Progesterone decreases sevoflurane requirement in male mice: a dose–response study

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Background. Progesterone has long been known to have central effects, by reduced anaesthetic requirements as measured by minimum alveolar concentration (MAC) in various settings. However, other studies have contradicted these findings. Therefore, we compared the effect of progesterone on anaesthetic requirements in a mouse model.

Methods. Male C57BL/6 mice were treated with either progesterone (37.5 or 75 mg kg\(^{-1}\)) or the olive oil vehicle, 1 h before each experiment. Animals were placed in a revolving cylinder (4 rev min\(^{-1}\)) and supplied with oxygen and stepwise increasing concentrations of sevoflurane. The number of complete rollovers during revolution of the chamber was counted as a measure of anaesthetic requirement.

Results. S.C. administration of progesterone 75 mg kg\(^{-1}\) significantly reduced sevoflurane requirement (\(P<0.0001\)). Progesterone 37.5 mg kg\(^{-1}\) did not change sevoflurane requirement.

Conclusions. We conclude that administration of exogenous progesterone injection at higher concentrations decreases anaesthetic requirement as defined by rolling response.

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Progesterone, a potent positive allosteric modulator of \(\gamma\)-aminobutyric acid (GABA) type A receptors,\(^1,2\) has long been known to have activity against the function of the central nervous system, including anxiolysis,\(^3\) sedation,\(^4\) and analgesia.\(^5\) So far, several groups have studied the anaesthetic requirements in terms of minimum alveolar concentration (MAC) in both sexes, including humans and other animals,\(^6–9\) and in terms of bispectral index (BIS).\(^10\) Some studies showed that higher progesterone level had decreased MAC,\(^6\) but some studies did not support that phenomenon.\(^7–9\) Therefore, the action of progesterone on inhaled anaesthetic requirements has not been established and the action of exogenous progesterone on sevoflurane requirement in male mice in terms of immobilization has not been studied.

Methods

Animals

Subjects were male C57BL/6 mice (\(n=10\) in each trial, 6–10 weeks old weighing 25–32 g). Animals were housed no more than five in a cage and were maintained in a light- and temperature-controlled environment with free access to food and water. All procedures were carried out between 09:00 and 17:00 h and were performed by researchers blinded to the animal treatment. Two independent experiments were done. All animal procedures were approved by the University of Tsukuba Animal Care and Use Committee.

Drugs

Mice were treated with either progesterone (Sigma, St Louis, MO, USA; 37.5 or 75 mg kg\(^{-1}\) suspended in 0.1 ml olive oil) or the olive oil vehicle only (0.1 ml) by s.c. injection into the scruff of the neck. Injection took place 1 h before each experiment.

Apparatus

The rolling response described by Robbins\(^11\) was used to assess anaesthetic endpoint. A rotational cylinder (internal diameter of 14 cm) made of steel mesh was built in a 1.8 litre small chamber. Animals were placed in the
cylinder, and the cylinder was set in the chamber, so that it could be rotated. The closed chamber was then supplied with oxygen (0.5 litre min\(^{-1}\)) and sevoflurane of a designated concentration. The concentration of sevoflurane was controlled stepwise at 0.05% intervals below 1.0% and intervals of 0.1% above 1.0% by using a vaporizer (Penlon PPV\(_2\), Penlon, UK), and was measured using a multi-gas analyzer (Capnomac Ultima, Datex, Finland) pre-calibrated automatically. Each designated concentration was maintained for at least 10 min before the trial. The cylinder was being rotated at 4 rev min\(^{-1}\). Animals that were unable to maintain an upright position and rolled over completely and continuously for at least 15 s during any revolution of the chamber were considered to have a zero response. Others were scored 1. Three sets of five revolutions were carried out at intervals of \(~5\) min.

**Data analysis**

We analysed the reduction of sevoflurane concentration by using a multiple independent variable logistic regression model (SAS System for Windows, version 6.12; SAS Institute Inc., Cary, NC, USA) that included an interaction term for sevoflurane concentration and progesterone dose. The components of the main effects determined whether sevoflurane and progesterone independently affected the falling down. The interaction coefficient determined whether sevoflurane and progesterone interacted to affect the immobility. We used the maximum likelihood ratio test to determine which of the independent variables significantly affected the model. To determine the concentration of sevoflurane required to make 50% of the mice rollover, the probability to rollover was assigned the value 0.5, and the equation was solved for the sevoflurane concentration.

Likewise, to determine the concentration of sevoflurane required to rollover in 5% or 95% of mice (95% confidence interval), the probability to rollover was set at 0.05 or 0.95, and the equation was solved for the sevoflurane concentration.

Statistical comparisons were performed with \(\chi^2\) testing between the two groups (StatView Software, SAS Institute Inc.). \(P<0.05\) was considered the minimum level of statistical significance.

**Results**

The percentages of mice that had rolled over at designated sevoflurane concentrations are shown in Table 1. Sevoflurane concentrations determined by logistic regression are shown in Figure 1. Concentrations for complete rollover for control, progesterone 37.5 mg kg\(^{-1}\), and progesterone 75 mg kg\(^{-1}\) were 0.86%, 0.83%, and 0.76%, respectively. Administration of progesterone at 75 mg kg\(^{-1}\) significantly reduced sevoflurane requirement (\(P<0.0001\)), but progesterone 37.5 mg kg\(^{-1}\) had no effect on sevoflurane requirement.

**Discussion**

We showed that s.c. administration of progesterone 75 mg kg\(^{-1}\), but not 37.5 mg kg\(^{-1}\), significantly reduced sevoflurane requirement in male mice in terms of rolling response. This finding is in accordance with previous studies that suggested that progesterone decreases MAC. It has been known in animal studies that the requirement for inhaled anaesthetics is decreased by up to 40% during pregnancy.\(^{12}\) Chronically administered progesterone also decreased halothane requirement in ovariectomized rabbits.\(^6\) Decreased MAC has been also observed during early pregnancy\(^{12}\) and in the immediate postpartum period.\(^{14}\) Likewise, the sedative effects of progesterone and its metabolite allopregnanolone have been proposed.\(^{15–17}\) Erden and colleagues\(^{10}\) also reported that increased progesterone decreased anaesthetic requirement in terms of BIS in women.
Reddy and colleagues\textsuperscript{18} reported that progesterone treatment (50 and 75 mg kg\textsuperscript{-1}) had shown dose-dependent efficacy (anxiolytic action), but a lower dose (10 mg kg\textsuperscript{-1}) had no effect. Therefore, we considered that 75 mg kg\textsuperscript{-1} should have been enough to affect anesthetic requirement, and so we chose 75 mg kg\textsuperscript{-1} and its half dose, 37.5 mg kg\textsuperscript{-1}. Progesterone can influence behaviour through its metabolite allopregnanolone, and allopregnanolone is believed to exert its various actions through positive modulation of GABA\textsubscript{A} receptors.\textsuperscript{19, 20} Our experiments were started 1 h after injection because it had been assumed that the concentrations of both progesterone and allopregnanolone achieved the effective level by then.\textsuperscript{18}

Our study design focused on the rolling response, in which impaired balance control is observed. We showed that progesterone influenced balance control at relatively low sevoflurane concentrations. Balance disturbances are associated with pontine reticular formation,\textsuperscript{21} which is mediated by GABA and vestibular disturbances.\textsuperscript{22} Thus, it can be presumed that female patients with high plasma progesterone levels may be at high risk of balance disturbances, and that the impaired function increases the risk of falls. Therefore, special attention should be paid to early rising from a recumbent position and commencement of walking after recovery from anaesthesia, especially in cases of ambulatory surgery under general anaesthesia. A difference in immobility during anaesthesia could be observed. If plasma progesterone level is high enough, a female patient may be immobilized at considerably lower concentrations of volatile anaesthetics,\textsuperscript{21} which may cause adverse events such as anaesthesia awareness or paralysis with consciousness.

The limitations of our study are as follows. First, we did not measure the blood levels of either progesterone or allopregnanolone. Therefore, the argument whether the plasma levels of progesterone or allopregnanolone were high enough to activate the involved receptors is merely a speculation. Secondly, our experimental system does not determine MAC, a spinoally determined anaesthetic phenotype, or degree of sedation described by BIS, which reflects actions on higher centres. The effects of progesterone may have differing effects on such measures. Thirdly, our sample size may have been too small to see an effect at 37.5 mg kg\textsuperscript{-1}, that is, a type II error.

We conclude that progesterone injection at 75 mg kg\textsuperscript{-1} decreases sevoflurane requirement as defined by rolling response in male mice.

**Conflict of interest**

None declared.

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