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### Research Article

Alpha2 adrenergic agonists are used in the anesthetic management of the surgical patient for their sedative/hypnotic properties although the alpha2 adrenoceptor subtype responsible for these anesthetic effects is not known. Using a gene-targeting strategy, it is possible to specifically reduce the expression of the individual adrenoceptors expressed in the central nervous system and to thereby determine their role in hypnotic action. Stably transfected cell lines (PC 124D for rat alpha2A; NIH3T3 for rat alpha2C adrenoceptors) were exposed to 5 microM antisense oligodeoxynucleotides (ODNs) for alpha2A and alpha2C adrenergic receptor subtypes for 3 d. Individual receptor subtype expression, as determined by radiolabeled ligand binding, was selectively decreased only by the appropriate antisense ODNs and not by the "scrambled" ODNs. These antisense ODNs were then administered three times, on alternate days, into the locus coeruleus of chronically cannulated rats and their hypnotic response to dexmedetomidine (an alpha2 agonist) was determined. Only the alpha2A antisense ODNs significantly change the hypnotic response causing both an increase in latency to, and a decrease in duration of, the loss of righting reflex following dexmedetomidine; hypnotic response had normalized 8 d after stopping the ODNs. Therefore, the alpha2A adrenoceptor subtype is responsible for the hypnotic response to dexmedetomidine in the locus coeruleus of the rat.

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## Antisense Technology Reveals the $\alpha_{2A}$ Adrenoceptor to Be the Subtype Mediating the Hypnotic Response to the Highly Selective Agonist, Dexmedetomidine, in the Locus Coeruleus of the Rat

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### Abstract

$\alpha_2$  adrenergic agonists are used in the anesthetic management of the surgical patient for their sedative/hypnotic properties although the  $\alpha_2$  adrenoceptor subtype responsible for these anesthetic effects is not known. Using a gene-targeting strategy, it is possible to specifically reduce the expression of the individual adrenoceptors expressed in the central nervous system and to thereby determine their role in hypnotic action.

Stably transfected cell lines (PC 124D for rat  $\alpha_{2A}$ ; NIH3T3 for rat  $\alpha_{2C}$  adrenoceptors) were exposed to 5  $\mu$ M antisense oligodeoxynucleotides (ODNs) for  $\alpha_{2A}$  and  $\alpha_{2C}$  adrenergic receptor subtypes for 3 d. Individual receptor subtype expression, as determined by radiolabeled ligand binding, was selectively decreased only by the appropriate antisense ODNs and not by the "scrambled" ODNs. These antisense ODNs were then administered three times, on alternate days, into the locus coeruleus of chronically cannulated rats and their hypnotic response to dexmedetomidine (an  $\alpha_2$  agonist) was determined.

Only the  $\alpha_{2A}$  antisense ODNs significantly change the hypnotic response causing both an increase in latency to, and a decrease in duration of, the loss of righting reflex following dexmedetomidine; hypnotic response had normalized 8 d after stopping the ODNs. Therefore, the  $\alpha_{2A}$  adrenoceptor subtype is responsible for the hypnotic response to dexmedetomidine in the locus coeruleus of the rat. (*J. Clin. Invest.* 1996. 98:1076–1080.) **Key words:** antisense • oligodeoxynucleotides, antisense •  $\alpha_2$  adrenergic agonists • anesthesia • sleep

### Introduction

Despite the fact that general anesthetics have been in use for more than 150 yr, knowledge of the biochemical and neurochemical mechanisms underlying the anesthetic state is lacking. In an attempt to shed light on this problem, we and others

(1) have turned to receptor-specific agents, including  $\alpha_2$  adrenergic agonists, to dissect the discrete brain regions and molecular components which are involved in anesthetic responses. Using dexmedetomidine, a highly selective  $\alpha_2$  adrenergic agonist, we demonstrated that the hypnotic response is transduced in the locus coeruleus (LC)<sup>1</sup> (2) and involves an  $\alpha_2$  adrenoceptor coupled to a pertussis toxin-sensitive G protein (3) which inhibits adenylate cyclase (4). The resulting decrease in activation of cAMP-dependent protein kinase modulates various ion channels (5).

Clinical studies with  $\alpha_2$  adrenergic agonists have revealed its putative utility in the perioperative period (6), but its attendant cardiovascular side effects have curtailed widespread application of this class of drug to anesthesia. Responses to the clinically available  $\alpha_2$  agonists, both beneficial and unwanted, are mediated by activation of one or more of the three  $\alpha_2$  adrenoceptor subtypes since none of these compounds have subtype selectivity. Future drug development in this class will need to target the anesthesia-mediating subtype while avoiding activation of the other subtype(s); such a strategy may mitigate the cardiovascular side effects.

Molecular genetic cloning studies in humans, rats, and mice have shown that three genes encode distinct  $\alpha_2$  adrenergic receptor subtypes (Table I). Pharmacological studies have defined four subtypes named  $\alpha_{2A}$ ,  $\alpha_{2B}$ ,  $\alpha_{2C}$ , and  $\alpha_{2D}$ , with the  $\alpha_{2D}$  representing a species homologue of  $\alpha_{2A}$  (the term  $\alpha_{2A}$  will be used to designate the  $\alpha_{2A/D}$  subtype). The three  $\alpha_2$  adrenergic receptor subtypes are nonhomogeneously distributed in the central nervous system (7–15) with the  $\alpha_{2A}$  and  $\alpha_{2C}$  subtypes predominating while the  $\alpha_{2B}$  subtype is sparsely represented and only in the diencephalon (16). In this study, using antisense oligodeoxynucleotide (ODN) technology (17–21), we establish that the  $\alpha_{2A}$  is the subtype mediating the hypnotic response to dexmedetomidine in the LC.

### Methods

**Synthesis of oligonucleotides.** The phosphodiester ODNs were synthesized on ABI 394 and ABI 380B DNA Synthesizer by use of phosphoramidite chemistry (PAN Facility, Stanford, CA). The introduction of phosphorothioate linkages (S-ODNs) were achieved by using tetraethylthiuram disulfide reagent (Applied Biosystems, Inc., Foster City, CA). The S-ODNs were purified over NAP-10 (Pharmacia Biotech, Uppsala, Sweden) and quantitated by spectrophotometry.

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1. **Abbreviations used in this paper:** LC, locus coeruleus; LORR, loss of righting reflex; ODNs, oligodeoxynucleotides; S-ODNs, phosphorothioate linked ODNs.

Table I. Classification of  $\alpha_2$  Adrenoceptors

Pharmacologic nomenclature	$\alpha_{2A/D}$	$\alpha_{2B}$	$\alpha_{2C}$
Human genetic nomenclature	$\alpha_{2C10}$	$\alpha_{2C2}$	$\alpha_{2C4}$
Rat genetic nomenclature	RG20	RNG	RG10
Mouse genetic nomenclature	MHC10	MHC2	MHC4

The sequences in the region immediately downstream from the initiation codon of rat  $\alpha_{2A}$  and  $\alpha_{2C}$  adrenoceptor subtypes were targeted. "Scrambled" ODNs containing the same nucleotides, but rearranged, were used as controls. The sequences of ODNs used in this study were: rat  $\alpha_{2A}$  antisense, 5'-ATG,GGC,TCC,CTG,CAG,CCG,GAT-3'; rat  $\alpha_{2A}$  scrambled antisense, 5'-CGA,GTT,GCC,TCA,AGC,GGT,CGC-3'; rat  $\alpha_{2C}$  antisense, 5'-ATG,GCG,TCC,CCA,GCG,CT-3'; rat  $\alpha_{2C}$  scrambled antisense, 5'-GGC,CTC,ACT,GCG,ACG,TC-3'.

*In vitro exposure of ODNs to cells stably transfected with either  $\alpha_{2A}$  and  $\alpha_{2C}$  receptor subtypes.* PC 124D cells, stably transfected with rat  $\alpha_{2A}$  adrenoceptors, and NIH3T3 cells stably transfected with rat  $\alpha_{2C}$  adrenoceptors were kindly provided by Dr. Stephen M. Lanier (22). These cell lines were maintained in monolayer culture at 37°C, under an atmosphere of 5% CO<sub>2</sub>. For the PC124D cells, RPMI 1640 medium was supplemented with 10% dialyzed fetal bovine serum plus 5% horse serum. In NIH3T3 cells, Dulbecco's modified Eagle's medium (DME) was supplemented with 10% bovine calf serum. All media were additionally supplemented with penicillin (100 U/ml), streptomycin (100 mg/ml), and fungizone (0.25 mg/ml). The culture media with 5  $\mu$ M phosphorothioate ODNs (antisense and scrambled) or saline were changed twice a day for 3 d. On day 4, the cells were harvested for assessment of receptor expression.

*Radiolabeled ligand binding assays.* These were performed as described previously (23). Briefly, cells were rinsed twice with cold PBS and then 10 ml of ice-cold lysis buffer (10 mM Tris-HCl, pH 7.4, 1 mM EDTA) was added. Cells were scraped off the flask with a disposable "policeman" and collected. The flask was rinsed with an additional 5 ml of lysis buffer and the washings were pooled. Cells were homogenized by four 5-s bursts at full speed using a polytron. The nuclei were pelleted by centrifugation at 220 g for 5 min at 4°C and the supernatant was centrifuged at 16,000 rpm (Sorvall) in SS34 rotor for 30 min. The pelleted membranes were resuspended in an appropriate volume of binding buffer (75 mM Tris-Cl, 12.5 mM MgCl<sub>2</sub>, 1 mM EDTA, pH 7.4) aliquoted into 1-ml screw-capped Eppendorf tubes, and stored in liquid nitrogen.

Binding experiments were performed in 0.25-ml volumes of buffer for 90 min at 25°C, using [<sup>3</sup>H]atipamezole (82 Ci/mmol; Orion Farnos, Turku, Finland) as the  $\alpha_2$  ligand. The assays were performed on between 5 and 100  $\mu$ g of membrane protein (determined by Bio-Rad's dye-binding reagent). The bound radiolabeled ligand was separated from the free ligand by filtration through GF/C filters using a vacuum filtration manifold (Brandel Cell Harvester). Saturation binding isotherms were performed by incubating membranes with varying concentrations of the radioligand, and nonspecific binding was determined by adding 10  $\mu$ M rauwolscine. Equilibrium dissociation constants were determined from saturation isotherms using a nonlinear least-square curve-fitting technique (GraphPAD Software Inc., San Diego, CA). Protein content for the membrane preparations were assayed according to Lowry et al. (24). B<sub>max</sub> was calculated in fmol/mg protein and was expressed as percent B<sub>max</sub> of the control.

*In vivo studies.* The experimental protocol was approved by the Animal Care and Use Committee at the Veterans Affairs Palo Alto Health Care System. Male Sprague-Dawley rats, originating from the same litter, weighing 250–350 grams, were used. The rats were stratified to match the distribution of the weights in the groups as closely as possible. All tests were performed between 10 a.m. and 4 p.m. The number of animals for each experiment is listed in the legends.

The left LC was stereotactically cannulated with a 24 gauge stainless steel cannula according to the following coordinates: with the

bregma as the reference, 1.2 mm lateral, 9.7 mm posterior, and at a depth of 6 mm from the skull. The surgical procedure was performed with the rat under halothane anesthesia and the cannula was fixed in position with methylmethacrylate resin. Correct placement of the cannula at the superior border of the LC was by the appropriate hypnotic response to dexmedetomidine (2). Cannulated rats were injected three times, on alternate days (days 1, 3, and 5), with 5 nmol/0.2  $\mu$ l of phosphodiester antisense, its scrambled control ODNs, or 0.2  $\mu$ l saline. The hypnotic response was determined before antisense treatment, just after the treatment (on day 6), and 8 d after the last antisense treatment. The treatment times were predicated by the *t*<sub>1/2</sub> of 4 d for the  $\alpha_{2A}$  adrenoceptor in the rat cortex (25). Dexmedetomidine, 7.0  $\mu$ g in 0.2  $\mu$ l, was administered into the LC with a microinfusion pump (CMA, West Lafayette, IN). The hypnotic response was defined by the loss of the rat's righting reflex (LORR); both the latency (time from injection until the animal first lost its righting reflex) and duration (time from the rat's inability to right itself when placed on its back until the time that it spontaneously reverted, completely, to the prone position) were measured. The observer was blinded to the various treatments.

*Statistical analysis.* The data are expressed as mean  $\pm$  SEM. The results of the in vitro experiments are analyzed by one-way ANOVA, followed by Newman-Keul's test. The results of the in vivo experiments are analyzed by two-way ANOVA with repeated measures, followed by Bonferroni test. *P* < 0.05 is considered statistically significant.

## Results

*Effect of ODNs on receptor expression in vitro.* Compared with the saline control, the  $\alpha_{2A}$  antisense ODNs significantly (*P* < 0.05) reduced the mean B<sub>max</sub> value of  $\alpha_{2A}$  adrenoceptors on PC124D cells by > 30%; neither  $\alpha_{2A}$  scrambled ODNs (94.0%) nor  $\alpha_{2C}$  antisense ODNs (98.2%) had any significant effects on the mean B<sub>max</sub> value on PC124D cells (Fig. 1, *n* = 4).

Compared with the saline control, the  $\alpha_{2C}$  antisense ODNs significantly (*P* < 0.01) reduced the mean B<sub>max</sub> value of  $\alpha_{2C}$

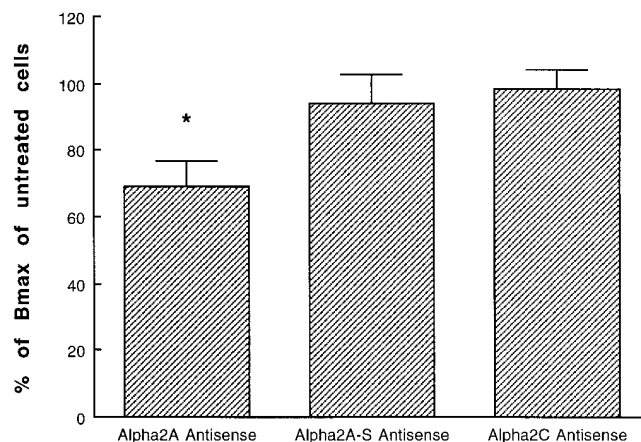
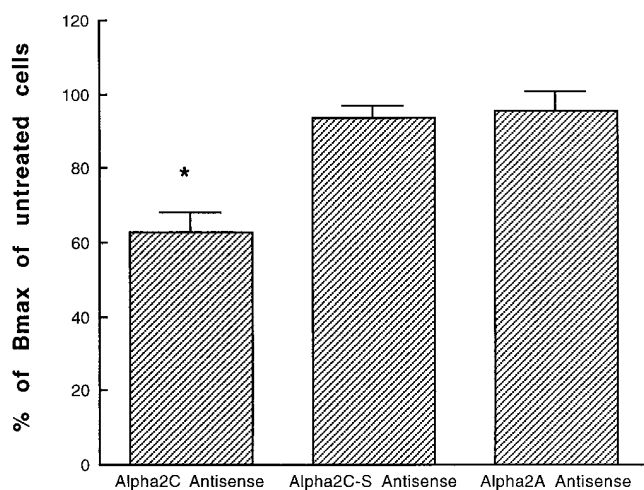


Figure 1. Effect of ODNs on expression of  $\alpha_{2A}$  adrenoceptors. PC124D cells stably transfected with rat  $\alpha_{2A}$  adrenoceptors were exposed to  $\alpha_{2A}$  antisense,  $\alpha_{2A}$  scrambled antisense (Alpha2A-S), or  $\alpha_{2C}$  antisense ODNs, 5  $\mu$ M daily for 3 d. Radiolabeled ligand binding with [<sup>3</sup>H]atipamezole was performed on plasma membranes prepared from the treated cells. Data (mean  $\pm$  SEM) are expressed as a percentage of the B<sub>max</sub> of cells unexposed to ODNs which was 16,310  $\pm$  844 fmol/mg protein. *K*<sub>d</sub> of the unexposed cells was 0.64  $\pm$  0.19 nM and was not different in the ODN exposed cells. *n* = 4; \**P* < 0.05.



**Figure 2.** Effect of ODNs on expression of  $\alpha_{2C}$  adrenoreceptors. NIH3T3 cells stably transfected with rat  $\alpha_{2C}$  adrenoreceptors were exposed to  $\alpha_{2C}$  antisense,  $\alpha_{2C}$  scrambled antisense (*Alpha2C-S*), or  $\alpha_{2A}$  antisense ODNs, 5  $\mu$ M daily for 3 d. Radiolabeled ligand binding with [<sup>3</sup>H]atipamezole was performed on plasma membranes prepared from the treated cells. Data (mean  $\pm$  SEM) are expressed as a percentage of the B<sub>max</sub> of cells unexposed to ODNs which was 3,051  $\pm$  144 fmol/mg protein. K<sub>d</sub> of the unexposed cells was 0.94  $\pm$  0.11 nM and was not different in the ODN exposed cells. *n* = 4; \**P* < 0.01.

adrenoreceptors on NIH3T3 cells by nearly 40%; neither  $\alpha_{2C}$  scrambled ODNs (93.4.0%) nor  $\alpha_{2A}$  antisense ODNs (95.7%) had any significant effects on the mean B<sub>max</sub> value on NIH3T3 cells. (Fig. 2, *n* = 4, respectively).

*Effect of ODNs on hypnotic response to dexmedetomidine.*

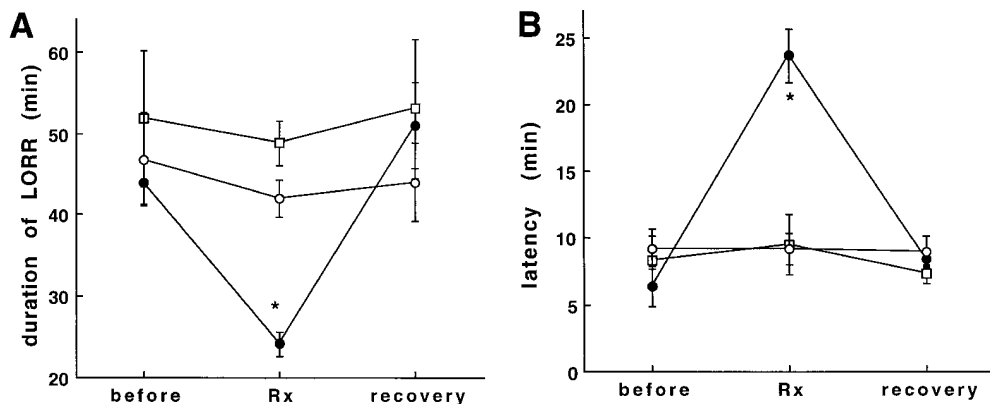
Immediately after  $\alpha_{2A}$  antisense treatment, duration of LORR was significantly decreased when compared with the values before and 8 d after antisense treatment (Fig. 3 A, *n* = 5). Conversely, no changes in the duration of LORR were noted in animals treated with either the saline vehicle or scrambled  $\alpha_{2A}$  antisense. The latency until LORR increased significantly immediately after  $\alpha_{2A}$  antisense treatment when compared with the values before and 8 d after antisense treatment (Fig. 3 B); again, no changes in the duration of LORR was noted in animals treated with either the saline vehicle or scrambled  $\alpha_{2A}$  antisense. Neither the duration (Fig. 4 A) nor the latency (Fig. 4

B) of LORR was affected by the  $\alpha_{2C}$  antisense ODNs or its scrambled  $\alpha_{2C}$  antisense ODNs.

**Discussion**

Antisense ODNs selectively and specifically decreased receptor subtype expression in vitro. Antisense ODNs for the  $\alpha_{2A}$  receptor subtype decreased the hypnotic response to dexmedetomidine in a reversible manner. Neither the scrambled  $\alpha_{2A}$  ODNs nor the antisense ODNs for the  $\alpha_{2C}$  receptor subtype affected the hypnotic response to dexmedetomidine. While we do not provide evidence that in vivo expression of the  $\alpha_{2A}$  receptor subtype is reversibly altered, our in vitro biochemical and in vivo behavioral findings strongly implicate the  $\alpha_{2A}$  receptor subtype in the mediation of the hypnotic response to  $\alpha_2$  agonists in the LC. Attempts to assess receptor expression in the minute LC were thwarted by the absence of appropriate antibodies to discriminate between the two  $\alpha_2$  receptor subtypes.

The antisense ODNs possess unique sequences relative to the entire genome. Therefore, antisense ODN technology provides a degree of specificity which is lacking in conventional pharmacologic or toxicologic probes (26). Inhibition of receptor expression by antisense ODNs relies on the ability of an ODN to bind a complementary sense mRNA sequence and prevent translation of the mRNA. Among the possible mechanisms whereby this interferes with protein expression is the presence of the ubiquitous H RNase which can digest the RNA-DNA duplex making the mRNA transcript unavailable for translation (27). The level of the expressed protein will decrease and therefore the function propagated by the protein will be lacking. The effect on protein expression is usually incomplete unless the ODNs are continuously delivered over a prolonged period of time, usually four times the *t*<sub>1/2</sub> of the protein. The turnover rate of the rat  $\alpha_{2A}$  adrenoreceptor is thought to be  $\sim$  4 d (28). If the remaining protein exceeds the threshold required to produce a functional response, the decrement in protein expression will not be functionally noticed. Such "redundancy" exists to varying degrees for each of the behavioral responses to  $\alpha_2$  agonists, as determined by *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ) studies (29). However, a 25%, or greater, decrement in receptor expression was found to be sufficient to attenuate the hypnotic response.



**Figure 3.** Effect of  $\alpha_{2A}$  antisense ODNs on hypnotic response to the  $\alpha_2$  agonist dexmedetomidine. Three cohorts of rat littermates were stereotactically cannulated, siting the tip of the needle in the LC. The duration (A) and the latency (B) of the LORR in response to dexmedetomidine, 7  $\mu$ g, LC, were assessed before, immediately after (*Rx*), and 8 d after (*recovery*), administering either  $\alpha_{2A}$  antisense (filled circles) (*n* = 5),  $\alpha_{2A}$  scrambled antisense (open boxes) (*n* = 4; 5

nmol/0.2  $\mu$ l), or saline (open circles) (*n* = 5; 0.2  $\mu$ l) three times, on days 1, 3, and 5. Data are expressed as mean  $\pm$  SEM. \**P* < 0.01 when compared with *before* and 8 d after (*recovery*) antisense treatment period.

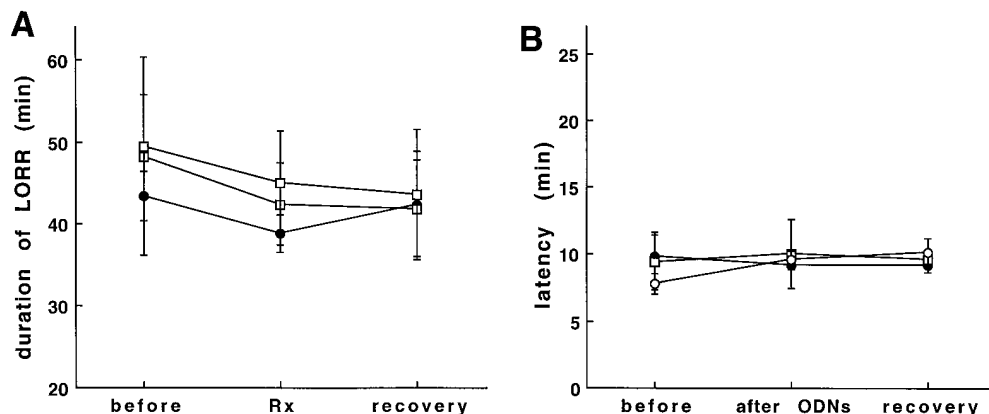


Figure 4. Effect of  $\alpha_{2C}$  antisense ODNs on hypnotic response to the  $\alpha_2$  agonist dexmedetomidine. Three cohorts of rat littermates were stereotactically cannulated, siting the tip of the needle in the LC. The duration (A) and the latency (B) of the LORR in response to dexmedetomidine, 7  $\mu$ g, LC, were assessed before, immediately after (Rx), and 8 d after (recovery), administering either  $\alpha_{2C}$  antisense (filled circles) ( $n = 5$ ),  $\alpha_{2C}$  scrambled antisense (open boxes) ( $n = 5$ ; 5 nm/0.2  $\mu$ l), or saline (open circles in A, open circles in B) ( $n = 5$ ; 0.2  $\mu$ l) three times, on days 1, 3, and 5. Data are expressed as mean  $\pm$  SEM.

A robust feature of an *in vivo* antisense ODN study is that the effect should be reversible over a time course predicated by the protein kinetics. The fact that the response is reversible assures one that the effect is not due to nonspecific antisense toxicity and provides a degree of specificity which is lacking in “knockout” or “transgenic” experiments unless complemented by subsequent breeding studies to reacquire the original phenotype. There are now several examples in which antisense ODNs have been successfully used *in vivo* to interfere with specific protein synthesis and its physiologic function (17–21).

Using a knockout gene-targeting strategy, work from Kobilka’s lab (30) has demonstrated that, unlike the wild-type, mice deficient in the  $\alpha_{2B}$  adrenoceptor subtype do not exhibit the acute hypertensive response to a bolus dose of dexmedetomidine. While knockout strategies have failed to yield mice deficient in the  $\alpha_{2A}$  adrenoceptor subtype, recent transgenic experiments have developed mice with “dysfunctional”  $\alpha_{2A}$  adrenoceptors. Data from behavioral studies on these mice also implicate this receptor subtype for the anesthetic action to dexmedetomidine (MacMillan, L.B., T.-Z. Guo, L.E. Limbird, and M. Maze, manuscript in preparation).

The  $\alpha_2$  adrenergic agonists are currently being used in the anesthetic management of the surgical patient for their sedative/hypnotic, anesthetic-sparing, analgesic, and sympatholytic properties. Each of the clinically available agents in this class has an imidazole ring which facilitates activation of nonadrenergic imidazoline binding sites; neither do they discriminate between the three  $\alpha_{2C}$  adrenoceptor subtypes. This relative nonspecificity and nonselectivity of the clinically available agonists may be the cause of troublesome side effects. For example, the vagally mediated bradycardia may be due, in part, to activation of the imidazoline-preferring receptor in the brain stem (31). Also, the acute hypertension that follows rapid bolus administration of  $\alpha_2$  agonists is probably due to activation of the  $\alpha_{2B}$  adrenoceptor subtype (30). Full realization of the clinical potential of this drug class for anesthesia will require the synthesis of novel ligands which have specificity for the receptor subtype(s) responsible for the salubrious effects while avoiding affinity for sites capable of producing side effects. Therefore, it is important to define, unequivocally, the receptor subtype(s) responsible for the anesthesia-related proper-

ties. This study taken together with the recent knockout and transgenic studies suggests that the  $\alpha_{2A}$  adrenoceptor subtype should be the target for subsequent drug development of  $\alpha_2$  agonists for anesthetic use.

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The authors extend special gratitude to Dr. Stephen Lanier for providing the stably transfected cell lines, and to Dr. Risto Lammin-tausta for providing dexmedetomidine and [<sup>3</sup>H]atipamezole.

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## References

- Tanelian, D.L., P. Kosek, I. Mody, and M.B. MacIver. 1993. The role of the GABA<sub>A</sub> receptor/chloride channel complex in anesthesia. *Anesthesiology*. 78:757–776.
- Correa-Sales, C., B.C. Rabin, and M. Maze. 1992. A hypnotic response to dexmedetomidine, an  $\alpha_2$  agonist is mediated in the locus coeruleus in rats. *Anesthesiology*. 76:948–952.
- Correa-Sales, C., K. Reid, and M. Maze. 1992. Pertussis toxin-mediated ribosylation of G proteins blocks the hypnotic response to an  $\alpha_2$  agonist in the locus coeruleus of the rat. *Pharmacol. Biochem. Behav.* 43:723–727.
- Correa-Sales, C., C. Nacif-Coelho, K. Reid, and M. Maze. 1992. Inhibition of adenylate cyclase in the locus coeruleus mediates the hypnotic response to an  $\alpha_2$  agonist in the rat. *J. Pharmacol. Exp. Ther.* 263:1046–1049.
- Nacif-Coelho, C., C. Correa-Sales, L.L. Chang, and M. Maze. 1994. Perturbation of ion channel conductance alters the hypnotic response to the  $\alpha_2$  adrenergic agonist dexmedetomidine in the locus coeruleus of the rat. *Anesthesiology*. 81:1527–1534.
- Mizobe, T., and M. Maze. 1995. Alpha<sub>2</sub>-adrenoceptor agonists and anesthesia. *Int. Anesthesiol. Clin.* 33:81–102.
- Ordway, G.A., S.M. Jaconetta, and A.E. Halaris. 1993. Characterization of the subtypes of alpha-2 adrenoceptors in the human brain. *J. Pharmacol. Exp. Ther.* 264:967–976.
- Wamsley, J.K., M.E. Alburges, M.A.E. Hunt, and D.B. Bylund. 1992. Differential localization of  $\alpha_2$ -adrenergic receptor subtypes in brain. *Pharmacol. Biochem. Behav.* 41:267–273.
- Nicholas, A.P., V. Pieribone, and T. Hokfelt. 1993. Distribution of mRNAs for alpha-2 adrenergic receptor subtypes in rat brain: an *in situ* hybridization study. *J. Comp. Neurol.* 328:575–594.
- Lawhead, R.G., H.S. Blaxall, and D.B. Bylund. 1992.  $\alpha_{2A}$  is the predominant  $\alpha_2$ -adrenergic receptor subtype in human spinal cord. *Anesthesiology*. 77: 983–991.
- Uhlen, S., and J.E.S. Wikberg. 1991. Rat spinal cord  $\alpha_2$  adrenoceptor are of the  $\alpha_{2A}$ -subtype: comparison with  $\alpha_{2A}$ - and  $\alpha_{2B}$ -adrenoceptors in rat spleen,

- cerebral cortex, and kidney using  $^3\text{H}$ -RX821002 ligand binding. *Pharmacol. Toxicol.* 69:341–350.
12. Zeng, D., and K.R. Lynch. 1991. Distribution of  $\alpha_2$ -adrenergic receptor mRNA in the rat CNS. *Mol. Brain Res.* 10:219–225.
  13. Handy, D.E., C.S. Flordellis, N.N. Bogdanova, M.R. Bresnahan, and H. Gavras. 1993. Diverse tissue expression of rat  $\alpha_2$ -adrenergic receptor genes. *Hypertension (Dallas)*. 21:861–865.
  14. De Vos, H., G. Vauquelin, J. De Keyser, J.P. De Backer, and I. Van Liefde. 1992. Regional distribution of  $\alpha_{2A}$ - and  $\alpha_{2B}$ -adrenoceptor subtypes in postmortem human brain. *J. Neurochem.* 58:1555–1560.
  15. Scheinin, M., J.W. Lomasney, D.M. Hayden-Hixson, U.B. Schambra, M.G. Caron, R.J. Lefkowitz, and R.T. Freneau, Jr. 1994. Distribution of alpha 2-adrenergic receptor subtype gene expression in rat brain. *Mol. Brain Res.* 21: 133–149.
  16. MacDonald, E., and M. Scheinin. 1995. Distribution and pharmacology of alpha<sub>2</sub>-adrenoceptors in the central nervous system. *J. Physiol. Pharmacol.* 46:241–258.
  17. Standifer, K.M., C.C. Chien, C. Wahlestedt, G.P. Brown, and G.W. Pasternak. 1994. Selective loss of delta opioid analgesia and binding by antisense oligodeoxynucleotides to a delta opioid receptor. *Neuron*. 12:805–810.
  18. Wahlestedt, C., E.M. Pich, G.F. Koob, F. Yee, and M. Heilig. 1993. Modulation of anxiety and neuropeptide Y-Y1 receptors by antisense oligonucleotides. *Science (Wash. DC)*. 259:528–531.
  19. Wahlestedt, C., E. Golanov, S. Yamamoto, F. Yee, H. Ericson, H. Yoo, C.E. Inturrisi, and D.J. Reis. 1993. Antisense oligodeoxynucleotides to NMDA-R1 receptor channel protect cortical neurons from excitotoxicity and reduce focal ischaemic infarctions. *Nature (Lond.)*. 363:260–263.
  20. Rossi, G., Y.X. Pan, J. Cheng, and G.W. Pasternak. 1994. Blockade of morphine analgesia by an antisense oligodeoxynucleotide against the mu receptor. *Life Sci.* 54:375–379.
  21. Akabayashi, A., C. Wahlestedt, J.T. Alexander, and S.F. Leibowitz. 1994. Specific inhibition of endogenous neuropeptide Y synthesis in arcuate nucleus by antisense oligonucleotides suppresses feeding behavior and insulin secretion. *Mol. Brain Res.* 21:55–61.
  22. Duzic, E., and S.M. Lanier. 1992. Factors determining the specificity of signal transduction by guanine nucleotide-binding protein-coupled receptors. *J. Biol. Chem.* 267:24045–24052.
  23. Mizobe, T., M. Maze, V. Lam, S. Suryanarayana, and B. Kobilka. 1996. Arrangement of transmembrane domains in adrenergic receptors. Similarity to bacteriorhodopsin. *J. Biol. Chem.* 271:2387–2389.
  24. Lowry, O.H., M.J. Rosebrough, A.L. Farr, and R.J. Randall. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* 193:265–275.
  25. Adler, C.H., E. Meller, and M. Goldstein. 1985. Recovery of  $\alpha_2$ -adrenoceptor binding and function after irreversible inactivation by *n*-ethoxycarbonyl-2-ethoxy-1,2-dihydroxyquinoline (EEDQ). *Eur. J. Pharmacol.* 116:175–178.
  26. Stein, C.A., and Y.C. Cheng. 1993. Antisense oligonucleotides as therapeutic agents — is the bullet really magical? *Science (Wash. DC)*. 261:1004–1012.
  27. Milligan, J.F., M.D. Matteucci, and J.C. Martin. 1993. Current concepts in antisense drug design. *J. Med. Chem.* 36:1923–1937.
  28. Mahan, L.C., R.M. McKernan, and P.A. Insel. 1987. Metabolism of  $\alpha$ - and  $\beta$ -adrenergic receptors in vitro and in vivo. *Annu. Rev. Pharmacol. Toxicol.* 27:215–235.
  29. Rabin, B.C., K. Reid, T.Z. Guo, E. Gustafsson, C. Zang, and M. Maze. 1996. The sympatholytic and MAC-sparing responses are preserved in rats rendered tolerant to the hypnotic and analgesic action of dexmedetomidine, a selective  $\alpha_2$  adrenergic agonist. *Anesthesiology*. In press.
  30. Link, R.E., K. Desai, L. Hein, M.S. Stevens, A. Chruscinski, D. Bernstein, G.S. Barsh, and B.K. Kobilka. 1996. Cardiovascular regulation in mice lacking alpha-2-adrenoceptor subtypes B and C. *Science (Wash. DC)*. In press.
  31. Bousquet, P., J. Feldman, E. Tibirica, G. Bricca, H. Greney, M. Dontenwill, J. Stutzmann, and A. Belcourt. 1992. Imidazoline receptors: a new concept in central regulation of the arterial blood pressure. *Am. J. Hypertens.* 5:47S–50S.