In recent years various classes of drugs have been studied as agents to aid in anesthesia and analgesia that were not originally designed for these therapeutic uses. One example is the use of α_2-adrenergic agonists as analgesic/anesthetic adjuvants (1). Since these drugs (e.g., clonidine) were primarily developed for the treatment of cardiovascular disorders, it is not surprising that cardiovascular effects can limit their use. Molecular cloning studies have indicated the existence of three α_2-adrenergic receptor subtypes (α_{2A}, α_{2B}, and α_{2C}), although the precise physiologic role of each of these subtypes has not yet been defined. Therefore, it is plausible to speculate that not all of the receptor subtypes will be involved in analgesic actions. More importantly, the subtypes mediating these actions might be distinct from those involved in the cardiovascular effects, and thus it might be possible to develop drugs with an improved efficacy/toxicity profile compared with presently available non–subtype-selective drugs.

Classically, the identification of receptor subtypes mediating a physiological response has relied on the use of subtype-selective antagonists and, to a lesser degree, agonists. Other information (radioligand binding, immunohistochemistry, or in situ hybridization) can provide evidence regarding the presence of a receptor subtype in a tissue or cell type of interest. However, such results are incomplete and potentially misleading in the absence of functional data. In the case of α_2-adrenergic receptors (and for that matter, many other classes of G protein–linked receptors for which multiple subtypes have been cloned) highly subtype-selective agents are not available and this has limited insight into the functional role of each subtype. The cloning of G protein–linked receptors and components of their signal transduction machinery provides a number of alternative approaches to define the functional role of receptor subtypes and signaling components. Such approaches include "chronic methods," such as the generation of transgenic animals, which overexpress or lack a certain receptor or signaling component, and "acute methods," whereby receptor expression is inhibited by antisense techniques. Recombinant DNA–based techniques have the potential to achieve a degree of specificity greater than that achieved by classical pharmacological methods, but this advantage can be offset by various limitations. For example, in the case of transgenic animals, factors such as gene dosage effects, inappropriate tissue targeting, inducibility versus constitutive expression, developmental effects of transgenes, and compensatory changes all may influence observed results.

In this issue of *The Journal*, Mizobe et al. (2) describe an antisense strategy with direct injection of oligonucleotide into the locus coeruleus of the rat to identify the α_2-adrenergic receptor subtype mediating the hypnotic response to the agonist dexmedetomidine. The data indicate that α_{2A}-adrenergic receptors (and not α_{2C}-adrenergic receptors) are primarily involved in this response. Whether these results can be taken as evidence that α_{2A}-adrenergic agonists may be effective analgesic agents without cardiovascular effects is doubtful for at least two reasons. First, it has been demonstrated (3) that intracerebroventricular application of α_{2A}-adrenergic antisense constructs to rats increases their blood pressure, while α_{2B}/α_{2C}-adrenergic antisense oligodeoxynucleotides are without effect (Mizobe et al. do not report blood pressure values in their antisense-injected animals). Second, oxymetazoline, which is used as a decongestant because of its vasoconstricting effects, has some selectivity for α_{2A}-adrenergic receptors (4), which suggests that agonists active at this subtype may have cardiovascular actions in peripheral tissues. Thus, additional work will be necessary to determine the utility of subtype-selective α_{2A}-adrenergic agonists as analgesic agents with fewer cardiovascular side effects than nonselective agents.

In addition, the work by Mizobe et al. highlights a number of potential problems in the use of antisense strategies for receptor studies, particularly when used in living animals. The key issues in the use of antisense techniques are effectiveness and specificity. Lack of effectiveness can result in falsely negative results, while lack of specificity can result in falsely positive results. Recent articles have reviewed some of the application of antisense techniques for studies of several classes of peptide or aminergic receptors (e.g., references 5–7). Effectiveness of antisense oligonucleotides will depend on several factors, including choice of sequence to suppress formation of the corresponding mRNA and protein, concentration and stability of the antisense oligonucleotide and its penetration into the target cell, turnover rate of the corresponding proteins, and properties related to the respective functional response (e.g., receptor reserve in the case of receptor-induced responses). More detailed descriptions of some of these issues are discussed elsewhere (8–10).

Choice of an appropriate sequence for use in vivo can generally be determined from in vitro studies with cell lines expressing the receptor of interest. However, in vivo effects may not always be predictable from in vitro studies, in part because of the potential for activation of the immune system by nucleotides. Mizobe et al. used cell lines which had been stably transfected with α_{2A}- or α_{2C}-adrenergic receptors. They demonstrate that these antisense oligonucleotides, when added at a concentration of 5 μM twice daily for 3 d, reduced receptor density in the cell lines, but only by 30–40%. This partial response is likely attributable to the relatively long half-life of the receptors in these cells.

With regard to oligonucleotide concentration, in most in vitro studies low micromolar concentrations are effective and the risk of obtaining nonspecific effects rises with increasing concentrations. Selection of a "correct" dose for in vivo experiments is more difficult than in vitro because compartmentalization, diffusion, and metabolic degradation are less predictable in vivo. Chemically modified oligonucleotides that vary with regard to lipophilicity and stability result in distinct pharmacokinetic profiles (11). Such pharmacokinetic considerations may be important in selecting appropriate oligonucleotide doses for in vivo studies, in particular when the oligonucleotides are delivered by bulk application to a tissue or brain region, as in the studies by Mizobe et al. Alternatively, antisense nucleotides can be delivered to the cell interior in a

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more specific manner using transfection or microinjection methods. For example, such methods have been used in vitro to define the role of various G protein subunits in the regulation of voltage-dependent calcium channels (12) and in the ability of protein kinase C isoforms to regulate arachidonic acid release (13). An example of in vivo studies is those that have involved the generation of transgenic animals that harbor constructs to express antisense RNA in an inducible and tissue-specific manner to define functional roles for the $G_{i\alpha 2}$-protein (e.g., reference 14).

When antisense oligonucleotides are directed at receptors, the degree of reduction in receptor number depends not only on correct delivery of an appropriate antisense oligonucleotide but also on the turnover of the corresponding protein. Thus, antisense strategies are expected to be most effective when receptor proteins are studied that have a low abundance and a rapid rate of turnover while antisense strategies are less likely to be successful for proteins with low turnover rates. As noted above, the latter factor probably explains why Mizobe et al. achieved only a 30–40% reduction in α_{2A}-adrenergic receptor expression in their studies with cells. A further complication comes from the possibility that the turnover rate of a protein may be different among cell types, and that in a given cell, in the case of receptors, turnover will depend on the presence of agonist or neurotransmitter and on compensatory changes in receptor expression after antisense treatment. Whether reductions of the magnitude seen by Mizobe et al. in their in vitro studies are sufficient to impair functional responses will depend on the receptor reserve ("spare receptors") for a given effect and the intrinsic activity of a given agonist. Since it is often difficult (or impossible) to predict the number of receptors necessary to maintain a response, this issue can be a major source of falsely negative data in antisense experiments. It should be noted that Mizobe et al. were unable to document effects of antisense oligonucleotide treatment on expression of the α_2 receptor protein in vivo because of the problems of protein abundance and lack of appropriate reagents.

Specificity is another potential problem in antisense experiments (8–10). To control for nonspecific effects of antisense constructs, sense or nonsense (scrambled) sequences are most frequently used, although other approaches, such as mismatched constructs or reversed sense constructs, have also been employed. Nonspecific effects of oligonucleotides may relate to their chemical backbone, but also may be sequence specific. While phosphorothioate oligonucleotides are less sensitive to nuclease digestion than their phosphodiester counterparts, phosphorothioate oligonucleotides may have effects that are not related to their sequence. These may include binding to membrane proteins, DNA polymerases, or induction of transcription factors. For example, studies on c-*myc* antisense oligomers have found multiple effects on cell growth that depend on the backbone composition of the oligonucleotide. These included those mediated by sequence-specific antisense inhibition, sequence-specific nonantisense (aptameric) inhibition, and non–sequence-specific, nonantisense inhibition (15). In addition, sequence-specific effects of control oligonucleotides have also been observed (e.g., reference 16).

In conclusion, antisense strategies, in particular use of antisense oligonucleotides, have the potential to be powerful tools to supplement classical pharmacological approaches for the identification of receptor subtypes and signal transduction pathways mediating physiological responses. The "acute" use of antisense oligonucleotides with cells in vitro and in tissues in vivo may provide complementary information to that obtained by the generation of transgenic animals. However, the limitations of the antisense approaches due to lack of effectiveness and/or specificity and consequences thereof should not be underestimated.

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