Minimal alveolar concentration of sevoflurane inhibiting the reflex pupillary dilatation after noxious stimulation in children and young adults

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Editor's key points

- Reflex pupillary dilatation (RPD) may be a useful measure of nociception in anaesthetized children.
- The effect of sevoflurane on inhibiting RPD was assessed in pre- and post-pubertal children.
- The minimal alveolar concentration for sevoflurane to inhibit RPD was higher and less consistent in pre-pubertal children.
- Neither heart rate nor bispectral index was reliably related to changes in RPD.

Background. In children, sevoflurane is the most commonly used anaesthetic. Its excellent haemodynamic tolerance gives it a wide therapeutic index. This halogenated agent can abolish movement [minimal alveolar concentration (MAC)] or haemodynamic responses (MACBAR) to noxious stimulus in children as in adults. Reflex pupillary dilatation (RPD) has been demonstrated as a very sensitive measure of noxious stimulation. In order to investigate the effect of sevoflurane on the RPD, a subcortical reflex, we determined the MAC of sevoflurane inhibiting the RPD in 50% of the subjects in response to skin incision (MACpup) in pre- and post-pubertal subjects.

Methods. We included 30 pre-pubertal children and 19 post-pubertal subjects. Patients received sevoflurane at preselected concentrations according to an ‘up and down’ design, and after a steady-state period, skin incision was performed. The RPD was considered as significant when the pupillary diameter increased by more than 100%. Heart rate (HR) and bispectral index (BIS) changes were analysed according to the pupillary response.

Results. The MACpup of sevoflurane was 4.8% (95% confidence interval, 4.6–5.1%) in pre-pubertal children vs 3.4% (3.5–3.3%) in post-pubertal subjects (P < 0.001). Inhibition of RPD was always associated with lack of significant HR response. In pre-pubertal children receiving high concentrations of sevoflurane, RPD in response to noxious stimulation was frequently associated with lack of HR response and low BIS values.

Conclusions. MACpup was higher than MAC and close to the MACBAR. Inhibition of RPD in pre-pubertal children required higher sevoflurane concentrations compared with post-pubertal subjects, suggesting that the relationship between the brain structure sensitivities may differ with brain maturation.

Keywords: anaesthesia; pain measurement; paediatrics; pupil; sevoflurane

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The concept of minimal alveolar concentration (MAC) was first described at the beginning of the 1960s, in order to be able to compare inhalation anaesthetic agents in terms of anaesthetic power. The MAC is a complex index based on the assessment of a motor response to a noxious stimulus such as skin incision, integrating the concepts of hypnosis, immobility, and analgesia. The amount of anaesthesia required to block autonomic responses [such as increased heart rate (HR) or arterial pressure] to noxious stimuli in 50% of the subjects is called ‘MACBAR’ (blocking autonomic responses).

Prediction of pain response is an important goal during anaesthesia. Unfortunately, methods based on the electroencephalogram, such as spectral edge frequency (SEF90), median power frequency, or the bispectral index (BIS), have failed to predict movement during surgery. Whether this is due to improper electroencephalographic processing or to other methodological issues related to pain processing remains debated. Thus, analgesia in anaesthetized patients is still generally evaluated by autonomic nervous system (ANS) responses through haemodynamic changes after surgical stimulation.

In the awake subject, the reflex pupillary dilatation (RPD) to painful stimulus is correlated with subjective pain perception. This reflex is regulated by the ANS and persists under anaesthesia. In adults, RPD has been demonstrated to be a very sensitive measure of noxious stimulation, correlated with opioid concentrations. This reflex is now well measured by specific devices including infrared cameras and automatic pupil size recordings. In children anaesthetized with sevoflurane, RPD is an age-independent and more sensitive measure of noxious stimulation than the commonly used variables such as HR, arterial pressure, and BIS.
In children, sevoflurane is the most commonly used agent today for anaesthesia by inhalation. Its inhibitory effects on haemodynamic (MACBAR) and motor (surgical MAC) response to nociceptive stimulation have been widely investigated. However, its inhibitory effects on RPD, a diencephalic reflex, remain unknown.

Therefore, the aim of our study was to determine the MAC of sevoflurane inhibiting RPD in 50% of the subjects after a standardized surgical skin incision (MACpup) in pre-pubertal children. As puberty acquisition may influence cerebral maturation, MACpup was also determined in post-pubertal subjects which were considered as young adults and served as a control group. In addition, HR and BIS changes induced by noxious stimulus were analysed according to the pupillary response.

**Methods**

After approval from our local ethics committee (Committee for the Protection of Persons in medical research of Saint-Antoine Hospital), and informed written consent of parents and children, we included prospectively 49 subjects, ASA I, aged 1–17 yr. They were undergoing elective limb surgery under general anaesthesia, requiring a 5 cm skin incision.

Patients were excluded if they showed one of the following criteria: neurological disorder such as cerebral palsy, peripheral neuromuscular disorders, eye disease, treatment with a drug interacting with the autonomic or central nervous system, obesity, or cardiac, renal, or hepatic disorder.

The subjects were allocated either to the pre-pubertal group (n=30) or to the post-pubertal group (n=19) according to the clinical observation of external secondary sexual characteristics (Tanner Stage 4) (Supplementary Fig. S1). Patients were fasted for 8 h before surgery and received no premedication. Standard monitoring was used throughout the study, including HR, pulse oximetry, non-invasive arterial pressure, BIS, and gas analysis (sevoflurane, N₂O, CO₂, and O₂). An i.v. cannula was placed during induction by inhalation with 6% inspired sevoflurane in 100% oxygen. After visualization of central pupils, tracheal intubation was performed and mechanical ventilation was started and adapted to maintain $eCO₂$ between 35 and 40 mm Hg. Before surgery, a steady-state period of 10 min was performed with a stable preselected value of expired concentration of sevoflurane ($F_{sevo}$); this value depended on the response of the previous included subject according to the Dixon method. Just after tracheal intubation, the pupillometer (VideoalgesiGraph, VAG, Synapsys, Marseille, France) was installed according to the recommendation of the manufacturer. At the end of the steady-state period, a standardized skin incision (5 cm) was performed on a limb.

From tracheal intubation up to the fourth minute after skin incision, HR, $F_{sevo}$ (AS5 collect, GE Health Care, UK), BIS value (BIS XP; Aspect Medical Systems, Natick, MA, USA; Winlog® software), and pupillary diameter were recorded every minute.

Data analysis including HR, BIS, and pupillary diameter was performed at baseline (just before skin incision) and every minute after skin incision up to the fourth minute. We used the Dixon ‘up and down method’ to determine the MACpup in our two populations. The response of the preceding patient determined the end-tidal concentration of sevoflurane given to the next patient in each group. We chose 2.5% and 2.3%, respectively, in pre- and post-pubertal subjects as the initial end-tidal concentration of sevoflurane, according to previous determinations of the MAC of sevoflurane. End-tidal concentration of sevoflurane was decreased by 0.2% in the case of RPD inhibition (RPD−) or increased by 0.2% in case of significant pupillary dilatation (RPD+). Based on our previous study, the pupillary dilatation was considered as significant when the pupillary diameter increased by more than 100%. Changes in pupillary diameter, HR, and BIS were assessed by comparing with baseline values taken just before skin incision.

**Statistical analysis**

MACpup values were calculated as the mean of six independent cross-overs of responses in which RPD− and RPD+ responses were paired up for each group (Fig. 1). Data were also analyzed using a logistic model to calculate the effective sevoflurane concentration required to block the pupillary response to skin incision in 50% and 95% (ED50 and ED95, respectively) of the patients (XLSTAT 2010).

Group data were analysed by Student’s t-test (StatView, 4.02; Abacus Concepts, Berkeley, CA, USA). In each group, HR and BIS changes induced by skin incision were compared between RPD− subjects (persistence of RPD) and RPD− subjects (inhibition of the RPD) using an ANOVA for repeated measures (StatView, 4.02; Abacus Concepts). Probability values <0.05 were considered statistically significant.

**Results**

Forty-nine patients completed the investigation. Two pre-pubertal children were excluded because of technical inability to collect data.

Data analysis was performed on recordings from 47 subjects: 28 pre-pubertal children [5.7 (2–12) yr, mean (range), 21.6 (8.3) kg, mean (sd)] and 19 post-pubertal subjects [13.7 (11–17) yr, 60.0 (9.4) kg].

**MAC calculation**

The up–down progression is shown in Figure 1. In the post-pubertal group, the stabilization of the cross-over responses was rapidly observed, allowing MACpup calculation. In contrast, inhibition of the pupillary dilatation response in the pre-pubertal group required higher concentrations of sevoflurane, exposing these subjects to the risk of EEG and clinical seizures. Indeed, two children demonstrated a brief episode of tonico-clonic movements (subject#26, $F_{sevo}$= 5.3%; subject#28, $F_{sevo}$= 5.7%). Given the risk of occurrence of clinical seizures at high concentrations of sevoflurane, we gave up the more precise determination of the MACpup in
pre-pubertal subjects and the study was stopped after obtaining six pairs of opposite responses, despite the absence of stabilization around an average value.

The calculated MACpup was clearly higher in pre-pubertal subjects compared with post-pubertal: 4.8% [95% confidence interval (CI) 4.6–5.1%] vs 3.4% (3.5–3.3%; \( P \), 0.001).

Because of the lack of the stabilized cross-over in the pre-pubertal group, the reliable calculation of \( E_D_{50} \) and \( E_D_{95} \) obtained from logistic regression analysis was possible only in the post-pubertal group: respectively, 3.43% (95% CI 3.18–3.73%) and 3.66% (3.52–6.29%).

Regarding the pupillary dilatation, the response was typically on/off (Fig. 2 and Table 1). The maximal response was observed in the first minute after skin incision in most of the subjects: 11/12 in post-pubertal and 17/24 in the pre-pubertal subjects, the others demonstrated a maximal response in the second minute.

**HR response**

HR responses in RPD\( + \) and RPD\( − \) are illustrated in Figure 3. In the post-pubertal group, 12 subjects showed pupillary
dilatation [increase of 196 (62)%]; among them, nine showed an increase in HR of over 15% [increase of 41 (24)%].

In the pre-pubertal group, 23 subjects showed pupillary dilatation [increase of 150 (31)%]; among them, eight showed an increase in HR of over 15% [increase of 24 (10)%].

On the other hand, inhibition of the pupillary response was always associated with a lack of significant HR response.

**BIS response**

The BIS values taken just before skin incision, in RPD+ and RPD–, are illustrated in Figure 4.

whatever the group, the BIS at T0 did not predict the pupillary response. Moreover, skin incision did not influence the BIS value, whatever the pupillary response and the group. In the pre-pubertal group, eight subjects showed pupillary dilatation, despite BIS values below 20.

**Discussion**

In this study, we have demonstrated that the MACpup of sevoflurane was markedly higher than the surgical MAC and close to the MACBAR. In addition, our results demonstrated that inhibition of the pupillary reflex to noxious stimulation required higher doses of sevoflurane in pre-pubertal subjects (children) compared with post-pubertal subjects (young adults).

The choice of the relevant threshold regarding significance of the RPD was based on our previous study assessing...
the pupillary dilatation in response to skin incision in subjects aged 2–16 yr, anaesthetized with 1 MAC of sevoflurane. We found that RPD reached more than 160% at 30 s and about 200% at the end of the first minute. In all cases, the dilation was >100%. Given the similar conditions of investigation in the current study, we chose to keep this criterion of 100% to consider significant pupillary dilatation. In accordance with this hypothesis, the average response in non-responders was around 5%, with a maximum around 13%. The lack of intermediate response between 10% and 100% suggested that the threshold of 100% was quite relevant in these standardized conditions.

The Dixon method is a useful statistical approach of MAC calculation, requiring moderate sample size of subjects. Each of them has the same weight in MAC calculation, and the finality of this method is to determine the value predicting a 50% probability of response. This is provided by the average of successive opposite responses (pairs). The reliability of the Dixon method increases with an increasing number of pairs; indeed, six pairs are considered as optimal for a clinical study. Logistical regression may be derived from the Dixon data, allowing wider calculation of probability of occurrence of positive response such as ED95.

In our study, at least six pairs of opposite responses were found in the two groups. Despite the noticeable variability in the pre-pubertal group, the substantial difference in terms of sensitivity of pupillary reflex observed between the two populations leads to significantly different MACpup calculated with the Dixon method. Indeed, in the younger population, higher and more variable concentrations were required, reflecting lower sensitivity and wider interindividual variability compared with the post-pubertal subjects. This large interindividual variability is frequently observed in young children and classically related to different rates of physiological maturation.

The surgical MAC of sevoflurane, resulting from inhibition of motor response to skin incision, may be estimated around 2.5% in children and 2.2% in young adults.
while the MACBAR resulting from inhibition of autonomic cardiovascular response to skin incision may be estimated around 1.9 MAC. These data demonstrate that inhibition of autonomic cardiovascular centres located in the medulla requires higher concentrations of sevoflurane than inhibition of the spinal cord. In our study, the values of MACpup corresponded to 1.9 and 1.5 MAC in, respectively, pre- and post-pubertal subjects, suggesting that the concentrations of sevoflurane required to block the pupillary reflex are markedly higher than those required to inhibit the spinal cord. The close values for the MACBAR and MACpup can be explained by the involvement of the ANS in both pupillary and cardiovascular responses to nociceptive stimulation.

Comparison between cardiac autonomic response and pupillary response may be investigated more precisely when looking at our HR data. Indeed, in pre-pubertal as in post-pubertal subjects, inhibition of pupillary reflex was always associated with lack of HR response to noxious stimulus. However, in some cases, pupillary dilatation was observed despite the lack of HR response, especially in pre-pubertal subjects in whom significant HR response disappeared from 4.1% of sevoflurane, whereas most of them showed RPD still. This dissociated response suggested that HR responses disappear before pupillary responses. These findings are in agreement with a previous study showing that RPD was more sensitive than haemodynamic parameters in response to skin incision in children anaesthetised with sevoflurane. Both reflexes result from subcortical structures located at two close but different levels: the midbrain for pupillary reflex and the lower half of the brainstem (medulla oblongata) for cardiovascular autonomic control. Thus, the dissociation between HR and pupillary responses observed under sevoflurane may be explained by different sensitivities of the brain structures involved in these processes.

Regarding BIS data, our results confirmed in the two groups the lack of ability for the BIS to predict response to noxious stimulation assessed by RPD as demonstrated previously. Moreover, the absence of significant changes in BIS during stimulation confirmed the weak influence of noxious stimulus on the EEG in subjects anaesthetized with sevoflurane in the studied concentration ranges. The poor performance of BIS to predict the response to pain has been known for many years. Katoh and colleagues in 1998 have shown that BIS predicted loss of consciousness but not the motor response to surgical incision in adult patients anaesthetized with sevoflurane.

Velly and colleagues’ study showed that in subjects anaesthetized with propofol or sevoflurane, the parameters collected by standard EEG (cortical) predicted loss of consciousness but not the motor response to laryngoscopy, whereas the parameters from the EEG recorded in the deep areas of the brain (subcortical) predicted the motor response to laryngoscopy but not unconsciousness. Thus, it appears that anaesthetics have different brain targets, cortical and subcortical, where different processes are integrated: loss of consciousness in the cortex and pain response in subcortical areas. Anaesthetics have dissociated effects on these two targets in accordance with the agents and doses. Finally, these two processes require different monitoring adapted to the targets (cortex and subcortex).

As a corollary of the different MACpup values highlighted in our two populations, we also observed significant differences in BIS values associated with the inhibition of reflex pupillary dilation. In post-pubertal subjects, this inhibition appeared from 3.3% sevoflurane, and the BIS values of subjects RPD—were around 30, except for one patient who demonstrated an isoelectric EEG trace at 3.5% of sevoflurane. All post-pubertal subjects showing significant RPD had a BIS value above 20. On the other hand, as the inhibition of RPD in pre-pubertal subjects required higher sevoflurane concentrations, lower values of BIS were expected. Interestingly, our BIS data recorded at steady state showed that from 4% of sevoflurane, two profiles of BIS values were observed: the first one occurring between 4% and 5% of sevoflurane, characterized by paradoxically high values over 40 that might reflect the influence of epileptoid signs on BIS calculation; and the second one occurring between 4.5% and 5.7% of sevoflurane characterized by very low BIS values and reflecting total or subtotal EEG cortical inhibition. The RPD+ and RPD—subjects were equally distributed in both types of BIS profiles. Interestingly, we can emphasize that seven children showed a BIS value below 10, while they still had a significant pupillary response to noxious stimulation. These findings suggested that pre-pubertal subjects anaesthetised with sevoflurane may present subcortical reactivity to noxious stimulus, despite major cortical inhibition attested by EEG. This dissociated response between cortical and subcortical area has already been demonstrated.

Mourisse and colleagues have demonstrated in adults that the blink reflex (brainstem function) was more sensitive to sevoflurane than BIS (forebrain function), while tetanic stimulus-induced withdrawal reflex (spinal cord) was less sensitive than BIS. These authors concluded that sevoflurane may act differently according to the effect site. To date, there are few paediatric data investigating the relative effect of anaesthetic on cortical and subcortical sites. A simple way to approach this question could be to compare the ratio between surgical MAC and a given target of cortical EEG, that is, a BIS value of 50; this was done by Davidson and colleagues, who have demonstrated an age-related discrepancy between MAC and EEG suppression with a less active EEG relative to MAC gradually as the child grows from 1 to 12 yr; these authors concluded that the relationship between the two differs with brain maturation. Our study goes a step further, using non-invasive devices, we assessed cortical (BIS derived from EEG) and subcortical (pupillary reflex) response to noxious stimulus in pre-pubertal children vs post-pubertal subjects. Our data revealed that sensitivity to sevoflurane of some subcortical structures (i.e. mesencephalic control of RPD) is lower in pre-pubertal children than in older subjects. These findings suggest that physiological changes associated with puberty acquisition may influence brain sensitivity to anaesthetic agents. In agreement with this hypothesis, hormonal changes observed
at puberty have been demonstrated to strongly influence cerebral maturation and neuronal plasticity especially on cortical and subcortical structures involved for instance in emotional control.27

In summary, we have shown that inhibition of the RPD (subcortical reflex) in response to noxious stimulus required higher concentrations of sevoflurane than inhibition of the motor response. Moreover, compared with post-pubertal subjects, pre-pubertal children demonstrated a higher MACpup. Our results suggest that the relationship between cortical and subcortical sensitivities to sevoflurane is modified by puberty. In addition, we demonstrated that the persistence of a pupillary response to noxious stimulation may be the only visible clinical sign of nociception in pre-pubertal children. This nociceptive response may not be abolished in the usual clinical range of sevoflurane concentrations. Our findings give a new approach to nociception assessment in anaesthetised children which might be more relevant than the classical haemodynamic parameters. Further studies are required to evaluate the clinical interest of pupillary diameter monitoring to guide analgesic administration during general anaesthesia.

Supplementary material
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Declaration of interest
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