Interaction of i.v. anaesthetic agents with 5-HT₃ receptors

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Summary

Using N1E-115 neuroblastoma cells as an experimental model, we have examined if four commonly used i.v. anaesthetic induction agents interact with 5-HT₃ receptors. Specifically, we tested the hypothesis that the antiemetic effects of propofol may result from 5-HT₃ receptor antagonism. Binding of tropisetron (a 5-HT₃ selective reference compound), etomidate, ketamine, thiopentone and propofol to 5-HT₃ receptors were assessed by measuring the displacement of [³H]BRL 43694 from whole N1E-115 cells. The rank order potency (Kᵢ) was tropisetron (1.7 (SEM 0.2) nmol litre⁻¹) > etomidate (83 (4) µmol litre⁻¹) > ketamine (97 (4) µmol litre⁻¹) > thiopentone (177 (9) µmol litre⁻¹) > propofol (819 (171) µmol litre⁻¹). With the exception of thiopentone these effects were outside the clinical range and suggest that anaesthetic agents are unlikely to interact directly with 5-HT₃ receptors, and that other mechanism(s) must underlie the antiemetic effects of propofol. (Br. J. Anaesth. 1995: 76: 271–273)

Materials and methods

Mouse neuroblastoma cells of the clone N1E-115 were cultured in Dulbecco’s modified Eagle’s medium. The growth medium was supplemented with 10 % fetal calf serum, with the antibiotics penicillin 100 u. ml⁻¹, streptomycin 100 µg ml⁻¹ and Fungizone 2.5 µg ml⁻¹. Cells were cultured to subconfluence in a humidified atmosphere containing 5 % carbon dioxide at 37 °C and were passaged every 5–7 days with re-feeding every third day.

Drugs used included: propofol (Zeneca Pharmaceuticals, Macclesfield, UK, lot: 71013/91A) solubilized in dimethylsulphoxide (DMSO); etomidate hydrochloride (Janssen Pharmaceuticals, Bucks, UK); sodium thiopentone (Rhone-Poulenc Rorer, Dagenham, UK, batch 62800) solubilized at 1 mmol litre⁻¹ in NaOH 1.0 mol litre⁻¹ (when this was added to the binding assay buffer, a 100 dilution, the pH remained unchanged at 7.4); ketamine hydrochloride (Sigma, Dorset, UK), ICS 205-930 (3-tropanylindole-3-carboxylate hydrochloride, tropisetron, Semat, St Albans, UK), [³H]BRL 43694 (specific activity 85.5 Ci mmol⁻¹, NEN Research Products, Herts, UK).

BINDING STUDIES

Binding of i.v. anaesthetic agents to 5-HT₃ receptors was studied using displacement of a fixed concentration (approximately 0.4 nmol litre⁻¹) of the high affinity 5-HT₃ selective antagonist, [³H]BRL...
43694. After removal of growth medium, cells were harvested, washed three times, and resuspended in Krebs–HEPES buffer, pH 7.4, of the following composition (mmol litre\(^{-1}\)): Na\(^+\) 143.3, K\(^+\) 4.7, Ca\(^{2+}\) 2.5, Mg\(^{2+}\) 1.2, Cl\(^-\) 125.6, H\(_2\)PO\(_4\)^{2-}\) 1.2, SO\(_4^{2-}\) 1.2, glucose 11.7 and HEPES 10. Cells were incubated at 37 °C for 60 min in 1-ml volumes of Krebs–HEPES buffer, in the presence of \([\text{H}]\)BRL 43694 and increasing concentrations of displacing drugs (i.v. anaesthetic agents and tropisetron). Bound and free ligand were separated by vacuum filtration onto Whatman GF/B filters using a Brandel cell harvester. Radioactivity retained on the filters was measured by liquid scintillation spectrophotometry. Non-specific binding was defined in the presence of excess tropisetron (10 μmol litre\(^{-1}\)) and amounted to 4.8 ± 1.6 % (n = 34).

**DATA ANALYSIS**

The concentration of displacer producing 50 % displacement (IC\(_{50}\)) was estimated by computer-assisted curve fitting (Graphpad-Prism). IC\(_{50}\) values were corrected for the competing mass of \([\text{H}]\)BRL 43694 according to Cheng and Prusoff [13] using a \(K_i\) (equilibrium dissociation constant) value for \([\text{H}]\)BRL 43694 of 0.3 nmol litre\(^{-1}\) [14] to yield \(K_i\)

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\[ K_i = \frac{IC_{50}}{[B]} + \frac{[B]}{EC_{50}} \]
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Statistical comparison of \(K_i\) values was made using Student’s unpaired \(t\) test and considered significant when \(P < 0.05\).

**Results**

Binding of \([\text{H}]\)BRL 43694 was displaced in a dose dependent manner by the 5HT\(_3\) selective reference compound, tropisetron. The calculated affinity (1.7 nmol litre\(^{-1}\)) was consistent with previous reports [15–17]. In addition, there was a dose-dependent displacement of \([\text{H}]\)BRL 43694 by the four anaesthetic agents tested. The curves shown in figure 1

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\text{Figure 1 Binding of [H]BRL 43694 to N1E-115 cells was displaced in a dose-dependent way by tropisetron (\(\bullet\)), etomidate (\(\square\)), ketamine (\(\bigcirc\)), thiopentone (\(\blacktriangle\)) and propofol (\(\triangleleft\)). Data are mean, \(\text{SEM} (n = 6–8)\).}
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and analysed in table 1 are based on maximum displacement of 100 %, although this was not achieved experimentally. The rank order potency was etomidate \(\geq\) ketamine \(>\) thiopentone \(>\) propofol. DMSO, the vehicle for propofol, produced < 5 % displacement of \([\text{H}]\)BRL 43694 at the highest concentration (300 μmol litre\(^{-1}\)) of propofol only. With the exception of thiopentone, these effects fell outside the clinically relevant serum concentrations [18–21].

**Discussion**

We have demonstrated that four commonly used i.v. anaesthetic induction agents exert little direct effect on endogenous 5-HT\(_3\) receptors in a cultured neuronal cell line, N1E-115. There was some overlap with the \(K_i\) for thiopentone and the observed peak serum concentration. However, these studies do not enable predictions to be made as to whether thiopentone is acting as an agonist (emetic) or antagonist (antiemetic). More importantly, our studies suggested that the reported antiemetic action of propofol [7–9] is unlikely to be caused by 5-HT\(_3\) receptor block as the \(K_i\) values of propofol exceeded the peak plasma and brain [22] anaesthetic concentrations. In addition, the \(K_i\) was approximately 25-fold greater than plasma propofol concentrations at subhypnotic doses, where a significant antiemetic action can be demonstrated [7–9]. However, it should be noted that there may be interspecies variation in the 5-HT\(_3\) receptor and consequently \(K_i\) values in mouse cells may not be a true reflection of those in humans.

Our results are at variance with the findings of a recent study of the action of i.v. anaesthetics with the 5-HT\(_3\) receptor by Barann and colleagues [23], who found that i.v. anaesthetics inhibited 5-HT\(_3\)-induced flux of \(^{14}\)C-guanidinium through the cation channel of the 5-HT\(_3\) receptor. The rank order of potency in their study was propofol \(>\) etomidate \(>\) thiopentone \(>\) ketamine (in our study we found propofol the least potent). This functional block of the 5-HT\(_3\) receptor by anaesthetic drugs may result from channel block rather than receptor antagonism, but suggests that direct 5-HT\(_3\) receptor antagonism may not be the only way to reduce emesis, in that “downstream” functional block may also be important. As the inhibition of \(^{14}\)C-guanidinium influx [23] was non-

Table 1 \(K_i\) values (mean (SEM)) for tropisetron and the four i.v. anaesthetic agents. Data are derived from the curves in figure 1.

<table>
<thead>
<tr>
<th>Agent</th>
<th>(K_i) (nmol litre(^{-1}))</th>
<th>Clinical conc(\ddagger) (μmol litre(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tropisetron</td>
<td>1.7 (0.2)</td>
<td>Not available</td>
</tr>
<tr>
<td>Etomidate</td>
<td>83.2 (3.8)</td>
<td>10 [18]</td>
</tr>
<tr>
<td>Ketamine</td>
<td>96.9 (3.5)</td>
<td>20 [19]</td>
</tr>
<tr>
<td>Thiopentone</td>
<td>177.3 (9.4) (\star) (\dagger)</td>
<td>380 [20]</td>
</tr>
<tr>
<td>Propofol</td>
<td>819.0 (171.3)</td>
<td>35 [21]</td>
</tr>
</tbody>
</table>

\(\dagger\) Peak at induction, not corrected for protein binding. Subhypnotic doses would be lower. \(\star\) \(P < 0.05\), less potent than ketamine and etomidate; \(\ddagger\) \(P < 0.05\), less potent than thiopentone, ketamine and etomidate.
competitive, this modifies the general conclusions of our study. While there does not appear to be 5-HT₃ receptor block, channel inhibition by anaesthetic agents appears to be a viable and potent [23] mechanism for producing antiemesis. However, thiopentone and etomidate do not appear to exert significant antiemetic action but still reduce 5-HT₃ receptor block, channel inhibition by anaesthetic agents appears to be a viable and potent [23] mechanism for producing antiemesis. However, thiopentone and etomidate do not appear to exert significant antiemetic action but still reduce 5-HT₃ function at clinically relevant doses.

References

12. Cheng YC, Prusoff WH. Relationship between the inhibitor constant (Kᵢ) and the concentration of inhibitor which causes 50 % inhibition (IC₅₀) of an enzymatic reaction. *Biochemical Pharmacology* 1973; 22: 3099–3108.