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# Molecular Genetic Technology Defines the Receptor Subtypes Responsible for Anesthetic and Cardiovascular Responses to alpha-2 Adrenergic Agonists

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**Abstract**

The analgesic effects of $\alpha_2$ adrenergic agonists are mediated by activation of $\alpha_2$ adrenoceptor in the locus coeruleus (LC) and spinal cord (SC). It is not known which of the three $\alpha_2$ adrenoceptor subtypes is responsible for the analgesic effects. Using a gene-targeting strategy, it is possible to determine their role in the analgesic action of dexmedetomidine, an $\alpha_2$ agonist. The guide cannulae were sited stereotactically in the LC or intrathecally in male Sprague-Dawley rats. The antinociceptive effect of dexmedetomidine was measured using the tail flick latency (TFL) response. Separate cohorts of rats were administered the ODNs directed against either the $\alpha_{2A}$ or $\alpha_{2C}$ adrenoceptor subtypes which are present in the CNS. Control rats were treated with scrambled ODNs or saline. The TFL response to dexmedetomidine was measured, before, immediately after, and 8 days after the ODN administration. Immediately following $\alpha_{2A}$ ODN treatment, the TFL response to dexmedetomidine, either LC or SC, was significantly attenuated; this recovered to the pretreatment value after 8 days. $\alpha_{2C}$ ODN treatment did not change the analgesic response. The analgesic response to morphine was unaffected by $\alpha_{2A}$ ODN treatment. These data strongly suggest that the $\alpha_{2A}$ adrenoceptor subtype mediates the analgesic responses to dexmedetomidine both in the LC and in the SC.

**Key words**: Adrenoceptor, $\alpha_2$ adrenergic agonists, Analgesia, Dexmedetomidine

## Introduction

$\alpha_2$ adrenergic agonists are efficacious, cardiovascular, anesthetic, and analgesic agents. However, the $\alpha_2$ agonists do produce a variety of other pharmacologic actions affecting almost every organ system, especially the cardiovascular system causing quite troubling side-effects including hypertension, hypotension, and bradycardia[1]. This plethora of $\alpha_2$ adrenoceptor-mediated responses is due both to the ubiquity of this receptor class as well as to the existence of different receptor subtypes[2,3].

More than twenty years ago, Langer[4] proposed that alpha adrenergic agonists such as clonidine were acting at a distinct presynaptic $\alpha_2$ adrenoceptor to produce its sympatholytic effect. More recently it has become apparent that $\alpha_2$ adrenoceptors can be located both pre and post-synaptically and that there are three distinct $\alpha_2$ adrenoceptor subtypes, initially demonstrated by pharmacologic means and subsequently confirmed by molecular genetic techniques[5].

Molecular genetic cloning studies in humans, rats and mice have shown that genes encode three distinct $\alpha_2$ adrenergic receptor subtypes (Table 1). While phar-

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Table 1 Classification of $\alpha_2$ adrenoceptors

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|---|---|---|---|
| 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 |

macological studies have defined four subtypes named $\alpha_{2A}$, $\alpha_{2B}$, $\alpha_{2C}$, and $\alpha_{2D}$, $\alpha_{2D}$ represents a species homologue of the human $\alpha_{2A}$[6](In this manuscript the term $\alpha_{2A}$ will be used to designate the $\alpha_{2A/D}$ subtype). While each of the three $\alpha_2$ adrenergic receptor subtypes are distributed in the central nervous system[7~15], the $\alpha_{2A}$ and $\alpha_{2C}$ subtypes predominate while the $\alpha_{2B}$ subtype is sparsely represented in the CNS and only in the diencephalon[16].

The therapeutic index of $\alpha_2$ agonists may be enhanced by developing strategies which avoid the unwanted actions while sustaining the desirable properties. If the salubrious properties are mediated by a different receptor subtype from that which mediates the undesirable effects, synthesis of subtype-selective $\alpha_2$ agonists may represent a strategy for enhancing the therapeutic index of $\alpha_2$ agonists. Pharmacologic[17] and molecular genetic[18] schemes represent two approaches that can be used to assign, unequivocally, an $\alpha_2$ action to a particular subtype. Unfortunately, there are no truly selective pharmacologic ligands, neither agonists nor antagonists, which can be used to probe the individual receptor subtypes.

Recently, we and others have reported on the assignment of receptor subtype to individual responses using gene-targeting strategies. Kobilka's group[19] has reported on cardiovascular studies of mice in which either the $\alpha_{2B}$ or $\alpha_{2C}$ adrenoceptor had been "knocked out." The $\alpha_{2B}$ knockout mice did not exhibit the acute hypertensive response to a bolus dose of dexmedetomidine. No cardiovascular phenotype was observed in the $\alpha_{2C}$ knockout mice. Using transgenic mice with dysfunctional $\alpha_{2A}$ adrenoceptors, Limbird's group was able to demonstrate that the $\alpha_{2A}$ adrenoceptor mediates the bradycardic and hypotensive effects[20].

We have addressed the anesthetic properties of the $\alpha_2$ agonist, dexmedetomidine, and reported that the $\alpha_{2A}$ adrenoceptor subtype mediated the hypnotic response in the locus coeruleus of the rat[18]. Subsequently, using transgenic mice we confirmed that the $\alpha_{2A}$ adrenoceptor subtype was the anesthetic site of action for this species too[21].

The $\alpha_2$ agonists exert their analgesic properties by activating $\alpha_2$ adrenoceptors in the neuraxis both supraspinally[22] and in the spinal cord[23], also $\alpha_2$ agonists can produce pain relief at sites outside of the central nervous system[24,25]. Unlike opioids, the $\alpha_2$ agonists do not exhibit potentially dangerous side-effects such as physical dependence and respiratory depression[26]. Using a gene-targeting strategy we now report on the $\alpha_2$ adrenoceptor subtype responsible for the analgesic properties of dexmedetomidine in the locus coeruleus and spinal cord of the rat.

## Methods

*Synthesis of oligonucleotides*

The following phosphodiester oligodeoxynucleotides (ODNs) were synthesized on ABI 394 and ABI 380B DNA Synthesizer by use of phosphoramidite chemistry (PAN Facility, Stanford, CA).

rat $\alpha_{2A}$ ODNs, 5'-ATG, GGC, TCC, CTG, CAG, CCG, GAT-3';

rat $\alpha_{2A}$ scrambled ODNs, 5'-CGA, GTT, GCC, TCA, AGC, GGT, CGC-3';

rat $\alpha_{2C}$ ODNs, 5'-ATG, GCG, TCC, CCA, GCG, CT-3';

rat $\alpha_{2C}$ scrambled ODNs, 5'-GGC, CTC, ACT, GCG, ACG, TC-3'

These represent sequences in the region immediately following the initiation codon of rat $\alpha_{2A}$, and $\alpha_{2C}$ adrenoceptor subtypes. "Scrambled" ODNs contained the same nucleotides, but rearranged, and were used as controls for a non-specific ODN effect. Oligodeoxynucleotides (ODNs), possess unique sequences relative to the entire genome and confer a degree of specificity which is lacking in conventional pharmacologic probes[27]. Earlier we showed that ODNs directed against the $\alpha_{2A}$ subtype resulted in a significant decrease in receptor expression (as defined by radiolabeled ligand binding) for $\alpha_{2A}$ with no

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decrease in expression for $\alpha_{2C}$[18]. "Scrambled" ODNs did not affect receptor expression. Similarly, ODNs directed against the $\alpha_{2C}$ subtype selectively decreased expression only of $\alpha_{2C}$ receptor subtype[18].

*Cannulation for drug injection*

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 250-350g 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.

Locus coeruleus (LC) and intrathecal cannulations were performed as described before[22]. Briefly, halothane-anesthetized rats were placed in a stereotactic apparatus and the left LC was penetrated with a 24-G stainless steel cannula using coordinates according to the atlas of Paxinos and Watson[28]. The cannula was fixed in position with methylmethacrylate resin, and the animal was allowed to recover for 3 days before the experiment. The correct placement of the cannula was established by demonstrating that dexmedetomidine, 3.5 $\mu$g / 0.2 $\mu$l, produced loss of righting reflex (LORR). Only rats in which the previous administration of dexmedetomidine through the cannula resulted in LORR were used for subsequent studies.

For intrathecal cannulation, animals were anesthetized with halothane, an incision was made over the cervical spine, and a small puncture made in the dura mater. Polyethylene tubing (0.28 mm internal diameter) was threaded into the intrathecal space, 8.5 cm, so that the tip of the catheter was positioned at the lumbar enlargement. The tubing was sutured in place, and the skin sutured together over the tubing. The animal was allowed to recover for 3 days before the definitive experiment was initiated.

*ODN administration*

LC-cannulated rats received 3 injections on alternate days (day 1, 3, 5), with 5 nmol/0.2 $\mu$l of phosphodiester ODNs, its "scrambled" control ODNs or 0.2 $\mu$l saline. Intrathecally-cannulated rats rceived 3 injections, on alternate days (day 1, 3, 5), with 5 nmol/10 $\mu$l of phosphodiester ODN, its "scrambled" control ODNs or 10 $\mu$l saline. These ODN treatment and recovery periods were selected based on the known half-life of the $\alpha_2$ adrenergic receptor in the cerebral cortex[29].

*Analgesia Testing*

Analgesic response was measured by the tail-flick latency response. A high intensity light was focused on the rat's tail and the time for the rat to move its tail out of the light beam was automatically recorded (Tail-flick apparatus, Columbus Instruments, Columbus, OH) and referred to as tail-flick latency. A different patch of the tail was exposed to the light beam on each trial to minimize the risk of tissue damage. The animals were placed on the heating blanket to maintain the body and tail temperature during the experiment. A cut-off time of 10 sec was predetermined, at which time the trial was terminated if no response occurred. Each tail flick latency data point consisted of a mean of three trials on an individual animal. Data are expressed as maximum percent effect (MPE) according to the following formula:

$$\text{MPE}\,(\%) = \frac{(\text{Postdrug latency}) - (\text{basal latency})}{(\text{Cut-off latency}) - (\text{basal latency})} \times 100\%$$

The analgesic response to an $ED_{50}$ dose of dexmedetomidine was performed on three separate occassions, namely before administration of ODNs, immediately after the last administration of ODNs, and 8 days after the last administration of ODNs.

To establish that the ODNs were acting at the receptor level to attenuate the analgesic response to dexmedetomidine, the analgesic effect of the opiate narcotic, morphine, was tested in rats treated with $\alpha_{2A}$ ODNs.

*Statistical analysis*

The data are expressed as mean $\pm$ SEM. The results are analyzed by two-way analysis of variance with repeated measures, followed by post-hoc Scheffe' test. $P < 0.05$ is considered statistically significant.

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## Results

Dexmedetomidine, 3.5 μg/0.2 μl, LC, produced a similar %MPE in each cohort before ODN treatment. Immediately after $\alpha_{2A}$ ODN treatment the % MPE was significantly attenuated (Figure 1); the responses in the other treatment groups were unchanged. Following an 8 day recovery period after $\alpha_{2A}$ ODN treatment, the analgesic response to dexmedetomidine had recovered to the pretreatment values. The analgesic response to dexmedetomidine, 3.5 μg / 0.2 μl, LC, did not change after $\alpha_{2C}$ ODN treatment (Figure 2).

Dexmedetomidine, 1 μg/10 μl, intrathecal, produced a similar %MPE in each cohort before ODN treatment. Immediately following $\alpha_{2A}$ ODN treatment the %MPE was significantly attenuated (Figure 3); the other treatment groups were unchanged. Following an 8 day recovery period after $\alpha_{2A}$ ODN treatment, the analgesic response to dexmedetomidine had recovered to the pretreatment values. The analgesic response to dexmedetomidine, 1 μg / 10 μm, intrathecal, did not change after $\alpha_{2C}$ ODN treatment (Figure 4).

The analgesic response to morphine, 0.65 μg/10 μl intrathecally (determined to be the $\approx ED_{50}$ dose in pilot studies), which had produced a similar %MPE in both cohorts before ODN treatment, was unaffected by $\alpha_{2A}$ ODN treatment (Figure 5).

## Discussion

$\alpha_{2A}$ ODNs selectively, and reversibly, attenuated the analgesic response to dexmedetomidine whether administered supraspinally (Figure 1), or spinally (Figure 3). These data suggest that the $\alpha_{2A}$ adrenoceptor subtype is involved in the analgesic

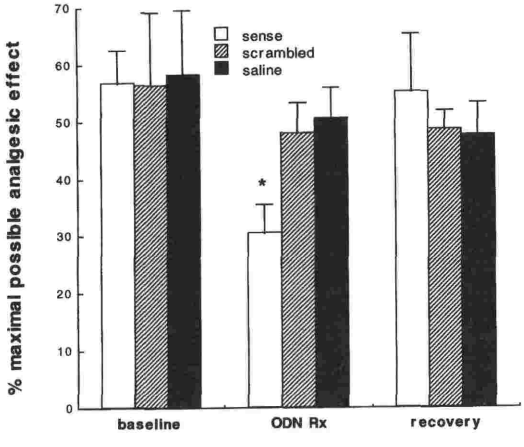


**Figure 1** Effect of oligodeoxynucleotides (ODNs) directed against the $\alpha_{2A}$ adrenoceptor subtype on antinociceptive response to dexmedetomidine in the locus coeruleus.

Three cohorts of rat littermates, were stereotactically-cannulated, siting the tip of the needle in the locus coeruleus (LC). The antinociceptive response was represented by the percent of maximum possible prolongation of the tail flick latency. The antinociceptive response to dexmedetomidine, 3.5 μg, LC, was assessed before (baseline), immediately after (ODN Rx) , and 8 days after (recovery) administering either $\alpha_{2A}$ ODN (n=5; 5 nmol/0.2 μl), $\alpha_{2A}$ "scrambled ODNs" (n=5; 5 nmol/0.2 μl), or saline (n=5; 0.2 μl) three times, on days 1, 3, and 5. Data are expressed as mean ± SEM. *p<0.05 when compared to "baseline" and "recovery" ODN treatment period.

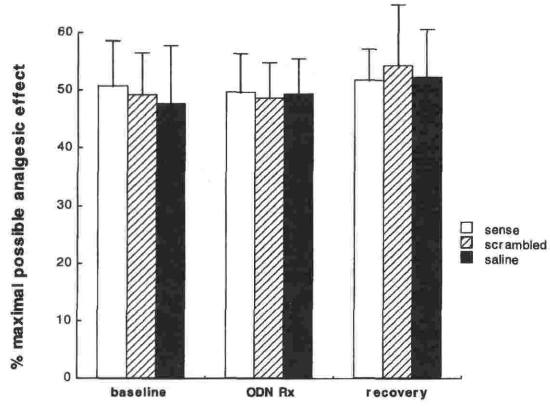


**Figure 2** Effect oligodeoxynucleotides (ODNs) directed against the $\alpha_{2C}$ adrenoceptor subtype on antinociceptive response to dexmedetomidine LC.

Three cohorts of rat littermates, were stereotactically-cannulated, siting the tip of the needle in the locus coeruleus (LC). The antinociceptive response was represented by the percent of maximum possible prolongation of the tail flick latency. The antinociceptive response to dexmedetomidine, 3.5 μg, LC, was assessed before (baseline), immediately after ( ODN Rx) , and 8 days after (recovery), administering either $\alpha_{2C}$ ODN (n=6; 5 nmol/0.2 μl), $\alpha_{2C}$ "scrambled" ODN (n=5; 5 nmol/0.2 μl), or saline (n=5; 0.2 μl) three times, on days 1, 3, and 5. Data are expressed as mean±SEM.

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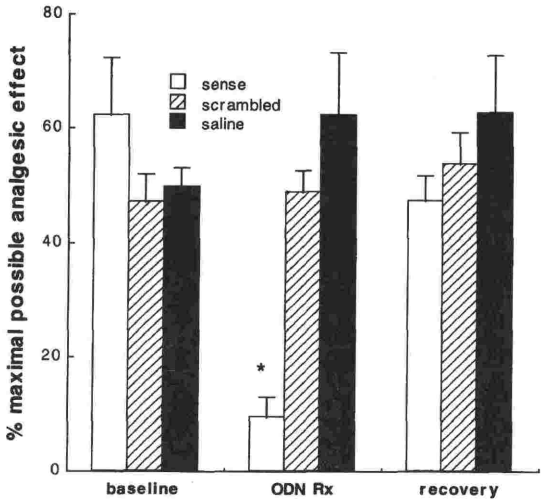


**Figure 3** Effect of oligodeoxynucleotides (ODNs) directed against the $\alpha_{2A}$ adrenoceptor subtype on antinociceptive response to dexmedetomidine in the spinal cord (SC).

Three cohorts of rat littermates, were stereotactically-cannulated, siting the tip of the catheter at the lumbar enlargement of the intrathecal space. The antinociceptive response was represented by the percent of maximum possible prolongation of the tail flick latency. The antinociceptive response to dexmedetomidine, 1.0 $\mu$g, SC, was assessed before (baseline), immediately after (ODN Rx) , and 8 days after (recovery), administering either $\alpha_{2A}$ ODN (n=5; 5 nmol/10 $\mu$l), $\alpha_{2A}$ "scrambled" (n=4; 5 nmol/10 $\mu$l), or saline (n=4; 10 $\mu$l) three times, on days 1, 3, and 5. Data are expressed as mean $\pm$ SEM. * $p<0.01$ when compared to "baseline" and "recovery" from ODN treatment period.

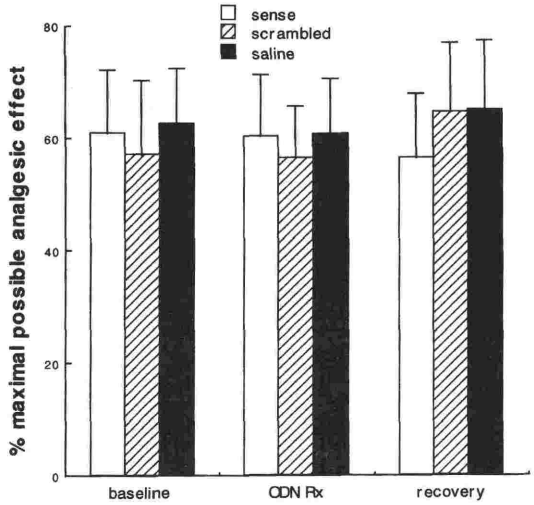


**Figure 4** Effect of $\alpha_{2C}$ oligodeoxynucleotides (ODNs) on antinociceptive response to dexmedetomidine in the spinal cord (SC).

Three cohorts of rat littermates, were stereotactically-cannulated, siting the tip of the catheter at the lumbar enlargement of the intrathecal space. The antinociceptive response was represented by the percent of maximum possible prolongation of the tail flick latency. The antinociceptive response to dexmedetomidine, 1.0 $\mu$g, SC, was assessed before (baseline), immediately after (ODN Rx) , and 8 days after (recovery), administering either $\alpha_{2C}$ ODN (n=5; 5 nmol/10 $\mu$l), $\alpha_{2C}$ "scrambled ODN" (n=5; 5 nmol/10 $\mu$l), or saline (n=5; 10 $\mu$l) three times, on days 1, 3, and 5. Data are expressed as mean $\pm$ SEM.

responses to $\alpha_2$ agonists both in the LC and in the spinal cord. We were unable to prove that treatment with the $\alpha_{2A}$ ODNs reversibly changed the expression of $\alpha_{2A}$ adrenoceptor subtype in the LC and in the spinal cord since neither subtype-selective radiolabeled ligands, nor subtype-selective antibodies are available to perform the appropriate confirmatory test, namely, receptor subtype binding studies and in situ quantitative immunocytochemistry, respectively.

In lieu of definitive evidence for altered expression of $\alpha_{2A}$ adrenoceptors following $\alpha_{2A}$ ODN treatment, there exists considerable circumstantial evidence. Firstly, we earlier demonstrated that these same ODNs selectively decreased receptor expression in vitro[18]. Secondly, only $\alpha_{2A}$ ODNs and not the "scrambled" ODNs diminished receptor expression in vitro and analgesic responses in vivo precluding the possibility that the behavioral changes are due to a non-specific ODN effect. Next, the behavioral effect are reversible over a time-course which is consistent with new receptor expression[29]. Also, morphine and $\alpha_2$ adrenergic agonists, which share the same signal transduction mechanism for analgesia in the spinal cord[30] are differentially affected by $\alpha_{2A}$ ODN treatment (Figures 1, 5). This final point suggests that the attenuated analgesic response to $\alpha_2$ agonists is due to an alteration in the $\alpha_{2A}$ adrenoceptor itself and not due to a change in its post-receptor signal transduction

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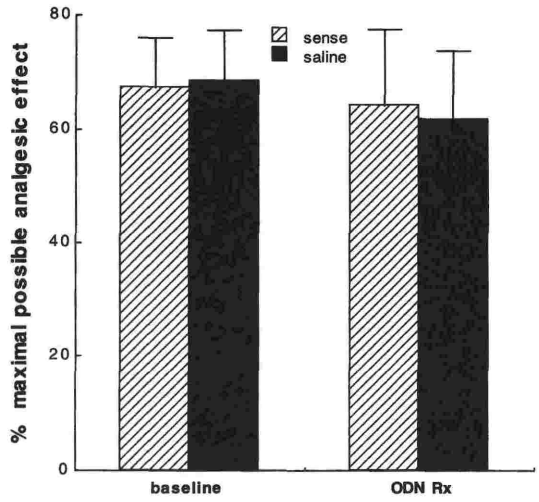


**Figure 5** Effect of $\alpha_{2A}$ oligodeoxynucleotides (ODNs) on antinociceptive response to morphine in the spinal cord (SC).

Two cohorts of rat littermates, were stereotactically-cannulated, siting the tip of the catheter at the lumbar enlargement of the intrathecal space. The antinociceptive response was represented by the percent of maximum possible prolongation of the tail flick latency. The antinociceptive response to morphine, 0.65 $\mu$g, SC, was assessed before (baseline), and immediately after (ODN Rx) administering either $\alpha_{2A}$ ODN (n=7; 5 nmol/10 $\mu$l), or saline (n=6; 10 $\mu$l) three times, on days 1, 3, and 5. Data are expressed as mean±SEM.

mechanism.

In studies using pharmacologic probes, Millan's group suggested that antinociceptive responses are mediated by $\alpha_{2A}$ adrenoceptors[31]. Although their experimental paradigm differed with respect to species (mice vs rats) and nociceptive test (writhing and hot-plate vs tail-flick latency), we have corrobrated their conclusions. Initially, Wikberg's group suggested that only the $\alpha_{2A}$ adrenoceptor subtype was expressed in the spinal cord[32], recently, Yaksh suggested that an additional $\alpha_2$ adrenergic receptor is also capable of mediating the antinociceptive response to $\alpha_2$ agonists[33]. Perl suggested that only $\alpha_{2A}$ adrenergic receptor subtype is expressed in dorsal root ganglion cells[34]. We can find no role for an antinociceptive response to dexmedetomidine via the $\alpha_{2C}$ adrenergic receptor subtype. This does not preclude the possibility that the antinociceptive effect of a different $\alpha_2$ agonist measured by a different paradigm may act via a different receptor subtype.

The $\alpha_2$ adrenergic agonists may be effective analgesics for both acute and chronic pain states. However, none of the clinically-available $\alpha_2$ agonists can discriminate between the three $\alpha_2$ adrenoceptor subtypes. This relative non-specificity may be the cause of troublesome side-effects such as hypertension. The acute hypertension that follows rapid bolus administration of $\alpha_2$ agonists is probably due to activation of the $\alpha_{2B}$ adrenoceptor[19], a subtype which is not found to any great extent in the neuraxis of rodents[12]. If our data are extrapolatable to humans then the full potential of this drug class for analgesia may only be realized once ligands which have specificity for the receptor subtype(s) responsible for the salubrious effects thereby avoiding sites responsible of producing side-effects. Therefore, it is important to define, unequivocally, the receptor subtype(s) responsible for the anesthesia-related properties. This current study, taken together with the recent "knockout" and "transgenic" studies, suggests that the $\alpha_{2A}$ adrenoceptor subtype could be the target for subsequent drug development of $\alpha_{2A}$ agonists for analgesic use.

## References

1) Dyck JB, Maze M, Haack C, et al : The pharmacokinetics and hemodynamic effects of intravenous and intramuscular dexmedetomidine hydrochloride in adult human volunteers. Anesthesiology 78 : 813-820, 1993
2) Mizobe T, Maze M : $\alpha_2$ adrenoceptor agonists and anesthesia. Int Anesthesiol Clin 33 : 81-102, 1995
3) Mizobe T, Maze M : Molecular pharmacology of $\alpha_2$ adrenoceptors: relevance to anesthesia. Annual of Anesthetic Pharmacology 1 : 1-26, 1997
4) Langer SZ : Presynaptic regulation of catecholamine release. Biochem Pharmacol 23 : 1793-1800, 1974
5) Bylund DB : Subtypes of alpha 2 adrenoceptors: Pharmacological and molecular biological evidence converge. Trends Pharmacol Sci 9 : 356-361, 1988
6) Renouard A, Widdowson PS, Millan MJ : Multiple alpha-2 adrenergic receptor subtypes. I. Comparison of [3H]-RX821002-labeled rat alpha-2A adrenergic receptors in cerebral cortex to human alpha-2A adrenergic receptor and other populations of alpha-2 adrenegic subtypes. J Pharmacol Exp Ther 270 : 946-957, 1994
7) Ordway GA, Jaconetta SM, Halaris AE : Characterization

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of the subtypes of alpha-2 adrenoceptors in the human brain. J Pharmacol Exp Ther 264：967-976, 1993
8) Wamsley JK, Alburges ME, Hunt MAE, et al：Differential localization of $\alpha_2$-adrenergic receptor subtypes in brain. Pharmacol Biochem Behav 41：267-273, 1992
9) Nicholas AP, Pieribone V, Hokfelt T：Distribution of mRNAs for alpha-2 adrenergic receptor subtypes in rat brain: An in situ hybridization study. J Comparative Neurol 328：575-594, 1993
10) Lawhead RG, Blaxall HS, Bylund DB：Alpha2A is the predominant $\alpha_2$-adrenergic receptor subtype in human spinal cord. Anesthesiology 77：983-991, 1992
11) Uhlen S, Wikberg JES：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 3H-RX821002 ligand binding. Pharmacol Toxicol 69：341-350, 1991
12) Zeng D, Lynch KR：Distribution of $\alpha_2$- adrenergic receptor mRNA in the rat CNS. Mol Brain Res 10：219-225, 1991
13) Handy DE, Flordellis CS, Bogdanova NN, et al：Diverse tissue expression of rat $\alpha_2$- adrenergic receptor genes. Hypertension 21：861-865, 1993
14) De Vos H, Vauquelin G, De Keyser J, et al：Regional distribution of $\alpha_{2A}$- and $\alpha_{2B}$- adrenoceptor subtypes in postmortem hunam brain. J Neurochem 58：1555-1560, 1992
15) Scheinin M, Lomasney JW, Hayden-Hixson DM, et al：Distribution of alpha 2-adrenergic receptor subtype gene expression in rat brain. Mol Brain Res 21：133-149, 1994
16) MacDonald E, Scheinin M：Distribution and pharmacology of alpha 2-adrenoceptors in the central nervous system. J Physiol Pharmacol 46：241-258, 1995
17) Nacif-Coelho C, Lee D, Guo TZ, et al：Dexmedetomidine-induced hypnosis is mediated by the adrenoceptor subtype in the locus coeruleus of the rat. (Abstract) Anesthesiology 79A：789, 1993
18) Mizobe T, Maghsoudi K, Sitwala K, et al：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. J Clin Invest 98：1076-1080, 1996
19) Link RE, Desai K, Hein L, et al：Cardiovascular regulation in mice lacking alpha-2-adrenoceptor subtypes B and C. Science 273：803-805, 1996
20) MacMillan LB, Hein L, Smith MS, et al：Central hypotensive effects of the alpha 2a-adrenergic receptor subtype. Science 273：801-803, 1996
21) Lakhlani PP, MacMillan LB, Guo TZ, et al：Substitution of a mutant alpha 2A-adrenergic receptor via "hit and run" gene targeting reveals the role of this subtype in sedative, analgesic, and anesthetic-sparing responses in vivo. Proc Natl Acad Sci U S A 94：9950-9955, 1997
22) Guo TZ, Buttermann AE, Jiang JY, et al：Dexmedetomidine injection into the locus coeruleus produces analgesia. Anesthesiology 84：873-881, 1996
23) Takano Y, Yaksh TL：Chronic spinal infusion of dexmedetomidine, ST-91 and clonidine: spinal alpha 2 adrenoceptor subtypes and intrinsic activity. J Pharmacol Exp Ther 264：327-335, 1993
24) Davis KD, Treede RD, Raja SN, et al：Topical application of clonidine relieves hyperalgesia in patients with sympathetically maintained pain. Pain 47：309-317, 1991
25) Khasar SG, Green PG, Chou B, et al：Peripheral nociceptive effects of alpha 2 adrenergic receptor agonists in the rat. Neuroscience 66：427-432, 1995
26) Jarvis DA, Duncan S, Segan IS：Ventilatory effects of clonidine alone, and the presence of alfentanil, in human volunteers. Anesthesiology 76：899-905, 1992
27) Stein CA, Cheng YC：Antisense oligonucleotides as therapeutic agents - is the bullet really magical? Science 261：1004-1012, 1993
28) Paxinos G, Watson C：The Rat Brain in Stereotaxic Coordinates. London, Academic Press, pp.54-59, 1986
29) Mahan LC, McKernan RM, Insel PA：Metabolism of a- and b- adrenergic receptors in vitro and in vivo. Ann Rev Pharmacol Toxicol 27：215-235, 1987
30) Kanjhan R：Opioids and pain. Clin Exp Pharmacol Physiol 22：397-403, 1995
31) Millan MJ, Bervoets K, Rivet JM, et al：Multiple alpha 2 adrenergic receptor subtypes. II. Evidence for a role of rat alpha 2A adrenergic receptors in the control of nociception, motor behavior and hippocampal synthesis of noradrenaline. J Pharmacol Exp Ther 270：958-972, 1994
32) Uhlen S, Wikberg JES：Rat spinal cord alpha2-adrenoceptors are of the alpha 2A subtype; Comparison of alpha 2A and alpha 2B adrenoceptors in rat spleeen, cerebral cortex, and kidney using [3H] 821002 ligand binidng. Pharmacol and Toxicol 69：341-350, 1991
33) Takano Y, Takano M, Yaksh TL：The effect of intrathecally administered imiloxan and WB4101: Possible role of alpha 2-adrenoceptor subtypes in the spinal cord. European J Pharmacol 219：465-468, 1992
34) Perl ER：A reevaluation of mechanisms leading to sympathetically related pain, Pharmacological Apporaches to the treatment of chronic pain: New Concepts and Critical Issues. Edited by Fields HL, Liebeskind JC. Series: Progress in Pain Research and Management. Volume 1. Seattle, IASP Press, pp.129-150, 1994

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