Neuropeptide Y response to tracheal intubation in anaesthetized children: effects of clonidine vs midazolam as premedication

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We have determined if tracheal intubation causes an increase in neuropeptide Y (NPY), a marker of major adrenergic activation, and investigated if rectal premedication with clonidine 2.5 µg kg−1 might be capable of attenuating the stress response to tracheal intubation compared with midazolam 300 µg kg−1, in 20 paediatric patients (1–9 yr). Prospective randomization was performed in a double-blind manner. After induction of anaesthesia with 1% isoflurane, tracheal intubation was performed, and norepinephrine, NPY concentrations and haemodynamic variables were recorded. Tracheal intubation did not increase NPY plasma concentrations, despite transient increases in norepinephrine concentrations, heart rate and arterial pressure. There was no significant difference between the two groups. We conclude that the adrenergic stress reaction in response to tracheal intubation in children was short-lived and of limited magnitude, as indicated by the lack of NPY release.

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Tracheal intubation in anaesthetized children has been shown to cause sympathetic nervous system activation with a similar haemodynamic and norepinephrine response to that observed in adults.1 In adults, sympathetic activation has been found to result in adverse organ effects, such as myocardial ischaemia,2 3 in predisposed individuals. In children, the sympathetic response caused by tracheal intubation is of short duration4 and adverse organ effects are rarely, if ever, seen, as children usually do not have limited organ function reserve as a result of factors such as clinical or subclinical arteriosclerosis. In order to determine the degree of sympathetic activation caused by tracheal intubation during general anaesthesia in children, we have studied neuropeptide Y (NPY) release.

NPY is co-localized with norepinephrine at the nerve endings of the sympathetic nervous system and has been found to be co-released together with norepinephrine in response to major or prolonged sympathetic activation (i.e. intense physical exercise and the cold pressure test).5 6 Contrary to major activation of the sympathetic system, minor activation causes only transient release of norepinephrine. Thus NPY can be considered a marker of more profound activation of the sympathetic nervous system.7

A secondary aim of our study was to investigate, in a double-blind, randomized manner, if clonidine, an α2 agonist reported to be capable of substantial attenuation of NPY release after major sympathetic activation,8 could modify potential NPY release associated with tracheal intubation in anaesthetized children compared with the widely used premedicant midazolam.

Patients and methods

The study was approved by the Local Ethics Committee and the Swedish Medical Products Agency. Written and oral informed consent were obtained from all parents.

We studied 20 ASA I patients, aged 16–98 months, weighing 10–20 kg, undergoing minor surgery (inguinal hernia repair, undescended testis or adenoidectomy). Patients were allocated randomly to one of two treatment groups using a prospective randomized design. Both patients and investigators were blinded to the premedicant and the code was broken after completion of the study. Patients in the first group received our standard premedication with rectal midazolam 300 µg kg−1 and atropine 40 µg kg−1. Patients in the second group received premedication with
rectal clonidine 2.5 µg kg⁻¹ and atropine 40 µg kg⁻¹. Based on previous experience, rectal premedication with clonidine 2.5 µg kg⁻¹ produces peak plasma concentrations after 40–50 min. Premedication was administrated approximately 45 min before induction of anaesthesia. EMLA cream (Astra, Södertälje, Sweden) was applied for 60 min to allow painless venous cannulation.

After insertion of an i.v. cannula, anaesthesia was induced with thiopental 5 mg kg⁻¹ i.v. and subsequently maintained with 1% isoflurane and 99% oxygen. The patient breathed spontaneously via a face mask; a modified Jackson–Rees anaesthesia system was used. After induction, a second i.v. cannula was inserted in the opposite arm for blood sampling. Tracheal intubation was performed by one of two paediatric anaesthetists after neuromuscular block with atracurium 0.5 mg kg⁻¹ and manual ventilation for 5 min with 1% isoflurane in oxygen aiming towards end-tidal normocapnia. In order to standardize intubation trauma, viewing of the vocal cords was sustained for 15 s, with the blade of the laryngoscope (MacIntoch No. 2) placed in the vallecula, before intubating the trachea.

After tracheal intubation, anaesthesia was maintained with 1% isoflurane and 66% nitrous oxide in oxygen at normocapnia.

**Sample handling and analysis of plasma concentrations of norepinephrine and NPY**

Venous blood was obtained from an indwelling catheter into prechilled tubes containing heparin immediately before, and at 3, 6, 9 and 30 min after the start of the intubation procedure. Blood samples were transported in ice water and centrifuged at +4°C within 20 min. Plasma was frozen and kept at −70°C until analysis. For analysing norepinephrine, heparinized plasma was extracted with a freeze–thaw procedure. Blood samples were transported in ice water and at 3, 6, 9 and 30 min after the start of the intubation procedure. Blood samples were transported in ice water and centrifuged at +4°C within 20 min. Plasma was frozen and kept at −70°C until analysis. For analysing norepinephrine, heparinized plasma was extracted with aluminium oxide. Then dihydroxybensylamine (DHBA) was added as an internal standard and norepinephrine was analysed using high-pressure liquid chromatography, as described previously. NPY was analysed in plasma after extraction using Sep-Pak cartridges (Waters), with a specific competitive radioimmunoassay using the antiserum N1, as described previously. NPY was analysed in plasma after extraction using Sep-Pak cartridges (Waters), with a specific competitive radioimmunoassay using the antiserum N1, as described previously.

**Haemodynamic measurements**

Heart rate and non-invasive arterial pressure were recorded using a Hewlett-Packard Monitoring System (M 1046–9001 B, Böblingen, Germany) immediately before intubation and at 3-min intervals for 30 min after intubation. After 30 min, the study was concluded and surgery was allowed to commence.

**Statistical analysis**

The size of the sample was based on previous investigations showing a statistically significant difference (P<0.05) in attenuation of the stress response to tracheal intubation between clonidine and diazepam. A reduction in the stress response to 50% or more was regarded as clinically important. Data are expressed as median (95% confidence intervals) or mean (SEM), as appropriate. Comparison of independent sample data was performed using the two-tailed Pitman randomization test which is based on the Mann–Whitney U test. Correlations were assessed using the Kendall rank correlation test. P<0.05 was considered statistically significant.

**Results**

There were no significant differences between the two groups in patient characteristics, arterial pressure, or norepinephrine or NPY concentrations at baseline (immediately before induction of anaesthesia). Patients in the clonidine group were found to have a lower heart rate before intubation compared with patients receiving midazolam (P<0.05) (Table 1). Haemodynamic state, norepinephrine and NPY responses after tracheal intubation are shown in Table 2. The NPY response after intubation did not change compared with baseline when the entire population was analysed together (Fig. 1). The NPY response did not differ between the two subgroups (Table 2). There was no difference in stress response between the two different intubators (data not shown).

**Discussion**

We were unable to identify any increase in NPY plasma concentrations, despite transient increases in norepinephrine concentrations, after a standardized tracheal intubation closely resembling routine clinical practice. The lack of increase in NPY release suggests that the sympathetic response to tracheal intubation in children during 1% isoflurane anaesthesia is not large.

In adults, the stress response to tracheal intubation can cause adverse organ effects, such as myocardial ischaemia, but such organ dysfunction is not observed in otherwise healthy children. Despite this, we have observed a trend among colleagues to transpose the adult practice of attempting to attenuate the tracheal stress response into the paediatric setting, most frequently using opioids. However, when using the modern paediatric concept of light general anaesthesia combined with central or peripheral nerve block,
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Table 2 Haemodynamic data (heart rate (HR), arterial pressure (AP)), norepinephrine (NE) and neuropeptide Y (NPY) responses to tracheal intubation after clonidine or midazolam premedication. Data are mean (SEM) percentage change from pre-induction baseline values. nd = Not determined.

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Clonidine group</th>
<th>Midazolam group</th>
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<tbody>
<tr>
<td></td>
<td>HR (5.3)</td>
<td>HR (1.6)</td>
</tr>
<tr>
<td>3</td>
<td>12.1</td>
<td>9.5</td>
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<tr>
<td>6</td>
<td>8.1 (3.9)</td>
<td>9.4 (1.4)</td>
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<tr>
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<td>7.1 (4.0)</td>
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<td>30</td>
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<td>1.4 (2.6)</td>
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Fig 1 Percentage change in neuropeptide Y concentrations compared with baseline (whole study population).

As greater concentrations of isoflurane would have substantially modified or even abolished the stress response to tracheal intubation, we chose to perform intubation under only light anaesthesia (1%). This is slightly below what is generally accepted as the incision MAC value for isoflurane (approximately 1.4%) in this age group.15 16 Depth of anaesthesia after 5 min of controlled ventilation with 1% isoflurane would thus have allowed sufficient time for sympathetic activation in response to the intubation procedure to occur.

Contrary to what might have been expected, we were unable to identify any significant increase in plasma NPY concentrations after tracheal intubation in the overall patient population (Fig. 1). Neither could we detect any significant difference in NPY release between the clonidine and midazolam groups (Table 2). The lack of NPY release after tracheal intubation in our study indicates that this procedure is not associated with major activation of the sympathetic nervous system in anaesthetized children.

As it is not possible to use organ dysfunction secondary to substantial sympathetic nervous system activation as an end-point in children, some relevant physiological surrogate has to be studied in order to determine the degree of sympathetic stress response after tracheal intubation in anaesthetized children. Co-release of norepinephrine and NPY from sympathetic nerve endings has been identified as a possible discriminator of intense or prolonged sympathetic activation from a more limited response.5 6 The use of NPY as a marker of more pronounced stress response has recently been described in children and increased plasma concentrations of NPY have been identified after prenatal asphyxia14 and in association with discomfort caused by blood sampling.14 Therefore, we chose to use NPY release as the surrogate end-point.

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Mikawa and colleagues have demonstrated previously slight but statistically significant attenuation of the haemodynamic response to tracheal intubation in older children after premedication with oral clonidine 4 µg kg⁻¹, given 90–120 min before induction of anaesthesia compared with oral diazepam.4 In our study, we compared rectal premedication with clonidine 2.5 µg kg⁻¹ and midazolam 0.3 mg kg⁻¹ which, according to our previous clinical experience, produces equipotent sedation and anxiolysis, but were unable to reproduce this attenuation of the haemodynamic stress response to tracheal intubation.

It is somewhat surprising that other studies, with limited patient populations, have been able to demonstrate a difference in haemodynamic stress response after tracheal intubation in paediatric patients premedicated with clonidine compared with benzodiazepines. Calculations based on the pronounced inter-individual variation in haemodynamic stress response and concomitant norepinephrine release seen in our study indicate the need for 60–400 patients in order to demonstrate such a difference, depending on the
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haemodynamic variable and time point chosen. It should also be noted that such a difference would be in favour of the midazolam group. The discrepancies between our results and those of Mikawa and colleagues4 may be a result of a substantial difference in study design (e.g. intubation time, timing of premedication, differences in potency between midazolam and diazepam, and differences in clonidine doses).

In summary, the adrenergic stress response to routine tracheal intubation in anaesthetized children, aged 1–9 yr, was associated with marked inter-individual variation. The response was short-lived and of a limited magnitude, as indicated by lack of NPY release. The indication to routinely attempt to attenuate the tracheal intubation stress response in otherwise healthy children might thus be debatable. Rectal premedication with clonidine did not result in attenuation of the stress response compared with midazolam.

Acknowledgements

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References

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