Serotonergic receptor antagonists alter responses to general anaesthetics in rats

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Serotonergic neurotransmission is involved in controlling arousal levels in humans and other animals. Here, the effects of serotonergic receptor antagonists on the induction and depth of anaesthesia produced by three different general anaesthetics were investigated. Rats were pre-treated (i.p.) with either methiothepin (1.5 mg kg\(^{-1}\)), mianserin (5 mg kg\(^{-1}\)), ketanserin (7 mg kg\(^{-1}\)) or saline. Subsequently, successive, cumulative doses (i.p.) of either ketamine (final, cumulative dose of 350 mg kg\(^{-1}\)), sodium pentobarbital (final dose 77 mg kg\(^{-1}\)), or chloral hydrate (final dose 600 mg kg\(^{-1}\)) were administered. The response to the anaesthetic was measured using a behavioural test battery assessing nociceptive reflexes and hypnotic state. Pre-treatment with methiothepin enhanced responses to all three anaesthetics; mianserin enhanced responses to chloral hydrate. These results show that some serotonergic receptor antagonists change anaesthetic requirements, resulting in enhanced anaesthesia to hypnotics with different mechanisms of action.

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The neurotransmitter serotonin (5-hydroxytryptamine, 5-HT) plays an important role in the modulation of activity and arousal levels. Further, 5-HT is implicated in a variety of neurological and psychiatric diseases, and drugs that interact with the 5-HT system are commonly used in medical practice.\(^1\) It is likely that altered serotonergic functioning, resulting from disease or serotonergic therapies, can influence responses to general anaesthetics. In rats, the anaesthetics ethyl ether, chloral hydrate, or urethane produce deeper anaesthesia, as judged by EEG responses to noxious stimuli, when they are combined with one of several 5-HT receptor antagonists.\(^2\) Also, in vitro post-synaptic responses to 5-HT are reduced by some general anaesthetics, and this effect is potentiated by the addition of a 5-HT antagonist.\(^3\) Thus, several lines of evidence suggest that, in the presence of 5-HT antagonists, the actions of anaesthetics are enhanced.

Here, a behavioural test battery was used to measure the responses to anaesthetics (ketamine, sodium pentobarbital, chloral hydrate), given to rats pre-treated with one of several serotonergic receptor antagonists. The antagonists used were methiothepin (which blocks a wide range of 5-HT receptors), mianserin (a more selective 5-HT\(_{1A}\) blocker), and ketanserin (a highly selective 5-HT\(_{2}\) antagonist), chosen based on their effectiveness to reduce EEG signs of arousal (see above). It was hypothesized that measures of behavioural arousal and responsiveness can demonstrate that rats with reduced serotonergic tone show an enhanced response to anaesthetics.

Methods and results

All experimental procedures were performed in accordance with guidelines of the Canadian Council on Animal Care and approved by the local Animal Care Committee. Male, adult (300–400 g) Long-Evans rats were brought from the animal colony (12–12 h light-dark cycle, testing done during the light phase) to a test room where they were left to habituate to the novel environment for 30 min. Subsequently, rats received injections (pre-treatment; i.p.; volume of 1 ml kg\(^{-1}\)) of one of the following compounds: saline, mianserin (5 mg kg\(^{-1}\)), methiothepin (1.5 mg kg\(^{-1}\)), or ketanserin (7 mg kg\(^{-1}\); doses based on published\(^2\) and unpublished experiments). Drugs were obtained from Research Biochemicals Inc. Canada, and dissolved in saline. Twenty-five minutes after pre-treatment, and continuing at 25 min intervals, different groups of rats received successive, cumulative doses of one of the following anaesthetics: ketamine (RBI; 7×50 mg kg\(^{-1}\), total of 350 mg kg\(^{-1}\)); sodium pentobarbital (5×11, followed by 22 mg kg\(^{-1}\), total of 77 mg kg\(^{-1}\)); and chloral hydrate (Fisher Scientific; 4×100, followed by 200 mg kg\(^{-1}\), total of 600 mg
cotton tip of a Q-tip; absence/presence of a pinna reflex in response of touching the pinna with the wooden end of a Q-tip; presence/absence of limb or body movements in response to noxious stimulation in form of a tail pinch (attaching an alligator clip, sharp metal parts covered with electrical tape to avoid skin damage, 1 cm from the tip of the tail for 10 s). The presence or absence of a behavioural response were scored as a 0 and 1, respectively. These scores were then averaged across the five measures, and the resulting score was taken as the ‘Anaesthesia Score’ with higher scores indicating a deeper level of anaesthesia. Behavioural ratings were performed by an observer who was blind to the pre-treatment drug given to individual rats. Body temperature was monitored intermittently (every 10 min) with a rectal thermometer and maintained at 36–37°C by a heat lamp. Data are presented as mean (SEM) and were analysed by analyses of variance (ANOVA). In cases where ANOVA indicated a significant main effect of pre-treatment drug (i.e. saline or 5-HT antagonist), simple effect tests were computed to directly compare the two pre-treatment groups at each cumulative concentration of the anaesthetic (simple effects tests examine the effect of one factor, pre-treatment in this case, at one level of the other factor, anaesthetic concentration; see4). All statistical analyses were performed using the software package CLR Anova (Clear Lake Research Inc.).

Successive injections of ketamine produced a dose-dependent reduction in spontaneous and evoked behavioural responses (Fig. 1A; F1,91=21.1, P<0.001). Rats pre-treated with methiothepin (1.5 mg kg⁻¹) showed a more rapid onset and deeper level of anaesthesia than rats pre-treated with saline, as indicated by an ANOVA main effect of pretreatment (Fig. 1A; F1,13=4.9, P<0.045). Pre-treatment with mianserin (5 mg kg⁻¹) or ketanserin (7 mg kg⁻¹) did not affect the rats’ subsequent response to ketamine relative to saline pre-treatment (Fig. 1A; main effects of pre-treatment F1,12=0.8 and 1.6, respectively, P>0.2).

The results obtained with pentobarbital were similar to those described above (Fig. 1B). Pre-treatment with methiothepin produced a deeper level of anaesthesia relative to saline following pentobarbital administration (F1,14=8.5, P=0.011), but neither mianserin nor ketanserin produced this effect (F1,13=0.3 and 1.2, respectively, P>0.25).

For chloral hydrate (Fig. 1C), both methiothepin (F1,13=11.3, P=0.005) and mianserin pre-treatment (F1,14=7.9, P=0.014) resulted in deeper anaesthesia relative to saline; ketanserin did not produce this effect (F1,13=0.4, P>0.5). None of the pre-treatment drugs alone produced obvious indicators of anaesthesia.

**Comment**

The data presented here show that some 5-HT receptor antagonists can interact with different types of general anaesthetics. Information on interactions among 5-HT antagonists and anaesthetics is desirable to better predict
potential adverse effects of anaesthetics in patients receiving serotonergic therapies.

In the present study, a behavioural test battery was used to assess the depth of anaesthesia. Results in agreement to those described here have been obtained using EEG methods in rats to assess anaesthetic status; EEG arousal responses to noxious stimuli (tail pinch) can be elicited under urethane or chloral hydrate anaesthesia, but they are reduced or absent if rats are given a combination of the anaesthetic and a 5-HT antagonist (e.g. ketanserin, ritanserin, methiothepin). Thus, both behavioural and EEG measures suggest that 5-HT antagonists can enhance the action of general anaesthetics.

In addition to their serotonergic effects, the antagonists used here can interact with non-serotonergic receptors in the central and peripheral nervous system. Methiothepin and ketanserin both share an affinity for adrenergic and histaminergic receptors. It is difficult to rule out contributions of non-serotonergic actions to the effects seen here; however, the fact that ketanserin was ineffective in potentiating the action of anaesthetics suggests that adrenergic and histaminergic binding did not contribute critically to this effect.

The present results are preliminary and a more thorough investigation of interactions between serotonergic antagonists and anaesthetics is required. For example, the mechanisms by which 5-HT antagonists interact with anaesthetics are not known, and it is not clear to what extent pharmacokinetic and metabolic factors contribute to the effects observed in the present study. It is of interest to note that a potentiation of anaesthetic effectiveness by a 5-HT antagonist has been observed in vitro. This suggests that metabolic or pharmacokinetic changes alone may not account for the interactions between anaesthetics and 5-HT antagonists. Also, for all three anaesthetics tested, potentiation was seen during the earlier and/or intermediate, but not late stages of cumulative dosing (see Fig. 1). This observation provides indirect support for the hypothesis that differential clearing of the anaesthetic, or differences in the half-life of the 5-HT antagonists, both of which can be expected to have more pronounced effects in the later stages of a cumulative dosing experiment, may not be the sole, critical factors underlying enhanced anaesthesia with concurrent 5-HT antagonist treatment.

In summary, the available evidence suggests that serotonergic status can alter responses to general anaesthetics. Clinically, serotonergic antagonists (e.g. mianserin) are widely used to treat disorders as diverse as migraine, nausea, and depression. An enhanced understanding of the interactions among serotonergic antagonists and anaesthetics should improve the anaesthetic management of patients receiving such drug therapies.

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References