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ORIGINAL ARTICLE

Blockade of 5-HT_{2A} and/or 5-HT_{2C} receptors modulates sevoflurane-induced immobility

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Abstract
Purpose Blockade of 5-hydroxytryptamine $(5\text{-HT})_{2A}$ receptors reportedly mediates or modulates the ability of isoflurane to produce immobility during noxious stimulation and would thereby influence MAC (the minimum alveolar concentration required to suppress movement in response to noxious stimulation in 50% of subjects). However, no data are yet available regarding the role of this receptor in the immobilizing action of sevoflurane. In this study, we examined how prior intraperitoneal administration of either the 5-HT_{2A} receptor antagonist altanserin or the 5-$HT_{2C/2B}$ receptor antagonist SB 206553 might affect sevoflurane MAC in rats.
Methods Three groups of six male Wistar rats weighing 250–350 g each received one of the following drugs dissolved in dimethyl sulfoxide intraperitoneally 30 min before MAC testing: (1) altanserin 10 mg/kg; (2) SB 206553 10 mg/kg; (3) no drug (vehicle control). MAC was defined as the average of the concentrations that just prevented or just permitted movement in response to clamping the tail for 1 min.
Results The rank order of MAC values obtained after intraperitoneal drug pretreatment and sevoflurane exposure was altanserin < SB 206553 < vehicle control.
Conclusion Considering the low levels of 5-HT_{2B} receptors within the CNS, this result suggests that 5-HT_{2A} and the 5-HT_{2C} receptors are present within the neural circuitry influencing sevoflurane MAC. Blockade of 5-HT_{2A} and/or 5-HT_{2C} receptors may modulate the immobility produced by sevoflurane during noxious stimulation.

Keywords Sevoflurane · Immobility · Serotonin

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Introduction

It has previously been demonstrated in in vitro receptor expression systems that volatile anesthetics can block the effects of serotonin on serotonin receptors including G protein-coupled 5-HT_{2A} receptors [1, 2]. Moreover, when administered intrathecally and intravenously, ketanserin (given as a 5-HT_{2A} receptor antagonist) has been shown to decrease isoflurane MAC, the minimum alveolar concentration required to suppress movement in response to a noxious stimulus in 50% of human subjects [3]. However, several problems remain to be resolved concerning the possible role of the 5-HT_{2A} receptor in immobility induced by volatile anesthetics. First, no data are yet available concerning the role of this receptor in the immobilizing action of sevoflurane, a representative volatile anesthetic. Second, ketanserin, which was used as a 5-HT_{2A} receptor blocker in the previous study [3], also has affinities for other relevant receptors, including 5-HT_{2B} and 5-HT_{2C} receptors [4, 5]. Third, no information is available concerning the possible roles of the other two members of the 5-HT_2 receptor family, i.e., 5-HT_{2B} and/or 5-HT_{2C} receptors, in the immobility produced by volatile anesthetics during noxious stimulation.

An alternative candidate for a specific 5-HT_{2A} receptor antagonist would be altanserin because of its high affinity for the 5-HT_{2A} receptor (its K_i value is 0.13 nM) [6].

Further, a candidate for a selective and potent 5-$HT_{2C/2B}$ receptor antagonist is SB 206553, which exhibits high affinities for both the 5-HT_{2C} and the 5-HT_{2B} receptors and has a 100-fold or greater selectivity over all other receptors tested [7].

In the present study, to clarify the roles of 5-HT_{2A}, 5-HT_{2B}, and 5-HT_{2C} receptors in immobility produced by sevoflurane during noxious stimulation, we examined the effects of intraperitoneally injected altanserin or SB 206553 on sevoflurane MAC in rats.

Methods

With the approval of the Committee on Animal Research of Teikyo University Chiba Medical Center, we studied 18 male Wistar rats weighing 250–350 g that had been obtained from Charles River Laboratories (Yokohama City, Japan). The rats were housed 2 per cage in our animal care facility for at least 1 week before the study under a 12-h light/dark cycle and had continuous access to standard rat chow and tap water until the study.

On the day of the study, the laboratory investigation was launched at 1300. Sevoflurane MAC was determined concurrently in two rats at a time. Each rat was placed in a gas-tight clear plastic cylinder capped at each end with a rubber stopper pierced with holes to allow the passage of oxygen. The gases entered at the head end of the cylinder and exited at the tail end. To determine MAC, sevoflurane was introduced into the oxygen that was being delivered at a flow that allowed a delivery of 1 l/min to each cylinder from a conventional vaporizer, starting with a concentration of 1.5%. The sevoflurane concentration in the cylinder was monitored with the aid of an infrared analyzer (Capnomac Ultima; Datex, Finland). This instrument had been calibrated, according to the manufacturer's guidelines, using anesthetic mixtures of known concentration before the experiment.

Animals were equilibrated with the sevoflurane for 30 min, then a tail clamp was applied for 1 min or until the animal moved. In every case, the tail was stimulated proximal to any previous test site. Only the middle third of the tail was used for tail-clamping. The animal was considered to have moved if it made a gross purposeful muscular movement, usually of the hindlimb or the head, or both. To determine sevoflurane MAC, the anesthetic concentration was increased (or decreased) in steps of 0.2% until the positive motor response disappeared (or appeared), with 30 min for equilibration being allowed after each change in sevoflurane concentration. This procedure, which can achieve a steady-state F_I/F_A ratio of close to 1.0 [8], was repeated until a concentration was reached at which the animal did not move during tail-clamping.

After induction of general anesthesia, three groups of six rats each received one of the following drugs dissolved in dimethyl sulfoxide (DMSO) intraperitoneally 30 min before testing: (1) altanserin (Tocris Cookson, Ellisville, MO, USA) 10 mg/kg; (2) SB 206553 (Tocris Cookson) 10 mg/kg; or (3) no drug (as vehicle control). The dose of 10 mg/kg was selected for both altanserin and SB 206553 referred to in the previous studies using these drugs in rats [7, 9–11]. The injections were randomly distributed among the rats, and the investigators were blinded to the contents of the injection. Using the so-called up-down method [3, 12], the MAC for a given rat was taken as the average between the lowest concentration that prevented movement and the highest concentration that permitted movement in response to the stimulus. Rectal temperature was maintained between 36° and 38°C by external heating or cooling.

Data were analyzed by means of Bonferroni's multiple comparison tests after a one-way analysis of variance. In all tests, a value of $P < 0.05$ was considered statistically significant.

Results

The rank order of sevoflurane MAC values obtained after intraperitoneal drug pretreatment and sevoflurane exposure was altanserin < SB 206553 < vehicle control (Fig. 1). Compared with vehicle control, altanserin and SB 206553 reduced sevoflurane MAC by 36% and 9%, respectively.

Sevoflurane MAC was significantly lower in the altanserin group than in either the SB 206553 or vehicle control group. Moreover, sevoflurane MAC was significantly lower in the SB 206553 group than in the vehicle control group.

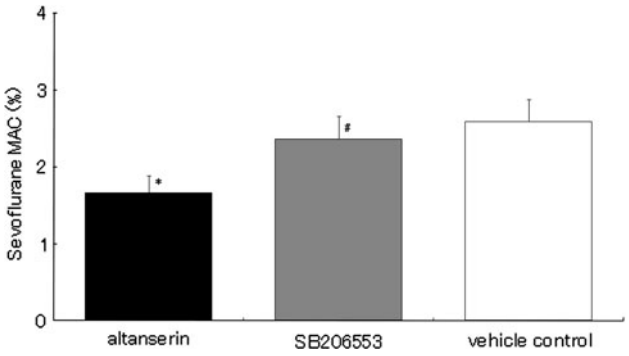

Fig. 1 Sevoflurane minimum alveolar concentration (MAC) in the three pretreatment groups. Blockade of 5-HT_{2A} receptors or of 5-HT_{2C} receptors decreased sevoflurane MAC in rats. Compared with vehicle control, altanserin and SB 206553 reduced sevoflurane MAC by 36% and 9%, respectively. $^{*}P < 0.001$ compared with vehicle control and SB 206553; $^{\#}P = 0.013$ compared with vehicle control

Discussion

The principal finding in the present study was that prior intraperitoneal injection of the 5-HT_{2A} receptor antagonist altanserin decreases sevoflurane MAC in rats. The 5-$HT_{2C/2B}$ antagonist SB 206553 also reduced sevoflurane MAC, whereas the reduction was smaller after SB 206553 than after altanserin.

In the present study, we used altanserin as a specific 5-HT_{2A} receptor antagonist to examine the effect of 5-HT_{2A} receptor blockade on sevoflurane MAC. Zhang and colleagues had previously demonstrated that ketanserin can decrease isoflurane MAC [3]. However, in terms of selectivity for the 5-HT_{2A} receptor over other relevant receptors (such as 5-HT_{1A}, α_1, D_1, and D_2), altanserin has been established as a more specific 5-HT_{2A} receptor antagonist than ketanserin [4–6]. Indeed, altanserin has a high affinity for the 5-HT_{2A} receptor ($K_i = 0.13$ nM), and its binding affinities for the aforementioned relevant receptors are more than 30 fold lower. Moreover, because altanserin has greater selectivity for 5-HT_{2A} receptors than 5-HT_{2C} receptors (300 fold) [6] than does ketanserin (20- to 30 fold) [13], altanserin can be used more reliably than ketanserin to discriminate the effects of 5-HT_{2A} and 5-HT_{2C} receptors. Thus, overall, altanserin is a better candidate as a specific antagonist of the 5-HT_{2A} receptor than ketanserin. Therefore, the reduction in sevoflurane MAC seen here after use of altanserin suggests that blockade of 5-HT_{2A} receptors may in part mediate or modulate the immobility produced by sevoflurane during noxious stimulation.

In addition to altanserin, we used the 5-$HT_{2C/2B}$ receptor antagonist SB 206553, which reportedly displays high affinity for both the 5-HT_{2C} and 5-HT_{2B} receptors, and it has a 100-fold or greater selectivity for these receptors compared to all other receptors tested [7]. For the reasons outlined next, the reduction in sevoflurane MAC observed here after SB 206553 administration may indicate that blockade of 5-HT_{2C}, rather than 5-HT_{2B}, receptors mediates or modulates part of the immobility produced by sevoflurane.

The 5-HT_2 receptors (i.e., 5-HT_{2A}, 5-HT_{2B}, and 5-HT_{2C} receptors) belong to the G protein-coupled metabotropic receptor family and facilitate the excitability of neurons. Although 5-HT_{2A} and 5-HT_{2C} receptors each have a widespread central nervous system (CNS) distribution [14], 5-HT_{2B} receptor expression is restricted to discrete brain nuclei in the rat [15]. Furthermore, the 5-HT_{2A} and 5-HT_{2C} receptors are widely distributed in the dorsal horn of the lumbar spinal cord, especially on deep dorsal horn neurons, whereas the distribution of 5-HT_{2B} receptors within the spinal cord is negligible [16]. Concerning their functions, the 5-HT_{2A} receptor system is reported to be implicated in pain processing, particularly in the processing of tonic pain [17], whereas 5-HT_{2C} receptors may be involved in the serotonergic control of catecholaminergic and cholinergic areas [14]. However, 5-HT_{2B} receptor activation is known only to cause contraction in the rat stomach fundus [7]. Therefore, our results, together with the paucity of 5-HT_{2B} receptors within the rat CNS [14, 15], may argue in favor of an involvement of the 5-HT_{2A} and/or the 5-HT_{2C} receptors, rather than the 5-HT_{2B} receptor, in the neural circuitry influencing sevoflurane MAC.

In the present study, the reduction in sevoflurane MAC was greater following altanserin than following SB 206553. Considering the actual occupancy at these receptors, it may be difficult to compare the potency of these receptor antagonists at a single dose. Therefore, we have some reservations regarding the order of potency of these two receptor antagonists.

In the present study, we chose intraperitoneal injection as the route of administration, whereas Zhang and associates administered ketanserin intrathecally and intravenously [3]. Although intraperitoneal injection may be slightly less effective because of hepatic first-pass removal [18], our intraperitoneal administration of altanserin produced a 36% reduction in sevoflurane MAC, a greater value than the 20–25% decrease in isoflurane MAC observed previously after intrathecal injection of ketanserin [3]. Although these findings may suggest that altanserin has a more powerful effect on supraspinal sites than on the spinal cord, further studies are needed to clarify this issue.

The MAC values we obtained after pretreatment with vehicle control are consistent with those previously determined in Wistar rats of a similar age [19]. Thus, intraperitoneal administration of DMSO apparently did not alter sevoflurane MAC in rats in the present study. Furthermore, any effects of changes in body temperature or circadian rhythm on the MAC can also be excluded. We believe that the present study is the first to indicate a possible mediation or modulation by blockade of 5-HT_{2A} and 5-HT_{2C} receptors in the immobility produced by sevoflurane during noxious stimulation.

In the present study, however, there are still several issues to be considered before concluding that sevoflurane-induced immobility results partially from blockade of 5-HT_{2A} and 5-HT_{2C} receptors. First, we have to refer to the possibility that systemic administration of these receptor antagonists alone affects general movement, the response to tail-clamping, and the arousal state in rats without sevoflurane. Using ketanserin as a 5-HT_{2A} receptor antagonist [9] and SB 206553 as a 5-$HT_{2C/2B}$ receptor antagonist [11], their effects on general movement and the arousal state have been excluded in awake rats. Moreover, using ketanserin as a 5-HT_{2A} receptor antagonist [20] and mesulergine as a 5-$HT_{2A/2C}$ receptor antagonist [21], their

effects on the response to tail-clamping have been excluded in awake mice. Second, the conclusion of our study may also be limited by a possible effect of sevoflurane, at its anesthetic doses, on the serotonin release, serotonin reuptake in the spinal cord, and/or activities of serotonergic neurons in the brainstem that send descending projections to the spinal cord. Such an effect of isoflurane has been suggested [22], whereas no data are available regarding the effects of sevoflurane.

In conclusion, pretreatment with altanserin or SB 206553 reduced sevoflurane MAC in rats. This finding, together with the relative paucity of 5-HT_{2B} receptors within the CNS, suggests that blockade of 5-HT_{2A} and 5-HT_{2C} receptors may modulate the ability of sevoflurane to produce immobility during noxious stimulation. Caution should be exercised in patients receiving either of these pharmacological interventions because of possible interactions of this type with volatile anesthetics.

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References

1. Franks NP, Lieb WR. Molecular and cellular mechanism of general aneaesthesia. Nature (Lond). 1994;367:607–14.
2. Minami K, Minami M, Harris RA. Inhibition of 5-hydroxytryptamine type 2A receptor-induced currents by a-alcohols and anesthetics. J Pharmacol Exp Ther. 1997;281:1136–43.
3. Zhang Y, Laster MJ, Eger EI II, Stabernack CR, Sooner JM. Blockade of 5-HT_{2A} receptors may mediate or modulate part of the immobility produced by inhaled anesthetics. Anesth Analg. 2003;97:475–9.
4. Sathi ZS, Anisuzzaman ASM, Morishima S, Suzuki F, Tanaka T, Yoshiki H, Muramatsu I. Different affinities of native α_{1B}-adrenoceptors for ketanserin between intact tissue segments and membrane preparations. Eur J Pharmacol. 2008;584:222–8.
5. Wouters W, Dun JW, Leysen JE, Laduron PM. Photoaffinity probe for serotonin and histamine receptors Synthesis and characterization of two azide analogues of ketanserin. J Biol Chem. 1985;260:8423–9.
6. Herth MH, Kramer V, Piel M, Palner M, Riss PJ, Knudsen GM, Rösch F. Synthesis and in vitro affinities of various MDL 100907 derivatives as potential ^{18}F-radioligands for 5-HT_{2A} receptor imaging with PET. Bioorg Med Chem. 2009;17:2989–3002.
7. Kennett GA, Wood MD, Bright F, Cilia J, Piper DC, Gager T, Thomas D, Baxter GS, Forbes IT, Ham P, Blackburn TP. In vitro and in vivo profile of SB 206553, a potent 5-HT_{2C}/5-HT_{2B} receptor antagonist with anxiolytic-like properties. Br J Pharmacol. 1996;117:427–34.
8. Vivien B, Langeron O, Coriat P, Riou B. Minimum alveolar anesthetic concentration of volatile anesthetics in normal and cardiomyopathic hamsters. Anesth Analg. 1999;88:489–93.
9. Dringenberg HC. Serotonergic receptor antagonists alter responses to general anaesthetics in rats. Br J Anaesth. 2000;85:904–6.
10. Kennett GA, Pittaway K, Blackburn TP. Evidence that 5-HT_{2C} receptor antagonists are anxiolytic in the rat Geller–Seifter model of anxiety. Psychopharmacology. 1994;114:90–6.
11. McCreary AC, Cunningham KA. Effects of the 5-$HT_{2C/2B}$ antagonist SB 206553 on hyperactivity induced by cocaine. Neuropsychopharmacology 1999; 20: 556-564
12. Nakata Y, Goto T, Ishiguro Y, Terui K, Niimi Y, Morita S. Anesthetic doses of sevoflurane to block cardiovascular responses to incision when administered with xenon or nitrous oxide. Anesthesiology. 1999;91:369–73.
13. Bonhaus DW, Bach C, DeSouza A, Salazar FH, Matsuoka BD, Zuppan P, Chan HW, Eglen RM. The pharmacology and distribution of human 5-hydroxytryptamine2B (5-HT_{2B}) receptor gene products: comparison with 5-HT_{2A} and 5-HT_{2C} receptors. Br J Pharmacol. 1995;115:622–8.
14. Pompeiano M, Palacios JM, Mengod G. Distribution of serotonin 5-HT_2 receptor family mRNAs: comparisons between 5-HT_{2A} and 5-HT_{2C} receptors. Mol Brain Res. 1994;23:163–78.
15. Duxon MS, Flanigan TP, Reavley AC, Baxter GS, Blackburn TP, Fone KCF. Evidence for expression of the 5-hydroxytryptamine-2B receptor protein in the rat central nervous system. Neuroscience. 1997;76:323–9.
16. Zao ZQ, Chiechio S, Sun YG, Zhang KH, Zhao CS, Scott M, Johnson RL, Deneris ES, Renner KJ, Gereau RW IV, Chen ZF. Mice lacking central serotonergic neurons show enhanced inflammatory pain and an impaired analgesic response to antidepressant drugs. J Neurosci 2007;27:6045–6053.
17. Kupers R, Frokjaer VG, Naert A, Christensen R, Budtz-Joergensen E, Kehlet H, Knudsen GM. A PET [^{18}F]altanserin study of 5-HT_{2A} receptor binding in the human brain and responses to pain heat stimulation. NeuroImage. 2009;44:1001–7.
18. Michiels M, Monbaliu J, Meudermans W, Hendriks R, Geerts R, Woestenborghs R, Heykants J. Pharmacokinetics and tissue distribution of ketanserin in rat, rabbit, and dog. Arzneimittelforschung. 1988;38:775–84.
19. Kashimoto S, Furuya A, Nonaka A, Oguchi T, Koshimizu M, Kumazawa T. The minimum alveolar concentration of sevoflurane in rats. Eur J Anaesthesiol. 1997;14:359–61.
20. Alhaider AA. Antinociceptive effect of ketanserin in mice: involvement of supraspinal 5-HT_2 receptors in nociceptive transmission. Brain Res. 1991;543:335–40.
21. Chojnacka-Wójcik E, Kłodzińska A, Dereń-Wesołek A. Involvement of 5-HT_{2C} receptors in the m-CPP-induced antinociception in mice. Pol J Pharmacol. 1994;46:423–8.
22. Mukaida K, Shichino T, Koyanagi S, Himukashi S, Fukuda K. Activity of the serotonergic system. Anesth Analg. 2007;104:836–9.