This has provoked two parallel lines of investigation to elucidate which function each of the three subtypes mediates and to define the distribution of the subtypes. The study by Ongjoco et al. in this issue of Anesthesiology sheds further light on this issue. The rationale for this approach is to identify a single receptor subtype target that mediates the desired analgesic effect and is incapable of producing the undesirable pharmacologic properties. Armed with this information, chemists can then direct a “target-based” drug-design program to synthesize a novel, subtype-selective, \( \alpha_2 \)-adrenoceptor agonist. The genes for each of these receptor subtypes have been cloned, making it possible to investigate their transcription, expression, and their role in physiologic and pathologic settings. The mRNA transcription patterns of these receptor subtypes can be elucidated qualitatively (e.g., by in situ hybridization) and quantitatively (e.g., by northern assay, ribonuclease protection assay or reverse-transcription polymerase chain reaction [RT-PCR] assay). Furthermore, recombinant DNA methodology has made it possible to address the role of the \( \alpha_2 \)-adrenoceptor subtypes by creating genetically modified reagents with dysfunctional (“transgenic”) or deficient (“knockout”) subtypes. Resolution of the protein sequence from the genome has facilitated the production of antibodies directed against the cognate proteins, which facilitates immunologic-based investigations (e.g., qualitative immunohistochemistry assay and quantitative Western assay).

Early pharmacologic studies showed that the activation of \( \alpha_2 \)-adrenoceptor mediated the analgesic effect of \( \alpha_2 \)-adrenoceptor agonists. A prazosin-sensitive \( \alpha_2 \)-adrenoceptor subtype was shown to inhibit neurotransmitter release from spinal cord preparations, suggesting a role for the \( \alpha_2 \text{H} \) or \( \alpha_2 \text{M} \)-adrenoceptor subtypes in antinociception. In support of this finding, ST-91, an \( \alpha_2 \text{C} \)-subtype-prefering agonist, was shown to induce antinociception in rats when it was administered intrathecally, and this effect was blocked by prazosin. More sophisticated analysis of the subtypes involved could not be accomplished by classic pharmacologic approaches because of a lack of subtype-selective agonists or antagonists. The development of molecular biologic techniques has provided more subtype-specific approaches. Studies using D79N mice, in which the gene for \( \alpha_2 \text{A} \)-adrenoceptor subtype has been rendered dysfunctional, established a role for \( \alpha_2 \text{A} \) subtype in antinociception, at least in the development of thermal analgesia. Yet the D79N mice exhibited some residual \( \alpha_2 \text{A} \)-adrenoceptor agonist-induced effects on spinal analgesia in the substance P-induced pain test. Studies using other transgenic and knockout models have shown that the \( \alpha_2 \text{A} \) subtype is responsible for the hypotensive and bradycardic actions of \( \alpha_2 \)-adrenoceptor agonists, whereas the \( \alpha_2 \text{B} \) subtype is responsible for its vasoconstrictive properties and the \( \alpha_2 \text{C} \) subtype for hypothermic action and modulation of dopaminergic activity. A recent study showed that intrathecally administered \( \alpha_2 \text{C} \), antisense oligodeoxynucleotides decreased mechanical antinociception induced by clonidine and other antinociceptive agents in the rat, suggesting that the \( \alpha_2 \text{C} \) subtype is also involved in antinociception. However, studies of genetically modified reagents may also provide misleading information. In the case of the
knockout models, these animals not only lack the protein of interest, but the organisms have had an opportunity to adapt to the deficiency during their development. These putative adaptive responses to the knockout remain largely undocumented. Therefore, in the situation in which the null mutation yields no loss of the investigated function, one cannot yet conclude that the molecule is not necessary for that function.\textsuperscript{11} The mechanism whereby antisense technology interferes with expression of the protein of interest in fully developed animals ("knockdown") remains poorly understood. In fact, the administration of sense oligonucleotides have also resulted in altered behavioral function,\textsuperscript{12} which underscores the necessity for demonstrating that expression of the cognate protein (e.g., by western assay) has been reduced before ascribing a functional role to that protein.

Previously, detailed descriptions of the distribution of $\alpha_2$-adrenoceptor subtypes had not been possible by conventional approaches using radiolabeled ligand binding assays because of the lack of subtype selective ligands. Recently, immunohistochemical analysis of specific protein expression and \textit{in situ} hybridization assay of gene transcription have been used to overcome this problem. Studies using rats and mice have shown that $\alpha_{2A}$ is the predominant receptor subtype in the brain, which is widely distributed throughout the brain. In the rat spinal cord, $\alpha_{2A}$ and $\alpha_{2C}$ subtypes have been identified by \textit{in situ} hybridization; $\alpha_{2A}$ are diffusely distributed, whereas the $\alpha_{2C}$ is located mainly in the cells of the dorsal root ganglia.\textsuperscript{13,14} In contrast, Smith \textit{et al.},\textsuperscript{15} using \textit{in situ} hybridization, previously demonstrated that $\alpha_{2A}$ and $\alpha_{2B}$ subtypes predominate in human spinal cord, with the $\alpha_{2C}$ subtype only sparsely represented. This discrepancy between rodents and humans instigated this current study regarding $\alpha_2$-adrenoceptor subtype distribution in human dorsal root ganglia. Because of the small size of dorsal root ganglia, they implemented a more sensitive quantitative RT-PCR assay. Data from these studies showed that mRNAs for $\alpha_{2B}$ and $\alpha_{2C}$ subtypes were almost equally transcribed in the dorsal root ganglia, but that the $\alpha_{2A}$ mRNA transcript was relatively deficient in this location. This distribution differs from those found in rodents and that present in the human spinal cord. There are possible methodologic reasons to explain these discrepancies. Quantitative RT-PCR assay provides less precise quantitation than that obtained by either ribonuclease protection assay or northern assay. An additional concern relates to degradation of the tissue mRNA over time because the material was harvested from autopsies performed from 2.5 to 20 h after death.

Are we to assume that the mRNAs of three receptor subtypes degrade to the same degree over time? Notwithstanding the possible technical concerns, RT-PCR assay does not provide qualitative information about its localization on different cell types in the dorsal root ganglia; such information necessitates immunohistochemical and \textit{in situ} hybridization assays. For examples, it is possible that, even though there is a relative deficiency of $\alpha_{2A}$ mRNA, the transcripts may all be present on important nociceptive neurons. Furthermore, there is not necessarily a 1:1 stoichiometric relation between the amount of transcript (mRNA) and the amount of protein subsequently translated and functionally expressed in the plasma membrane.

Although no one technique is likely to be the panacea for all our questions about something as complex as the molecular basis for behavior, the approach by Ongjoco \textit{et al.}\textsuperscript{1} exploits the profound advances that have been made in the neurosciences in the last decade. These data challenge the unproven assumption that data in rodents can be extrapolated to humans. Still, these molecular genetic techniques, and especially the information that they provide, have reduced the mechanistic questions, which have plagued our clinical discipline, into tractable segments.

\textbf{Mervyn Maze, M.B., Ch.B., F.R.C.P., F.R.C.A.}

Sir Ivan Magill Professor of Anaesthetics

m.maze@ic.ac.uk

\textbf{Masahiko Fujinaga, M.D.}

Senior Lecturer

Magill Department of Anaesthetics

Chelsea and Westminster Campus

Imperial College of Science, Technology, and Medicine

University of London

London, United Kingdom

m.fujinaga@ic.ac.uk

\textbf{References}

1. Ongjoco RRS, Richardson CD, Runder XL, Stafford-Smith M, Schwino DA: $\alpha_2$-Adrenergic receptors in human dorsal root ganglia: Predominance of $\alpha_{2B}$ and $\alpha_{2C}$ subtype mRNAs. \textit{Anesthesiology} 2000; 92:968–76


4. Hunter JC, Fontara DJ, Hedley LR, Jasper JR, Lewis R, Link RE,


10. Aley KO, Levine JD: Multiple receptors involved in peripheral α2, μ, and Δ4 antinociception, tolerance, and withdrawal. J Neurosci 1997; 17:735-44


Lippincott Williams & Wilkins, Inc.

Ion Channels Take Center Stage

Twin Spotlights on Two Anesthetic Targets

ONE of the fundamental ideas behind the science of anesthesiology throughout the majority of the twentieth century was the idea that there exists a "unitary site" of action for all general anesthetics.1 As our knowledge of the underlying neurobiology has grown, together with the database of potential anesthetic target sites, it has become increasingly obvious that this simplistic notion is incorrect.2 It now seems unlikely that general anesthetics interact with a single common target site because the function of a variety of membrane proteins has been shown to be altered within the clinically relevant range of anesthetic concentrations.3 Not only do multiple potential anesthetic targets exist, but the array of susceptible targets varies among different classes of anesthetic (review of Krasowski and Harrison4). For example, clinical concentrations of pentobarbital inhibit depolarization mediated via AMPA- and kainate-type glutamate receptors, enhance and prolong γ-aminobutyric acid (GABA)-mediated inhibition via an action at GABAA receptors, and inhibit the function of neuronal nicotinic acetylcholine receptors (nAChRs), whereas ketamine has no effect at GABAA receptors, but inhibits the function of the N-methyl-D-aspartate (NMDA) subtype of glutamate receptors (reviews of Franks and Lieb5 and Krasowski and Harrison4). As the century draws to a close, this "multiple alternate target" hypothesis gains further support from a study published in this issue of Anesthesiology.5 In this study,

This Editorial View accompanies the following article: de Sousa SLM, Dickinson R, Lieb WR, Franks NP: Contrasting synaptic actions of the inhalational general anesthetics isoflurane and xenon. Anesthesiology 2000; 92:1055-66.

Accepted for publication November 19, 1999.

Key words: Binding; GABA; isoflurane; NMDA; Xenon.

The author is not supported by, nor maintains any financial interest in, any commercial activity that may be associated with the topic of this article.

Anesthesiology, V 92, No. 4, Apr 2000