Aging decreases the sensitivity of the GABA carrier to propofol and etomidate†

H. KEITA, S. LASOCKI, D. HENZEL-ROUELLÉ, J.-M. DESMONTS AND J. MANTZ

Summary
The influence of aging on the pharmacodynamics of anaesthetic agents in the central nervous system remains poorly understood. As α-aminobutyric acid (GABA)-mediated neurotransmission appears to be an important target for anaesthetics in the brain, we hypothesized that aging could alter the sensitivity of the GABA carrier to anaesthetics. We have examined the effects of etomidate and propofol on the uptake of [3H]-GABA (5 min, 37 °C) into striatal synaptosomes of rats aged 2, 18 and 24 months. In 2-month-old rats, [3H]-GABA uptake was inhibited by nipecotic acid, a competitive inhibitor of the GABA carrier (IC50 = 3.6 ± 0.3 μM). Etomidate and propofol markedly reduced the activity of the GABA carrier, with IC50 values 58 (sd 3) and 46 (sd 3) μmol litre−1, respectively. Aging increased IC50 values for these anaesthetics. Nipecotic acid was unaffected. These data suggest that aging selectively alters the action of etomidate and propofol in the mammalian CNS. (Br J Anaesth. 1998; 81: 249–250)

Keywords: aging; anaesthetics, i.v.; propofol; anaesthetics, i.v.; etomidate; nerve, neurotransmitters, GABA

Methods and results
Handling procedures, as written in the Guide for the Care and Use of Laboratory Animals, were followed throughout. Experiments were performed on male Sprague–Dawley rats (Iffa-Credo, France) aged 2, 18 and 24 months housed on a 12-h light–dark cycle with food and water ad libitum. Animals were killed by stunning and decapitation, and striata quickly removed from the brains and placed into ice-cold sucrose 0.32 mol litre−1 containing EDTA 1 mmol litre−1, dithiothreitol 0.25 mmol litre−1 and adjusted to pH 7.4. Synaptosomes were isolated and purified on a four-step Percoll gradient and diluted to protein of 0.16 mg ml−1 with artificial cerebrospinal fluid (CSF) at 4 °C. The composition of the CSF was, in mmol litre−1: NaCl 126.5; NaHCO3 27.5; KCl 2.4; KH2PO4 0.5; CaCl2 1.1; MgCl2 0.83; Na2SO4 0.5; glucose 11.8; aminooxyacetic acid an inhibitor of GABA catabolism, (Sigma, France) 0.1 and β-alanine (an inhibitor of the GABA carrier in glial cells, Calbiochem, USA) 1; the pH was adjusted to 7.3 with a 95:5 v/v oxygen–carbon dioxide mixture.

Uptake was initiated by adding [2,3 3H]-GABA (Amersham, UK, 60 Ci mmol−1, 20 nM) to the synaptosomal suspension at 37 °C. This was performed in either the absence of any pharmacological or anaesthetic agent (control), or both, or the presence of nipecotic acid (a specific competitive inhibitor of neuronal GABA uptake), etomidate and propofol (10−6–10−3 mol litre−1). Etomidate and propofol were dissolved in dimethylsulphoxide (Merck, Germany). After 5-min incubation, the mixture was vacuum filtered through glass filters (Whatman, GF/F, 0.70–0.74 μm retention capacity) and washed with 10 ml of ice-cold CSF. The radioactivity retained on the filters was extracted with NaOH 0.1 mol litre−1 and measured by liquid scintillation in Aquasol 2 (New England Nuclear, USA). Specific [3H]-GABA uptake was defined by the difference between the radioactivity measured in both the presence and absence of nipecotic acid (10−3 mol litre−1).

The kinetics of anaesthetic-induced inhibition of [3H]-GABA uptake were investigated in 2-month-old rats by incubating synaptosomes (37 °C, 5 min) with increasing concentrations of [3H]-GABA (20–200 nmol litre−1) as previously reported. The reaction was stopped by addition of ice-cold CSF followed by vacuum filtration, and the radioactivity retained on the filters measured. Kinetic variables of the reaction (maximum velocity, Vmax; Michaelis–Menten constant, Km) were determined by genera-


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tion of double reciprocal plots in the absence (control) and presence of a fixed concentration of anaesthetic corresponding to the IC₅₀ value.

Concentration-response curves were generated by computer and IC₅₀ values for inhibition of [³H]-GABA uptake calculated using the GraphPAD software (USA). Results were considered reliable only if they had been reproduced in at least four independent experiments (each of them run in triplicate). Statistical comparison of the IC₅₀ values in 2-, 18- and 24-month-old rats was performed by the Kruskal–Wallis test followed by the Mann–Whitney U test. P<0.05 was considered the threshold for significance.

Synaptosomal [³H]-GABA uptake increased linearly with time and protein concentration. Mean value was 0.90 (SD 0.07) pmol litre⁻¹ mg protein⁻¹ after 5-min incubation. Non-specific uptake was always less than 5% of the total filter retained radioactivity.

Nippecotic acid, etomidate and propofol induced a concentration-related inhibition of [³H]-GABA uptake. In the 2-month-old animals, IC₅₀ values were mean 3.6 (SD 0.3), 46 (3) and 58 (3) µmol litre⁻¹ for nipeptic acid, propofol and etomidate, respectively. Dimethylsulphoxide did not affect [³H]-GABA uptake. Etomidate and propofol inhibition was non-competitive in that the Kₘ remained constant but there was a significant decrease in Vmax observed between control experiments and those containing these anaesthetics: V max (µmol mg protein⁻¹ 3 min⁻¹)=30 (4.1) for control and 22 (2.6) and 21.3 (3.3), P<0.05, for propofol and etomidate, respectively; Kₘ (µmol litre⁻¹)=0.2 (0.7) for control, and 5.4 (0.9) and 5.8 (0.8) for propofol and etomidate, respectively.

The influence of aging on the inhibition by anaesthetics of [³H]-GABA uptake is displayed in table 1.

There was a significant increase in the values for IC₅₀ of both propofol and etomidate in the 24-month-old rats. This value was found significantly increased for propofol, but not etomidate, in the 18-month-old animals. No significant change in the inhibitory effect of nipeptic acid was observed whichever the subpopulation of rats examined.

Comment

This study shows for the first time that aging induces functional alterations of neurotransmitters relevant to anaesthesia and subsequently produces changes in the pharmacodynamics of anaesthetics in the CNS. Indeed, the sensitivity of the GABA carrier to propofol and etomidate, two agents currently used, is decreased in old compared with young rats, as assessed by a significant increase in the IC₅₀ values for propofol (at 18 and 24 months) and etomidate (at 24 months).

The IC₅₀ values reported here for the inhibition of GABA uptake by nipeptic acid, propofol and etomidate and the kinetic analysis of this inhibition are consistent with our previous results. These anaesthetic concentrations are slightly greater than those required to produce anaesthesia. However, they can be considered clinically relevant, because they are in the range of those found in the plasma of anaesthetized humans. Therefore, the present data confirm and extend the observation that propofol and etomidate inhibit the activity of the GABA carrier in a non-competitive fashion.

The mechanisms underlying the aging-related decrease in sensitivity to propofol and etomidate remain to be elucidated. An aging-related neuronal loss has been reported in some species, but the functional relevance of this phenomenon remains controversial. Whether anaesthetics interact via the lipid environment of the GABA carrier or directly with the protein cannot be inferred from our data. Interestingly, and in contrast with propofol and etomidate, the potency of nipeptic acid to block GABA uptake was not affected by aging. Therefore, it is likely that propofol and etomidate do not share the same mechanism for blocking GABA uptake as nipeptic acid, and that aging selectively affects the anaesthetic-carrier interaction.

A major consequence of blocking GABA uptake is to increase the background levels of GABA activating extrasynaptic GABA₁ or even GABA₂-mediated synaptic transmission. This may contribute to the production of the anaesthetic state. The clinical relevance of the aging-related decrease in sensitivity to propofol and etomidate of the GABA carrier remains to be determined. It can be speculated that these or other related pharmacodynamic changes might contribute to the modifications in anaesthetic requirements that are observed in the elderly.

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References


Table 1 Influence of aging on the inhibition of [³H]-GABA uptake by anaesthetics (mean (SD)). **p<0.01, ***p<0.001 vs 2 months. n refers to the number of independent experiments.

<table>
<thead>
<tr>
<th>Age (months)</th>
<th>IC₅₀ values (µmol litre⁻¹)</th>
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<tbody>
<tr>
<td>2 (n=6)</td>
<td>3.6±(0.3)</td>
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<td>18 (n=6)</td>
<td>5.6±(0.8)</td>
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<td>24 (n=6)</td>
<td>3.9±(0.7)</td>
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<tr>
<th>IC₅₀ Propofol</th>
<th>Etomidate</th>
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<tr>
<td>46 ± (3)</td>
<td>58 ± (0.8)</td>
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<tr>
<td>225 ± (21)***</td>
<td>44 ± (4)</td>
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<td>161 ± (16)***</td>
<td>149 ± (14)**</td>
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