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## A novel mechanism for sevoflurane preconditioning-induced neuroprotection

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The concept “ischemic preconditioning” was introduced in the literature in 1986.<sup>1</sup> It describes a phenomenon in which short episodes of ischemia prior to a prolonged episode of ischemia provide protection against the detrimental effects of the prolonged ischemia. Subsequently, various stimuli, in addition to short episodes of ischemia, have been shown to induce a preconditioning effect.<sup>2,3</sup> It is specifically interesting to anesthesiologists that volatile anesthetics can also induce a preconditioning effect in various organs and systems including the central nervous system.<sup>4–7</sup> Isoflurane, sevoflurane and desflurane, currently used volatile anesthetics, have been shown to precondition the brain against ischemia in animal studies.<sup>6–9</sup> Various intracellular signaling molecules have been implicated in the volatile anesthetic preconditioning-induced neuroprotection.<sup>6–9</sup> In this issue of *Anesthesiology*, Xiong’s group<sup>10</sup> showed that activation of the canonical Notch signaling pathway may be involved in sevoflurane preconditioning-induced neuroprotection in rat brain. Since involvement of the Notch signaling pathway in volatile anesthetic effects on the brain is not known previously, this study helps identify this pathway as a novel mechanism for sevoflurane preconditioning-induced neuroprotection as well as other sevoflurane effects on the brain in a broader term.

The Notch signaling system is complex. There are 4 Notch proteins/receptors (Notch1 to Notch 4) and multiple Notch ligands in mammalian cells.<sup>11</sup> The Notch receptors exist in a heterodimer when they are in the plasma membrane. The ligands are often trans-membrane proteins. Two main types of ligands are called delta-like ligand and Jagged, each of which has subtypes. Once the receptors are bound with the ligands presented by a neighboring cell, the receptors are activated. Activated receptors are subjected to two cleavages in series: first by a disintegrin and metalloproteinase and then by  $\gamma$ -secretase. This process ultimately produces the Notch intracellular domain (NICD) that then travels to the nucleus to associate with the DNA-binding protein (RBP-J). This complex regulates the expression of target proteins, such as Hes and Hey that are transcription factors.<sup>11,12</sup> Thus, the Notch signaling pathway is unique because it does not require a separate intracellular signaling molecule to transmit the signaling to the nucleus. Also, activation of the Notch signaling pathway may require physical interaction between two neighboring cells because Notch ligands are often trans-membrane proteins and are not secretory proteins or peptides.

This Editorial View accompanies the following article:

Yang Q, Yan W, Li X, Hou L, Qiang D, Hailong Dong W, Wang S, Zhang X, Xiong L: Activation of canonical Notch signaling pathway is involved in the ischemic tolerance induced by sevoflurane preconditioning in mice. *Anesthesiology* 2012;XXX:XXX-XXX

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The Notch signaling system is a highly conserved pathway existing in many cell types. This pathway has various functions including causing increase or decrease of cell proliferation, death and differentiation processes.<sup>11,13</sup> Recent studies have shown that Notch signaling pathway participates in angiogenesis by regulating the conversion of cells contacting a tip cell to stalk cells in the formation of vascular network.<sup>14-16</sup> Tip cells are at the tip of angiogenic sprouts. The stalk cells neighboring to the tip cells proliferate to form the vessel wall. Importantly, dysregulation of the Notch signaling pathway is involved in many human diseases, such as cancer, congenital diseases and adult-onset diseases. Congenital diseases include Alagille syndrome, Tetralogy of Fallot and familial aortic valve disease.<sup>11,17</sup> Adult-onset diseases include multiple sclerosis and cerebral autosomal dominant arteriopathy with sub-cortical infarcts and leukoencephalopathy.<sup>11,13,18</sup> Thus, understanding the regulation of the Notch signaling pathway is very clinically relevant.

In the study performed by Xiong's group,<sup>10</sup> C57BL/6 mice were exposed to 2.5% sevoflurane for 1 h on each day for 5 consecutive days. The mice were subjected to a 60-min middle cerebral arterial occlusion (MCAO) at 24 h after the last sevoflurane exposure. MCAO is a classical focal brain ischemia model. The mice exposed to sevoflurane prior to MCAO had smaller brain infarct volumes and better neurological functions than the mice exposed only to the carrier gas (oxygen) or air when the assessment was performed at 3 days after the MCAO. These results clearly showed sevoflurane preconditioning-induced neuroprotection. The investigators then showed that sevoflurane preconditioning increased NICD proteins and Hes messenger RNA in the ischemic penumbral brain tissues. A  $\gamma$ -secretase inhibitor, N-[N-(3,5-difluorophenacetyl)-l-alanyl]-S-phenylglycine t-butyl ester, inhibited the sevoflurane preconditioning-induced NICD increase and neuroprotection. Sevoflurane induced a preconditioning effect in the brains of mice that had one intact and one disrupted allele of the *RBP-J* gene in their neurons (the *RBP-J*<sup>+/-</sup> mice). However, sevoflurane exposure did not induce a preconditioning effect in the brains of *RBP-J* deficient mice (the *RBP-J*<sup>-/-</sup> mice, both alleles of the *RBP-J* gene in these mice were disrupted). Collectively, these results suggest that activation of the Notch signaling pathway is needed for sevoflurane preconditioning-induced neuroprotection.

The results of Xiong's group<sup>10</sup> indicate that sevoflurane can activate the Notch signaling pathway. It is not known yet how sevoflurane activates this pathway. The possible mechanisms include enhancing the binding between the Notch receptors and ligands, increasing the activity of proteases that cleave Notch receptors and facilitating the binding of NICD to RBP-J. The investigators also have not identified the proteins downstream of NICD for sevoflurane preconditioning-induced neuroprotection. Since the Notch signaling pathway is involved in a broad range of biological functions,<sup>11,13</sup> alteration of this pathway can affect functions of many cells. Thus, one would think that regulating the proteins that are downstream of the Notch signaling pathway to induce neuroprotection may be more specific and potentially safer than activating the whole Notch signaling pathway. This rationale signifies the importance of identifying the effectors for sevoflurane preconditioning-induced neuroprotection.

There is one significant caveat in the study of Xiong and his colleagues.<sup>10</sup> The *RBP-J*<sup>-/-</sup> mice had smaller brain infarct volumes, fewer apoptotic cells in the ischemic penumbra and better neurological functions than wild-type mice after the MCAO. These beneficial effects appeared with sevoflurane preconditioning in wild-type and *RBP-J*<sup>+/-</sup> mice. However, exposure of the *RBP-J*<sup>-/-</sup> mice to sevoflurane worsened the neurological outcome to the level of wild-type mice without sevoflurane preconditioning after the MCAO. These results may be difficult to understand. The reasons for these findings are not clear from the study. However, the results suggest that the Notch signaling pathway can be harmful after brain ischemia/reperfusion. Thus, the timing may be critical in determining whether activation of

the Notch signaling pathway is translated into protective or harmful effects after brain insults. If this reasoning is correct, it is possible that blocking the beneficial effects of sevoflurane preconditioning via Notch signaling pathway in the RBP-J<sup>-/-</sup> mice makes the potentially harmful effects of repeated sevoflurane exposures to show up in these mice.

Sevoflurane is one of the most commonly used volatile anesthetics. Although sevoflurane preconditioning-induced neuroprotection is known for a few years,<sup>8</sup> Xiong's group now has suggested a novel mechanism for this protection. More studies are needed to confirm this finding and define how this mechanism leads to neuroprotection. In addition, the Notch signaling pathway may turn out to be a mediator for many other effects of sevoflurane on the brain.

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