In Vivo Gene Modification Elucidates Subtype-Specific Functions of $\alpha_2$-Adrenergic Receptors

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ABSTRACT

Mice with altered $\alpha_2$-adrenergic receptor genes have become important tools in elucidating the subtype-specific functions of the three $\alpha_2$-adrenergic receptor subtypes because of the lack of sufficiently subtype-selective pharmacological agents. Mice with a deletion (knockout) of the $\alpha_{2A}$-, $\alpha_{2B}$-, or $\alpha_{2C}$-gene as well as a point mutation of the $\alpha_{2A}$-gene ($\alpha_{2A}^{D79N}$) and a 3-fold overexpression of the $\alpha_{2C}$-gene have been generated. Studies with these mice indicate that most of the classical functions mediated by the $\alpha_2$-adrenergic receptor, such as hypotension, sedation, analgesia, hypothermia, and anesthetic-sparing effect, are mediated primarily by the $\alpha_{2A}$-subtype. The $\alpha_{2C}$-subtype is the principal mediator of the hypertensive response to $\alpha_2$-agonists, appears to play a role in salt-induced hypertension, and may be important in developmental processes. The $\alpha_{2C}$-subtype appears to be involved in many central nervous system processes such as the startle reflex, stress response, and locomotion. Both the $\alpha_{2A}$- and $\alpha_{2C}$-subtypes are important in the presynaptic inhibition of norepinephrine release and appear to have distinct regulatory roles. The ability to study subtype-specific functions in different mouse strains by altering the same $\alpha_2$-adrenergic receptor in different ways strengthens the conclusions drawn from these studies. Although these genetic approaches have limitations, they have significantly increased our understanding of the functions of $\alpha_2$-adrenergic receptor subtypes.
Mice with Genetically Engineered α₂-Adrenergic Receptor Subtypes

Several recent reviews have discussed the methods, advantages, and limitations of genetic engineering techniques (Wei, 1997; Rohrer and Kobilka, 1998; Yanez and Porter, 1998). There are now published reports on five mouse strains with genetic alterations of α₂-adrenergic receptor expression. Mice with a deletion of the α₂A- (α₂A-knockout [KO]), α₂B- (α₂B-KO), or α₂C-gene (α₂C-KO) have been generated (Link et al., 1995, 1996; Altman et al., 1999). More recently, the double knockout mice (α₂AC-KO), in which both the α₂A- and the α₂C-genes have been deleted, have been produced (Hein et al., 1999). Mice have also been developed with a point mutation of the α₂A-gene (α₂A-D79N) (Macmillan et al., 1996). This mutation of the aspartate to an asparagine residue at position 79 in the second transmembrane domain of the α₂A-adrenergic receptor selectively uncouples the receptor from the activation of K⁺ channels in vitro, although coupling to Ca²⁺ channels and adenyllyl cyclase activity is maintained (Surprenant et al., 1992). It was expected that the expression of this mutation in the intact animal would provide insight into the signal transduction mechanisms mediating the effects of α₂A-adrenergic receptor stimulation. However, α₂A-D79N mice showed an approximately 80% reduction in α₂A-adrenergic receptor binding despite normal mRNA levels. The receptors that were expressed showed the expected pharmacological characteristics but were unable to couple to K⁺ or Ca²⁺ channels (Lakhiani et al., 1997). Thus, the α₂A-D79N receptor expressed in vivo exhibits distinct characteristics compared with its expression in vitro, and this has served as a functional knockout. All four of the mouse strains described above are viable and appear grossly normal. Apparently, none of the α₂-adrenergic receptor subtypes are absolutely required for embryonic development or adult survival, although one or more of the subtypes may play a role in normal development. In addition to knockout strategies, transgenic techniques have also been applied to α₂-adrenergic receptors, and a strain of mice has been generated in Kobilka’s laboratory with approximately 3-fold overexpression (OE) of the α₂C-gene (α₂C-OE) under the control of its homologous promotor (Sallinen et al., 1997).

Results from experiments using mice with genetic alterations of α₂-adrenergic receptor expression are summarized in Tables 1 and 2. Several complicating factors should be kept in mind when interpreting the results from these experiments. Compensatory changes, such as the up- or down-regulation of another component of a signaling pathway, could offset the loss of a functional receptor in a genetically engineered mouse. These compensatory changes could also be the cause of a phenotype. A phenotype could result from developmental changes rather than from altered expression of a receptor in the adult, or the altered receptor expression could be a distant cause in a complex chain of physiological events. Some of the data obtained with particular animals, however, argue against compensatory changes occurring at least after manipulation of the α₂A-adrenergic receptor subtype (Janumpalli et al., 1998). There can also be remarkable differences in inbred mouse strains, necessitating the use of appropriate wild-type strains in experiments with KO and transgenic mice. Altered expression of a receptor could cause different phenotypes in young and old mice, males and females, different genetic backgrounds, or different environments. Crabbe and coworkers (1999) recently reported that different behavioral phenotypes were found by different laboratories using the same mouse strains, even different phenotypes in the same laboratory at different times, indicating that behavioral experiments in genetically altered mice are particularly vulnerable to variability. Thus, reproducibility is crucial for one to have confidence in the results from modifications of gene expression.

The α₂A-Subtype Mediates the Classical Effects of α₂-Adrenergic Receptor Agonists

Through experiments with the α₂A-KO and α₂A-D79N mice, most of the classical effects of α₂-adrenergic receptor agonists can be attributed to the α₂A-subtype. Mice with a mutated or deleted α₂A-subtype do not exhibit the hypoten-

TABLE 1
Physiological effects of altering α₂-adrenergic receptor gene expression in mice

The α₂A-subtype mediates most of the classical effects of α₂-adrenergic receptor agonists.

<table>
<thead>
<tr>
<th>Physiological Effect</th>
<th>Genetic Alterations</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>α₂A-D79N</td>
</tr>
<tr>
<td>Hypotensive effects of α₂-adrenergic receptor agonist</td>
<td>X</td>
</tr>
<tr>
<td>Bradycardic effects of α₂-adrenergic receptor agonist</td>
<td>↓</td>
</tr>
<tr>
<td>Hypertensive effects of α₂-adrenergic receptor agonist</td>
<td>↓</td>
</tr>
<tr>
<td>Cardiovascular effects of imidazoline agonist</td>
<td>X</td>
</tr>
<tr>
<td>Resting heart rate</td>
<td></td>
</tr>
<tr>
<td>Resting blood pressure</td>
<td></td>
</tr>
<tr>
<td>Salt-induced hypertension</td>
<td></td>
</tr>
<tr>
<td>Sedative effects of dexmedetomidine</td>
<td>X</td>
</tr>
<tr>
<td>Antinociceptive effects of α₂-adrenergic receptor agonist</td>
<td>X/↑</td>
</tr>
<tr>
<td>Antinociceptive effects of moxazidine</td>
<td>↓</td>
</tr>
<tr>
<td>Adrenergic-opiod synergy in spinal antinociception</td>
<td>X</td>
</tr>
<tr>
<td>Anesthetic-sparing effects of dexmedetomidine</td>
<td>X</td>
</tr>
<tr>
<td>Hypothermic effects of dexmedetomidine</td>
<td>X</td>
</tr>
<tr>
<td>Antiepileptogenic effects of endogenous norepinephrine</td>
<td>X</td>
</tr>
<tr>
<td>Presynaptic inhibition of norepinephrine release</td>
<td></td>
</tr>
<tr>
<td>Autoinhibition of locus coeruleus</td>
<td>X</td>
</tr>
</tbody>
</table>

X, abolished; –, no effect; ↑, accentuated; ↓, attenuated; and blank, not studied.

a Depending on site of agonist administration.

b Mice were heterozygous (+/−) for α₂A-null mutation.

c Extent of attenuation depended on test used.

d In α₂AC-double KO mice.
sive, sedative, antinociceptive, anesthetic-sparing, or hypothermic effects in response to \( \alpha_2 \)-adrenergic agonists.

**Hypotensive Effects.** \( \alpha_2 \)-Adrenergic agonists activate \( \alpha_2 \)-receptors in the rostral ventrolateral medulla, decreasing sympathetic outflow, which causes a reduction in arterial blood pressure and heart rate (Guyenet, 1997). In addition to these centrally mediated responses, there is a transient hypertensive response caused by \( \alpha_2 \)-adrenergic receptor-mediated vasoconstriction of vascular smooth muscle. The hypothesis of \( \alpha_2 \)-adrenergic receptor involvement in the centrally mediated cardiovascular responses was based on \( \alpha_2 \)-adrenergic receptor expression in the rostral ventrolateral medulla (Nicholas et al., 1996), and \( \alpha_2 \)-adrenergic receptor involvement was confirmed in \( \alpha_2 B \)-D79N mice. The hypertensive response to administration of \( \alpha_2 \)-adrenergic receptor agonists was abolished, demonstrating that the \( \alpha_2 A \)-subtype plays a principal role in this response (Macmillan et al., 1996). The bradycardic response to agonist also was blunted in \( \alpha_2 A \)-D79N mice (Macmillan et al., 1996). These results have been confirmed in both \( \alpha_2 A \)-D79N and \( \alpha_2 A \)-KO mice (Altman et al., 1999; Zhu et al., 1999). Furthermore, the hypertensive response was abolished in \( \alpha_2 B \)-KO mice, and the hypertensive effect was immediate and accentuated. The bradycardic response in \( \alpha_2 A \)-KO mice was normal, and \( \alpha_2 B \)-KO mice showed no differences from wild-type strains in their hypertensive, hypotensive, and bradycardic effects (Link et al., 1996). The \( \alpha_2 A \)-subtype appears to play a role in vasoconstriction at least in some vascular compartments because the hypertensive response in \( \alpha_2 A \)-D79N mice was absent when the agonist was administered through the femoral artery (Macmillan et al., 1996). These results demonstrate that the \( \alpha_2 A \)-adrenergic receptor mediates the hypertensive and bradycardic effects of \( \alpha_2 \)-adrenergic agonists. In contrast, the \( \alpha_2 B \)-adrenergic receptor appears to be the main mediator of the pressor response that results from \( \alpha_2 \)-adrenergic agonist administration.

Considering the role of this receptor in cardiovascular function, it was surprising that \( \alpha_2 A \)-D79N mice do not show any cardiovascular abnormalities. Recent evidence has indicated that \( \alpha_2 A \)-D79N mice do retain some \( \alpha_2 \)-adrenergic receptor function. In contrast to \( \alpha_2 A \)-D79N mice, \( \alpha_2 A \)-KO mice have tachycardia, higher systolic blood pressure, and higher plasma norepinephrine levels (Altman et al., 1999; Makaritsis et al., 1999b). Propranolol, a \( \beta \)-adrenergic receptor antagonist, eliminated the difference in heart rate between \( \alpha_2 A \)-KO and wild-type mice, demonstrating that the tachycardia in \( \alpha_2 A \)-KO mice was due to increased sympathetic tone, presumably resulting from increased norepinephrine release because of the absence of \( \alpha_2 \)-adrenergic presynaptic inhibition (Altman et al., 1999).

**Sedative Effects.** The sedative effects of dexmedetomidine were examined in \( \alpha_2 A \)-D79N mice by Rotarod, loss of righting reflex (Lakhiani et al., 1997), and spontaneous locomotor activity tests (Hunter et al., 1997). In all cases, \( \alpha_2 A \)-D79N mice showed no sedation in response to dexmedetomidine, indicating that the \( \alpha_2 A \)-adrenergic receptor mediates the sedative effects of \( \alpha_2 \)-agonist administration. In contrast, both the \( \alpha_2 A \)-KO and \( \alpha_2 C \)-KO mice showed dose-dependent reductions in locomotor activity in response to dexmedetomidine that were indistinguishable from wild-type mice (Hunter et al., 1997). \( \alpha_2 \)-Adrenergic agonists appear to induce sedation by activating autoreceptors in the locus coeruleus, reducing its spontaneous rate of firing (Nacif-Coelho et al., 1994). Several lines of evidence have implicated the \( \alpha_2 \)-subtype in this action, including the prominent expression of \( \alpha_2 \)-receptor mRNA and protein in the locus coeruleus seen with in situ hybridization and immunohistochemical studies (Nicholas et al., 1993; Rosin et al., 1993; Wang et al., 1993; Scheinin et al., 1994). In \( \alpha_2 A \)-D79N mice, \( \alpha_2 \)-adrenergic receptor agonists were unable to alter the spontaneous firing rate of locus coeruleus neurons, confirming the role of the \( \alpha_2 \)-subtype (Lakhiani et al., 1997).

**Antinociceptive Effects.** Another therapeutic use of \( \alpha_2 \)-adrenergic receptor agonists is analgesia (Eisenach et al., 1996). The antinociceptive effect of dexmedetomidine has been studied in the ramped hot-plate test as well as in hot-water immersion and intense light tail-flick latency tests. In all of these tests, \( \alpha_2 A \)-D79N mice showed no antinociceptive response to dexmedetomidine (Hunter et al., 1997; Lakhiani et al., 1997). In contrast, dexmedetomidine induced normal dose-dependent antinociception in \( \alpha_2 B \)-KO and \( \alpha_2 C \)-KO mice in the tail immersion test (Hunter et al., 1997). Spinal analgesia was examined in \( \alpha_2 A \)-D79N mice using tail-flick latency tests and the Substance P behavioral test, which uses inhibition of Substance P-induced behaviors as an indirect measure of antinociception. In the tail-flick latency test, both intrathecal brimonidine and clonidine induced dose-depen-

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**TABLE 2**

<table>
<thead>
<tr>
<th>Behavior</th>
<th>Genetic Alteration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Startle reflex</td>
<td>( \alpha_2 C )-KO ↓, ( \alpha_2 C )-OE ↓</td>
</tr>
<tr>
<td>Prepulse inhibition of startle reflex</td>
<td>( \alpha_2 C )-KO ↓, ( \alpha_2 C )-OE ↓</td>
</tr>
<tr>
<td>Latency to attack after isolation</td>
<td>( \alpha_2 C )-KO ↓, ( \alpha_2 C )-OE ↓</td>
</tr>
<tr>
<td>General aggression</td>
<td>( \alpha_2 C )-KO ↓, ( \alpha_2 C )-OE ↓</td>
</tr>
<tr>
<td>Locomotor stimulation of ( \alpha )-amphetamine</td>
<td>( \alpha_2 C )-KO ↓, ( \alpha_2 C )-OE ↓</td>
</tr>
<tr>
<td>1,5-hydroxytryptophan-induced serotonin syndrome</td>
<td>( \alpha_2 C )-KO ↓, ( \alpha_2 C )-OE ↓</td>
</tr>
<tr>
<td>1,5-hydroxytryptophan-induced head twitches</td>
<td>( \alpha_2 C )-KO ↓, ( \alpha_2 C )-OE ↓</td>
</tr>
<tr>
<td>Performance in T-maze</td>
<td>( \alpha_2 C )-KO ↓, ( \alpha_2 C )-OE ↓</td>
</tr>
<tr>
<td>Working memory enhancement of ( \alpha_2 )-adrenergic receptor agonist in T-maze</td>
<td>( \alpha_2 C )-KO ↓, ( \alpha_2 C )-OE ↓</td>
</tr>
<tr>
<td>Performance in Morris water maze</td>
<td>( \alpha_2 C )-KO ↓, ( \alpha_2 C )-OE ↓</td>
</tr>
<tr>
<td>Forced-swim stress and behavioral despair test</td>
<td>( \alpha_2 C )-KO ↓, ( \alpha_2 C )-OE ↓</td>
</tr>
<tr>
<td>Learning and memory in Morris water maze</td>
<td>( \alpha_2 C )-KO ↓, ( \alpha_2 C )-OE ↓</td>
</tr>
<tr>
<td>Anxiety in open field test</td>
<td>( \alpha_2 C )-KO ↓, ( \alpha_2 C )-OE ↓</td>
</tr>
<tr>
<td>Stimulus-response learning in passive avoidance test</td>
<td>( \alpha_2 C )-KO ↓, ( \alpha_2 C )-OE ↓</td>
</tr>
<tr>
<td>Cortical electroencephalogram (arousal)</td>
<td>( \alpha_2 C )-KO ↓, ( \alpha_2 C )-OE ↓</td>
</tr>
</tbody>
</table>

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\( \rightarrow \), no effect; ↑, accentuated; ↓, attenuated; and blank, not studied.
dent antinociception in wild-type but not α_2A-D79N mice (Stone et al., 1997; Fairbanks and Wilcox, 1999). In the Substance P behavioral test, the antinociceptive effect of intrathecal α_2-adrenergic agonists was blunted in α_2A-D79N compared with wild-type mice. Presumably, the remaining antinociceptive effect in α_2A-D79N mice is due to residual α_2A-adrenergic receptor activity, although a small effect due to another subtype cannot be ruled out. Thus, the α_2A-subtype is the predominant subtype involved in the analgesic effects of α_2-adrenergic receptor agonists.

α_2-adrenergic receptors also interact with opioid receptors in mediating the antinociception produced by nitrous oxide. In the tail-flick latency test, nitrous oxide produced dose-dependent antinociception in both wild-type and α_2A-D79N mice. The α_2-adrenergic antagonist yohimbine, the α_2B/α_2C-selective antagonist prazosin, and the opiate antagonist naloxone all inhibited the antinociceptive effect of nitrous oxide in both types of mice (Guo et al., 1999). Thus, the α_2B- and/or α_2C-subtypes seem to mediate the antinociceptive effects of nitrous oxide in conjunction with opioid receptors, although the α_2A-subtype may play a small role. Studies are needed in the α_2B-KO and α_2C-KO mice to determine the role of the α_2B- and α_2C-subtypes in this response.

A possible role for the α_2B- and/or α_2C-adrenergic receptor also has been suggested in moxonidine-induced spinal analgesia. Intrathecal moxonidine (an agonist at both the α_2A- and I_1 receptors) induced dose-dependent antinociception in α_2A-D79N and wild-type mice in both the tail-flick and Substance P tests. However, moxonidine was 2-fold less potent in α_2A-D79N mice. Both the α_2A-adrenergic receptor-selective antagonist SK&F 86466 and the I_1/α_2-adrenergic receptor antagonist efaroxan dose dependently inhibited the antinociceptive effects of moxonidine in α_2A-D79N mice (Fairbanks and Wilcox, 1999). These data suggest that moxonidine antinociception requires α_2-adrenergic receptors presumably of the α_2B- and/or α_2C-subtypes. However, a possible role for putative I_1 receptors cannot be ruled out. In addition, a possible role for α_2A-adrenergic receptors cannot be ruled out completely especially because α_2A-D79N mice retain some α_2A-adrenergic receptor-mediated functions (Altman et al., 1999).

**Other Effects.** Presynaptic inhibition of norepinephrine release is a classic α_2-adrenergic function. Dexametomidine potently inhibited neurotransmitter release in the vasa deferentia of α_2A- and α_2B-KO, and α_2C-KO mice. This inhibitory effect, however, was greatly attenuated in α_2A-KO mice, and the stimulatory effect of the α_2-adrenergic antagonist yohimbine was attenuated as well (Altman et al., 1999). Similar results have been found in the brain (hippocampus and occipito-parietal cortex) and the heart (atrium) of α_2A-KO mice (Trendelenburg et al., 1999). These data indicated that the α_2A-subtype is the most important in mediating presynaptic α_2-adrenergic receptor inhibition of neurotransmitter release, although a role for at least one other subtype seemed probable. Recent studies on the sympathetic nerves in the heart of α_2A-KO and α_2C-KO mice as well as in mice lacking both the α_2A- and the α_2C-subtypes (double knockout; α_2AC-KO) have confirmed and extended these conclusions. In the α_2A-KO but not the α_2C-KO mouse, the maximal inhibitory effect of brimonidine on norepinephrine release was significantly reduced but not eliminated as compared with the wild type. In the α_2AC-KO mouse, however, the inhibitory effect of brimonidine was completely abolished (Hein et al., 1999). Further experiments in these mice indicate that the α_2A-receptor inhibits transmitter release at high stimulation frequencies, whereas the α_2C-subtype regulates release at lower levels. The regulation at both high and low frequencies appears to be physiologically important (Hein et al., 1999).

In humans, α_2-adrenergic agonists are used as adjuncts to anesthesia because they permit the reduction of the dose of other anesthetic agents (Maze and Tranquilli, 1991). In α_2A-D79N mice, dexmedetomidine did not reduce the amount of halothane required to produce anesthesia (loss of righting reflexes), whereas in wild-type mice the amount of halothane was significantly reduced. These data indicate that the α_2A-subtype mediates the anesthetic-sparing effects of α_2-adrenergic agonists (Lakhiani et al., 1997). The role, if any, of the α_2B- and α_2C-subtypes has not been carefully examined.

Reduced body temperature is another consequence of α_2-adrenergic receptor activation. α_2A-D79N mice showed no hypothermic effect in response to varying doses of dexmedetomidine, whereas both α_2B- and α_2C-KO mice showed dose-dependent reductions in body temperature indistinguishable from those in wild-type animals (Hunter et al., 1997). In contrast, Sallinen et al. (1997) reported a slight attenuation of the hypothermic response in α_2C-KO mice. Thus, the α_2A-receptor also seems to be the primary mediator of the hypothermic effects of α_2-adrenergic agonists, although the α_2C-subtype may play a small role.

The α_2A-adrenergic receptor also mediates the antiepileptogenic actions of norepinephrine in the kindling model of epileptogenesis. Compared with wild-type mice, α_2A-D79N mice achieved kindling more rapidly and exhibited a 2-fold increase in the duration of their electrographic seizures. This accelerated pattern of kindling development in α_2A-D79N mice was indistinguishable from that seen in wild-type mice treated acutely with the α_2-adrenergic receptor antagonist idazoxan, whereas idazoxan treatment did not alter the pattern of kindling development in α_2B-D79N mice (Janumpalli et al., 1998). These data suggest that compensatory changes do not accompany mutation of the mouse genome with the α_2A-adrenergic receptor antagonist idazoxan, whereas idazoxan treatment did not alter the pattern of kindling development in α_2A-D79N mice, because the epileptogenic phenomena in these mice are indistinguishable from those in wild-type mice treated acutely with the α_2-adrenergic receptor antagonist idazoxan. These data also suggest that the α_2A-adrenergic receptor subtype is the principal mediator of the antiepileptogenic effect because idazoxan treatment of the mutant α_2A-D79N mice produced no further enhancement of epileptogenesis.

**The α_2B- and α_2C-Subtypes: Fewer Defined Functions**

**α_2B-Subtype.** In comparison with the α_2A-subtype, relatively less has been discovered about the functions of the α_2B- and α_2C-subtypes through knockout experiments. As noted above, the α_2B-subtype appears to have a dominant role in eliciting the vasoconstrictor response to α_2-adrenergic agonists because this response is lacking in α_2B-KO mice (Link et al., 1996). The α_2B-adrenergic receptor has also been implicated in salt-induced hypertension. When subjected to subtotal nephrectomy followed by dietary salt loading, the increase in blood pressure was much greater in α_2C-KO and wild-type mice as compared to α_2B-KO mice (Makaritsis et
al., 1999a). The significance of this effect is enhanced by the fact that it was obtained with heterozygous α2B-KO mice (due to the difficulty in breeding homozygous mice because their survival is limited), and thus the authors conclude that a full complement of α2-adrenergic receptor genes is necessary to raise blood pressure in response to dietary salt loading. Although the role, if any, of the α2A-subtype cannot be determined from these studies, the data imply that the α2B-but not the α2C-subtype is prominently involved in the development of salt-induced hypertension.

The α2B-adrenergic receptor may be important in developmental processes, although the role it plays is currently unknown. Because all α2-adrenergic receptor KO mice survive and are viable, no single subtype of α2-adrenergic receptor is absolutely necessary for development. However, homozygous α2B-KO mice are recovered from heterozygous crosses at less than the predicted Mendelian ratios, and homozygous α2B-KO mice do not breed well (Link et al., 1996; Makaritsis et al., 1999a), which indicates some developmental or reproductive role for the α2B-adrenergic receptor gene. In support of this is the reported inability to produce either α2B- or α2BC-double knockout mice, whereas the α2AC-double knockout mice are viable (Hein et al., 1999). Studies to detect possible changes in the developing brain and other tissues of KO mice will likely provide further insight into the function of the α2-adrenergic receptor subtypes during development.

**α2C-Subtype.** Unlike its counterparts, the α2C-subtype does not appear to play a major role in cardiovascular regulation or the other classical effects of α2-adrenergic receptors. The cardiovascular and sedative effects of dexmedetomidine were normal in α2C-KO mice. Sallinen and coworkers (1997) reported small, but opposite, changes in the hypothermic effect of dexmedetomidine in α2C-KO and α2C-OE mice, indicating that the α2C-subtype may play a role in this effect secondary to the prominent role of the α2A-subtype. In both α2C-KO and α2C-OE mice, dexmedetomidine induced dose-dependent reductions in monoamine turnover indistinguishable from those in wild-type animals. However, α2C-OE mice showed slightly increased basal levels of dopamine and its metabolite homovanillic acid, whereas α2C-KO mice showed slightly decreased levels of metabolites of dopamine (homovanillic acid), norepinephrine (3-methoxy-4-hydroxyphenylglycol), and serotonin (5-hydroxyindoleacetic acid) (Sallinen et al., 1997). The opposite findings for homovanillic acid in α2C-KO and α2C-OE mice point to a possible role for α2C-adrenergic receptors in the regulation of dopamine systems in the brain.

In mice, expression of the α2C-subtype seems to be restricted to the central nervous system, and the effect of altered α2C-adrenergic receptor expression has been evaluated in several different behavioral paradigms (see Table 2). Relative to wild-type mice, α2C-KO mice showed increased locomotor activity in response to amphetamine, whereas α2C-OE mice showed decreased activity in response to the drug (Sallinen et al., 1998a). 5-Hydroxytryptophan, a serotonin precursor, elicits a range of behaviors in rodents due to serotonin receptor activation, including head twitches and five behaviors that constitute the “serotonin syndrome” that is mediated mainly by the 5-HT1A receptor. Neither α2C-KO nor α2C-OE mice showed significant differences from wild-type strains in head twitches in response to dexmedetomi-
cerning their functional significance. For example, what is the role of each of the subtypes in development? Because the α2A-subtype mediates most of the classical effects of α2-adrenergic agonists, it is doubtful that an α2A-selective agonist would have a substantially better clinical profile than the currently available agents. On the other hand, because the α2B-subtype has not yet been shown to be important in cognitive functions, whereas the α2C-subtype does appear to play a role in these functions, it may turn out that selective α2A-agents may have fewer central nervous system side effects than nonselective agents. Drugs acting at α2A- or α2C-adrenergic receptors are likely to have fewer of the classical α2-adrenergic side effects than α2α-selective agents. However, because the functions of these subtypes are not as clear as those of the α2A-subtype, the therapeutic value of α2A- and α2C-selective drugs is also unclear. It would appear likely, however, that α2C-selective agents may be useful in at least some central nervous system disorders.

A comparison of the studies published to date using mice with altered expression of α2-adrenergic receptors reveals some inconsistencies such as the role, if any, of the α2A- or α2C-subtypes in α2-adrenergic-mediated spinal analgesia. The recent development of α2A/CO−“double-knockout” mice may help answer these questions (Hein et al., 1999). However, for some responses, such as suppression of epileptogenesis, this time-limited effects to be studied as they develop. However, for some responses, such as suppression of epileptogenesis, this time-intensive and expensive experimental strategy may not be practical. Nevertheless, the role of each of the subtypes in development? Because the α2A-subtype has not yet been shown to be important in cognitive functions, whereas the α2C-subtype does appear to play a role in these functions, it may turn out that selective α2A-agents may have fewer central nervous system side effects than nonselective agents. Drugs acting at α2A- or α2C-adrenergic receptors are likely to have fewer of the classical α2-adrenergic side effects than α2α-selective agents. However, because the functions of these subtypes are not as clear as those of the α2A-subtype, the therapeutic value of α2A- and α2C-selective drugs is also unclear. It would appear likely, however, that α2C-selective agents may be useful in at least some central nervous system disorders.

The ability to probe subtype-specific functions in mice by altering the same α2-adrenergic receptor (α2A-KO and α2A−D79N mice; α2C-KO and α2C-GE) and the general consistency of the results strengthens the conclusions drawn from these studies. Despite their acknowledged limitations, these genetic approaches have provided, and are expected to continue to provide, considerable insight into the functions of α2-adrenergic receptor subtypes.

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References


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