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Nitric Oxide and Minimum Alveolar Concentration

TKO or Knockout?

Several recent studies have suggested a role for nitric oxide in mediating central nociceptive pathways and a possible involvement in mechanisms of wakefulness and anesthesia.[1] Such studies have involved the administration of pharmacologic inhibitors of nitric oxide synthase (NOS) to *in vivo* animal models. The specificity of such inhibitors used in an *in vivo* preparation was necessarily left open to question. In this issue of ANESTHESIOLOGY, a report by Ichinose *et al.* employs a state-of-the-art molecular biology technique in combination with an *in vivo* pharmacologic investigation to specifically implicate the nitric oxide pathway and to confirm the specificity of previous pharmacologic studies showing the reduction in minimum alveolar concentration by NOS inhibitors.[2]

In this novel approach to investigating the role of neuronal nitric oxide in anesthesia, the technique of homologous recombination was employed to generate knockout mice showing no evidence of neuronal NOS gene expression. Knockout mice provide an excellent method for creating *in vivo* models of genetic disease and to determine the essential functions of important regulatory molecules. These mice are produced by gene-targeting in embryonic stem cells. The mice generated from these stem cells contain tissues that fail to express the targeted gene and are capable of passing on the trait to their offspring.[3]

The effect of the NOS inhibitor nitro^{G}-L-arginine methylester (L-NAME) on the minimum alveolar concentration of isoflurane and on the righting reflex response was compared between normal mice and the NOS knockouts. Administration of the NOS inhibitor significantly decreased the minimum alveolar concentration of isoflurane anesthesia in the normal mice but did not affect the minimum alveolar concentration of isoflurane in the knockout mice lacking neuronal NOS. Thus, the reduction of minimum alveolar concentration and inhibition of righting reflex by this inhibitor are clearly and specifically linked to an inhibition of neuronal NOS.

Of equal importance is the fact that the neuronal knockout mice exhibited a normal minimum alveolar concentration for isoflurane and demonstrated no obvious change in baseline consciousness. This implies the existence of a compensatory mechanism(s) to retain consciousness and pain responses. Such compensation is consistent with the presence of multiple pathways to maintain vital functions, a phenomenon evident throughout nature. Many knockout experiments with functionally important genes have given unexpected results, whereby mice carrying the disrupted gene either are phenotypically normal or produce a minimal phenotype change.[4] This generally indicates that either a given gene has no function in that particular tissue or the function is compensated for by another member of the gene family or another backup mechanism. The current observation of no change in baseline minimum alveolar concentration in the brain NOS knockout mice is consistent with the latter. It also supports the idea that multiple pathways are responsible for anesthesia. In other studies employing this strain of neuronal NOS knockout mice, an upregulation of endothelial NOS (eNOS) in neurons has been shown to compensate for lost responses (*e.g.*, long-term potentiation responses are maintained by eNOS). This cannot be the compensatory mechanism in the current study, because L-NAME inhibits both neuronal and endothelial NOS with approximately equal efficacy. Compensation must be occurring through a pathway not dependent or not totally dependent on nitric oxide. This further suggests that nitric oxide may be a modulatory or stimulatory factor in a more primary pathway(s) related to consciousness.

In the central nervous system, nitric oxide has been studied most commonly as a response to activation of excitatory N-methyl-D-aspartate (NMDA) receptors. Interaction with NMDA receptors and/or downstream signalling pathways has been studied frequently as a site of anesthetic action. Various intravenous and inhalational anesthetics have been shown to inhibit NMDA receptor activity, NMDA-stimulated nitric oxide or cyclic guanosine monophosphate production, and

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NMDA-stimulated or -inhibited electrical responses in neurons.[1] These multiple interactions with NMDA-stimulated pathways led Ichinose *et al.* to suggest that the decrease in minimum alveolar concentration by administration of NOS inhibitors involves the NMDA pathway. Multiple other neuronal pathways, however, may be involved in this response. Recent reports have demonstrated that nitric oxide is directly involved in γ-aminobutyric acid-stimulated neural transmission and may play a role in opioid, muscarinic, and α_2-adrenergic neuronal responses. Thus, the specific transduction pathway(s) where nitric oxide influences wakefulness, analgesia, or anesthesia remains unknown.

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References

1. Johns RA, Moscicki JC, DiFazio CA: Nitric oxide synthase inhibitor dose-dependently and reversibly reduces the threshold for halothane anesthesia. ANESTHESIOLOGY 77:779–784, 1992

2. Ichinose F, Huang PL, Zapol WM: Effects of targeted neuronal nitric oxide synthase gene disruption and nitro^{G}-L-arginine methylester on the threshold for isoflurane anesthesia. ANESTHESIOLOGY 83:101–108, 1995

3. Kulkarni AB, Karlsson S: Transforming growth factor-β1 knockout mice. Am J Path 143:3–9, 1993

4. Shastry BS: More to learn from gene knockouts. Mol Cell Biochem 136:171–182, 1994