CGRP拮抗剂对吸入性麻醉剂作用机转之探讨

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           □ 一年後可對外提供參考
           □ 二年後可對外提供參考

執行單位: 國立成功大學醫學院麻醉學科

中華民國1998年9月10日
Calcitonin Gene-Related Peptide May not Involved the Effects of Nitric Oxide on the Inhalational Anesthetics

**Background:** Calcitonin gene-related peptide (CGRP), a product of alternative splicing of RNA from the calcitonin gene, is widely distributed in the central nervous system. Several findings suggested that CGRP was involved in central regulation of nociception. Recent evidence shows that the production of nitric oxide (NO) by cultured dorsal root ganglia and nitric oxide synthase (NOS)-immunoreactive neurons rich in both neonatal and adult dorsal root ganglia implicated that NO possible act as an important signaling molecule in the dorsal horn of the spinal cord. Garry et al. (1994) demonstrated an increase in spinal CGRP releasing after the administration of sodium nitroprusside, a NO producer. It has also been proposed that CGRP receptors may be coupled to the production of cGMP, a second messenger whose level could be elevated by NOS activity. Previously, we used NOS inhibitor and NO scavenger demonstrated NO may effect through an independent pathway related to ‘arousal’. The inhibition of ‘arousal’ related pathway could influence the efficacy of isoflurane inducing anaesthesia. Our study aimed to evaluate the effect of sensory neuropeptide CGRP on inhalation anesthetics and also the coupling of the CGRP receptors to NOS.

**Methods:** Thirty-two adult male Wistar rats (315 ±15 gm) were randomly divided into either a control (saline) group or a CGRP antagonist (CGRP-8-37) group with 8 rats in each isoflurane or halothane. The aqueous solution of CGRP-8-37 (30 nmol/kg) was administered as an intravenous bolus dose. Control rats received the same volume of normal saline. The MAC of isoflurane and halothane were determined as described in our previous studies; these methods were originally adapted from those of Eger and colleagues. After MAC values were determined, the rats were killed by disarticulation and the cerebellum was immediately isolated, frozen in liquid nitrogen, and stored at −20°C until the NOS activity assays. NOS activity in rat cerebellum slices was determined as described previously. Briefly, NOS activity was measured as the ability of tissue homogenates to convert $^{3}$H-L-arginine to $^{3}$H-L-citrulline. Tissues were thawed and homogenated in a buffer (about 5 ml for 1 g wet tissue) containing Tris/HCl 50 mM, EDTA 0.1 mM, EGTA 0.1 M, sucrose 250 mM, leupeptin 2 M, pepstatin 1 M, and phenylmethyl sulphonyl fluoride 1 M (pH 7.4) under ice-cold. Samples were incubated at 37°C for 30 min with 100 μl assay buffer containing potassium 50 mM, NADPH 120 μM, L-citrulline 1.2 mM, L-valline 60 μM, CaCl₂ 1 mM, MgCl₂ 1.2 mM, DL-dithiothreitol 1 mM, and 10 μM $^{3}$H-L-arginine (63 Ci/mmol, Amersham) (being adding 18 μl tissue homogenate). Reaction was terminated by adding 1.5 ml 1:1 (V/V) H2O/Dowex-50W (200-400, 8% cross-link, Na+-form). The mix was left to settle for 10 min, and the newly formed $^{3}$H-L-citrulline in the supernatant (1 ml) was measured with a Beckman scintillation counter. The activity is expressed as pmol of formed citrulline per mg protein in 30 min. Protein content of the homogenates was measured using commercial kit (Protein Assay 500----6, Bio-Rad) through the method of Bradford.
Data analysis: All data are presented as mean ±SEM and calculated from each group with desired sample size (N). Statistical analysis was performed using unpaired t test with the two level of 0.05. Differences were considered significant at $p < 0.05$.

Results: The baseline value of isoflurane MAC was 1.48 ±0.18% and halothane MAC was 1.20 ±0.08 in the control group. The value of isoflurane MAC was 1.46 ±0.21 and halothane MAC was 1.13±0.20 in the study group. (P >0.05) (Table 1) The activity of NOS was respectively 26.48±3.64 pmol--mg protein$^{-1}$--min$^{-1}$ in isoflurane control group, and significantly increased (47.13±15.40 pmol--mg protein$^{-1}$--min$^{-1}$) in the isoflurane with CGRP-8-37 group (Table 2). However, NOS activity was significantly decreased in halothane group used CGRP-8-37 (61.02±18.34 vs. 3.06±0.87 pmol--mg protein$^{-1}$--min$^{-1}$) (Table 2). The hemodynamic data were no significantly changed with CGRP-8-37 treatment in both anesthetics (Table 3, 4). No apparent adverse effect was noted in rats treated with CGRP-8-37 with a single dose (30 nmole.kg$^{-1}$) in our study. No significant hypoxia, hypercapnia, or acidosis was observed in any rat.

Conclusions: Our results showed that using an antagonist of CGRP and CGRP-8-37 would not change the end-tidal threshold (MAC) of isoflurane or halothane. These implicated that CGRP may not involved in the mechanism of inhalation anesthetics. However, the cNOS activity increased in isoflurane group treated by CGRP-8-37 and decreased in halothane group treated by CGRP-8-37. The possible mechanisms of increasing or decreasing cNOS activity may be due to other interaction between the CGRP and NOS or just only the effect of inhalation anesthetics on NOS. In summary our study CGRP may not involved in the mechanism of NO on inhalation anesthetics and there may exist another interaction between the CGRP and NOS.
Table 1. Effects of CGRP-8-37 on isoflurane and halothane anesthesia in rats

<table>
<thead>
<tr>
<th></th>
<th>MAC of Iso (vol %)</th>
<th>N</th>
<th>MAC of Hal (vol %)</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group</td>
<td>1.48±0.18</td>
<td>8</td>
<td>1.20±0.08</td>
<td>8</td>
</tr>
<tr>
<td>CGRP-8-37 (30 nmole kg⁻¹)</td>
<td>1.46±0.21</td>
<td>8</td>
<td>1.13±0.20</td>
<td>8</td>
</tr>
</tbody>
</table>

MAC: minimal alveolar concentration, Iso: isoflurane; Hal: halothane.

Values are presented as mean±SD.

* p< 0.05 versus control value.

Table 2. Effects of CGRP-8-37 (30 nmole.kg⁻¹) on NOS activity of cerebellum in rats during inhalational anesthesia

<table>
<thead>
<tr>
<th></th>
<th>Control of Isoflurane</th>
<th>Isoflurane with CGRP-8-37</th>
<th>Control of Halothane</th>
<th>Halothane with CGRP-8-37</th>
</tr>
</thead>
<tbody>
<tr>
<td>cNOS (pmol . mg protein⁻¹ . min⁻¹)</td>
<td>26.48±3.64</td>
<td>47.13±15.40*</td>
<td>61.02±18.34</td>
<td>3.06±0.87*</td>
</tr>
</tbody>
</table>

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CGRP: Calcitonin gene-related peptide; cNOS: cerebellum nitric oxide synthase

Values are presented as mean±SEM.

*Significantly increase from control group (p<0.05), +Significantly decrease from control group (p<0.05)
Table 3. Effects of CGRP-8-37 on hemodynamic responses during isoflurane anesthesia in rats

<table>
<thead>
<tr>
<th></th>
<th>SBP (mmHg)</th>
<th>DBP (mmHg)</th>
<th>MAP (mmHg)</th>
<th>HR (beats/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group</td>
<td>130±16</td>
<td>108±13</td>
<td>119±14</td>
<td>238±29</td>
</tr>
<tr>
<td>CGRP-8-37 (30 nmole.kg⁻¹)</td>
<td>131±17</td>
<td>105±15</td>
<td>118±16</td>
<td>224±28</td>
</tr>
</tbody>
</table>

Values are presented as mean ± SD.

* P < 0.05 versus control value. SBP: systolic blood pressure; DBP: diastolic blood pressure; MAP: mean artery pressure; HR: heart rate.

Table 4. Effects of CGRP-8-37 on hemodynamic responses during halothane anesthesia in rats

<table>
<thead>
<tr>
<th></th>
<th>SBP (mmHg)</th>
<th>DBP (mmHg)</th>
<th>MAP (mmHg)</th>
<th>HR (beats/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group</td>
<td>96±11</td>
<td>80±12</td>
<td>88±11</td>
<td>188±29</td>
</tr>
<tr>
<td>CGRP-8-37 (30 nmole.kg⁻¹)</td>
<td>97±10</td>
<td>85±5</td>
<td>92±7</td>
<td>185±19</td>
</tr>
</tbody>
</table>

Values are presented as mean ± SD.

* P < 0.05 versus control value. SBP: systolic blood pressure; DBP: diastolic blood pressure; MAP: mean artery pressure; HR: heart rate.
References:
6. Helen F. Galley, Ph.D., FIMLS, and Nigel R. Webster, Ph.D., FFARCS, FRCPE: Brain nitric oxide synthase activity is decreased by intravenous anesthetics Anesth Analg 83:591-4,1996