that 8-OH-DPAT injected centrally via the suboccipital route in the guinea pig increased respiratory rate but had no effect on the cough reflex (Stone and Chung, unpublished observations), supporting a peripheral site of action of subcutaneously-administered 8-OH-DPAT on the cough reflex. Low-dose 8-OH-DPAT may influence airway C-fibre endings or other vagal afferents to modulate the cough reflex, but there is no information available on this.

Changes in sensitivity of the cough reflex caused by higher doses of 8-OH-DPAT were not paralleled by increased ventilation, suggesting that both parameters are subject to serotoninergic influence but their control may not be directly linked. Kamei et al. (1991) did not observe potentiation but inhibition of cough-like responses at a maximal dose of 0.3 mg/kg 8-OH-DPAT, while we observed an enhancement of the cough reflex at that dose. It is to be noted that Kamei and colleagues were recording changes in airflow in and out of a body plethysmograph as a measure of 'coughs'. These events were not confirmed to be 'coughs', as we have in the present study. In addition, the effects of 8-OH-DPAT on ventilation were not presented in the manuscript. Further studies are necessary to dissociate the changes in ventilation with those occurring with the cough reflex, such as studying the effects of centrally-injected 8-OH-DPAT.

In summary, we have demonstrated that 8-OH-DPAT had both inhibitory and excitatory influences on the cough reflex but only excitatory effects on ventilation and behaviour. Our data suggests that such effects may be initiated via the post-synaptic stimulation of central serotoninergic neurons and/or of co-existing peripheral sites of action of 8-OH-DPAT.

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