Perspectives in Pharmacology

In Vivo Gene Modification Elucidates Subtype-Specific Functions of α_2-Adrenergic Receptors[1]

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Accepted for publication January 3, 2000   This paper is available online at http://www.jpet.org

ABSTRACT

Mice with altered α_2-adrenergic receptor genes have become important tools in elucidating the subtype-specific functions of the three α_2-adrenergic receptor subtypes because of the lack of sufficiently subtype-selective pharmacological agents. Mice with a deletion (knockout) of the α_{2A}-, α_{2B}-, or α_{2C}-gene as well as a point mutation of the α_{2A}-gene (α_{2A}-D79N) and a 3-fold overexpression of the α_{2C}-gene have been generated. Studies with these mice indicate that most of the classical functions mediated by the α_2-adrenergic receptor, such as hypotension, sedation, analgesia, hypothermia, and anesthetic-sparing effect, are mediated primarily by the α_{2A}-subtype. The α_{2B}-subtype is the principal mediator of the hypertensive response to α_2-agonists, appears to play a role in salt-induced hypertension, and may be important in developmental processes. The α_{2C}-subtype appears to be involved in many central nervous system processes such as the startle reflex, stress response, and locomotion. Both the α_{2A}- and α_{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 α_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 α_2-adrenergic receptor subtypes.

Adrenergic receptors mediate the physiological responses to the catecholamines, norepinephrine and epinephrine. They belong to the superfamily of G protein-coupled receptors containing seven putative transmembrane domains and are divided pharmacologically into α_1-, α_2-, and β-adrenergic receptor types (Bylund, 1988). α_2-Adrenergic receptors are implicated in diverse physiological functions particularly of the cardiovascular system and the central nervous system. α_2-Adrenergic receptor agonists are used clinically in the treatment of hypertension, glaucoma, and attention-deficit disorder, in the suppression of opiate withdrawal, and as adjuncts to general anesthesia. α_2-Adrenergic receptors have been divided into three subtypes (α_{2A}, α_{2B}, and α_{2C}) on the basis of pharmacological and molecular cloning evidence (Lomasney et al., 1991; Bylund et al., 1994; Hein and Kobilka, 1995).

Understanding the role of α_2-adrenergic receptor subtypes in these diverse functions is clearly important particularly from a pharmacological point of view. One line of evidence supporting differential functions of the subtypes is differences in their characteristics, such as their tissue distributions throughout development (Winzer-Serhan et al., 1997), and in the adult, their coupling to G proteins and regulation in response to agonist stimulation. Although in situ hybridization studies of α_2-adrenergic receptor subtype expression in mice during development and in adults (Wang et al., 1996) and rats (Nicholas et al., 1993; Rosin et al., 1993; Scheinin et al., 1994) can reveal where α_2-adrenergic receptor subtypes are expressed, these findings cannot definitively link particular subtypes to physiological functions. Furthermore, assigning the physiological functions of α_2-adrenergic receptors to specific subtypes in vivo has been difficult because of the lack of sufficiently subtype-selective agonists and antagonists. The ability to genetically manipulate α_2-subtypes provides an alternative approach to elucidating subtype-specific functions as demonstrated in recent experiments using mice with deletions, mutations, or overexpression of specific α_2-adrenergic receptor subtypes (MacDonald et al., 1997).

ABBREVIATIONS: KO, knockout; OE, overexpression; PPI, prepulse inhibition.

Mice with Genetically Engineered α_2-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 α_2-adrenergic receptor expression. Mice with a deletion of the α_{2A}- (α_{2A}-knockout [KO]), α_{2B}- (α_{2B}-KO), or α_{2C}-gene (α_{2C}-KO) have been generated (Link et al., 1995, 1996; Altman et al., 1999). More recently, the double knockout mice (α_{2AC}-KO), in which both the α_{2A}- and the α_{2C}-genes have been deleted, have been produced (Hein et al., 1999). Mice have also been developed with a point mutation of the α_{2A}-gene (α_{2A}-D79N) (Macmillan et al., 1996). This mutation of the aspartate to an asparagine residue at position 79 in the second transmembrane domain of the α_{2A}-adrenergic receptor selectively uncouples the receptor from the activation of K^+ channels in vitro, although coupling to Ca^{2+} channels and adenylyl 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 α_{2A}-adrenergic receptor stimulation. However, α_{2A}-D79N mice showed an approximately 80% reduction in α_{2A}-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 Ca^{2+} channels (Lakhlani et al., 1997). Thus, the α_{2A}-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 fertile and appear grossly normal. Apparently, none of the α_2-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 α_2-adrenergic receptors, and a strain of mice has been generated in Kobilka's laboratory with approximately 3-fold overexpression (OE) of the α_{2C}-gene (α_{2C}-OE) under the control of its homologous promoter (Sallinen et al., 1997).

Results from experiments using mice with genetic alterations of α_2-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 α_{2A}-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 α_{2A}-Subtype Mediates the Classical Effects of α_2-Adrenergic Receptor Agonists

Through experiments with the α_{2A}-KO and α_{2A}-D79N mice, most of the classical effects of α_2-adrenergic receptor agonists can be attributed to the α_{2A}-subtype. Mice with a mutated or deleted α_{2A}-subtype do not exhibit the hypoten-

TABLE 1

Physiological effects of altering α_2-adrenergic receptor gene expression in mice

The α_{2A}-subtype mediates most of the classical effects of α_2-adrenergic receptor agonists.

Physiological Effect	Genetic Alterations			
	α_{2A}-D79N	α_{2A}-KO	α_{2B}-KO	α_{2C}-KO
Hypotensive effects of α_2-adrenergic receptor agonist	X	X	↑	−
Bradycardic effects of α_2-adrenergic receptor agonist	↓	↓	−	−
Hypertensive effects of α_2-adrenergic receptor agonist	↓ [a]	−	X	−
Cardiovascular effects of imidazoline agonist	X			
Resting heart rate	−	↑	−	−
Resting blood pressure	−	−	−	−
Salt-induced hypertension			X [b]	−
Sedative effects of dexmedetomidine	X	−	−	−
Antinociceptive effects of α_2-adrenergic receptor agonist	X/↓ [c]		−	−
Antinociceptive effects of moxonidine	↓			
Adrenergic-opioid synergy in spinal antinociception	X			
Anesthetic-sparing effects of dexmedetomidine	X			
Hypothermic effects of dexmedetomidine	X		−	−/↓
Antiepileptogenic effects of endogenous norepinephrine	X			
Presynaptic inhibition of norepinephrine release	−	↓	−	↓ [d]
Autoinhibition of locus coeruleus	X			

X, abolished; −, no effect; ↑, accentuated; ↓, attenuated; and blank, not studied.
[a] Dependent on site of agonist administration.
[b] Mice were heterozygous (+/−) for α_{2B}-null mutation.
[c] Extent of attenuation depended on test used.
[d] In α_{2AC}-double KO mice.

TABLE 2
Effects of altered α_{2C}-adrenergic receptor gene expression on behavior

Behavior	Genetic Alteration	
	α_{2C}-KO	α_{2C}-OE
Startle reflex	↑	−
Prepulse inhibition of startle reflex	↓	↑
Latency to attack after isolation	↓	↑
General aggression	−	−
Locomotor stimulation of D-amphetamine	↑	↓
L-5-hydroxytryptophan-induced serotonin syndrome	↓	−
L-5-hydroxytryptophan-induced head twitches	−	−
Performance in T-maze	↓	
Working memory enhancement of α_2-adrenergic receptor agonist in T-maze	−	
Performance in Morris water maze		↓
Forced-swim stress and behavioral despair test	↓	↑
Learning and memory in Morris water maze		−
Anxiety in open field test		−
Stimulus-response learning in passive avoidance test		−
Cortical electroencephalogram (arousal)		−

−, no effect; ↑, accentuated; ↓, attenuated; and blank, not studied.

sive, sedative, antinociceptive, anesthetic-sparing, or hypothermic effects in response to α_2-adrenergic agonists.

Hypotensive Effects. α_2-Adrenergic agonists activate α_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 α_2-adrenergic receptor-mediated vasoconstriction of vascular smooth muscle. The hypothesis of α_{2A}-adrenergic receptor involvement in the centrally mediated cardiovascular responses was based on α_{2A}-adrenergic receptor expression in the rostral ventrolateral medulla (Nicholas et al., 1996), and α_{2A}-adrenergic receptor involvement was confirmed in α_{2A}-D79N mice. The hypotensive response to administration of α_2-adrenergic receptor agonists was abolished, demonstrating that the α_{2A}-subtype plays a principal role in this response (Macmillan et al., 1996). The bradycardic response to agonist also was blunted in α_{2A}-D79N mice (Macmillan et al., 1996). These results have been confirmed in both α_{2A}-D79N and α_{2A}-KO mice (Altman et al., 1999; Zhu et al., 1999). Furthermore, the hypertensive response was abolished in α_{2B}-KO mice, and the hypotensive effect was immediate and accentuated. The bradycardic response in α_{2B}-KO mice was normal, and α_{2C}-KO mice showed no differences from wild-type strains in their hypertensive, hypotensive, and bradycardic effects (Link et al., 1996). The α_{2A}-subtype appears to play a role in vasoconstriction at least in some vascular compartments because the hypertensive response in α_{2A}-D79N mice was absent when the agonist was administered through the femoral artery (Macmillan et al., 1996). These results demonstrate that the α_{2A}-adrenergic receptor mediates the hypotensive and bradycardic effects of α_2-adrenergic agonists. In contrast, the α_{2B}-adrenergic receptor appears to be the main mediator of the pressor response that results from α_2-adrenergic agonist administration.

Considering the role of this receptor in cardiovascular function, it was surprising that α_{2A}-D79N mice do not show any cardiovascular abnormalities. Recent evidence has indicated that α_{2A}-D79N mice do retain some α_{2A}-adrenergic receptor function. In contrast to α_{2A}-D79N mice, α_{2A}-KO mice have tachycardia, higher systolic blood pressure, and higher plasma norepinephrine levels (Altman et al., 1999; Makaritsis et al., 1999b). Propranolol, a β-adrenergic recep-

tor antagonist, eliminated the difference in heart rate between α_{2A}-KO and wild-type mice, demonstrating that the tachycardia in α_{2A}-KO mice was due to increased sympathetic tone, presumably resulting from increased norepinephrine release because of the absence of α_{2A}-adrenergic presynaptic inhibition (Altman et al., 1999).

Sedative Effects. The sedative effects of dexmedetomidine were examined in α_{2A}-D79N mice by Rotarod, loss of righting reflex (Lakhlani et al., 1997), and spontaneous locomotor activity tests (Hunter et al., 1997). In all cases, α_{2A}-D79N mice showed no sedation in response to dexmedetomidine, indicating that the α_{2A}-adrenergic receptor mediates the sedative effects of α_2-agonist administration. In contrast, both the α_{2B}-KO and α_{2C}-KO mice showed dose-dependent reductions in locomotor activity in response to dexmedetomidine that were indistinguishable from wild-type mice (Hunter et al., 1997). α_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 α_{2A}-subtype in this action, including the prominent expression of α_{2A}-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 α_{2A}-D79N mice, α_2-adrenergic receptor agonists were unable to alter the spontaneous firing rate of locus coeruleus neurons, confirming the role of the α_{2A}-subtype (Lakhlani et al., 1997).

Antinociceptive Effects. Another therapeutic use of α_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, α_{2A}-D79N mice showed no antinociceptive response to dexmedetomidine (Hunter et al., 1997; Lakhlani et al., 1997). In contrast, dexmedetomidine induced normal dose-dependent antinociception in α_{2B}-KO and α_{2C}-KO mice in the tail immersion test (Hunter et al., 1997). Spinal analgesia was examined in α_{2A}-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-

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 antinociception. 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_2-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. Dexmedetomine potently inhibited neurotransmitter release in the vasa deferentia of α_{2A}-D79N, 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, how-

ever, 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 (Lakhlani 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_{2A}-D79N mice (Janumpalli et al., 1998). These data suggest that compensatory changes do not accompany mutation of the mouse genome with the 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 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 α_{2B}-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 α_{2B}-receptor genes is necessary to raise blood pressure in response to dietary salt loading. Although the role, if any, of the α_{2A}-subtype cannot be determined from these studies, the data imply that the α_{2B}- but not the α_{2C}-subtype is prominently involved in the development of salt-induced hypertension.

The α_{2B}-adrenergic receptor may be important in developmental processes, although the role it plays is currently unknown. Because all α_2-adrenergic receptor KO mice survive and are viable, no single subtype of α_2-adrenergic receptor is absolutely necessary for development. However, homozygous α_{2B}-KO mice are recovered from heterozygous crosses at less than the predicted Mendelian ratios, and homozygous α_{2B}-KO mice do not breed well (Link et al., 1996; Makaritsis et al., 1999a), which indicates some developmental or reproductive role for the α_{2B}-adrenergic receptor gene. In support of this is the reported inability to produce either α_{2AB}- or α_{2BC}-double knockout mice, whereas the α_{2AC}-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 α_2-adrenergic receptor subtypes during development.

α_{2C}-Subtype. Unlike its counterparts, the α_{2C}-subtype does not appear to play a major role in cardiovascular regulation or the other classical effects of α_2-adrenergic receptors. The cardiovascular and sedative effects of dexmedetomidine were normal in α_{2C}-KO mice. Sallinen and coworkers (1997) reported small, but opposite, changes in the hypothermic effect of dexmedetomidine in α_{2C}-KO and α_{2C}-OE mice, indicating that the α_{2C}-subtype may play a role in this effect secondary to the prominent role of the α_{2A}-subtype. In both α_{2C}-KO and α_{2C}-OE mice, dexmedetomidine induced dose-dependent reductions in monoamine turnover indistinguishable from those in wild-type animals. However, α_{2C}-OE mice showed slightly increased basal levels of dopamine and its metabolite homovanillic acid, whereas α_{2C}-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 α_{2C}-KO and α_{2C}-OE mice point to a possible role for α_{2C}-adrenergic receptors in the regulation of dopamine systems in the brain.

In mice, expression of the α_{2C}-subtype seems to be restricted to the central nervous system, and the effect of altered α_{2C}-adrenergic receptor expression has been evaluated in several different behavioral paradigms (see Table 2). Relative to wild-type mice, α_{2C}-KO mice showed increased locomotor activity in response to amphetamine, whereas α_{2C}-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-HT$_{1A}$ receptor. Neither α_{2C}-KO nor α_{2C}-OE mice showed significant differences from wild-type strains in head twitches in response to dexmedetomi-

dine. However, dexmedetomidine failed to attenuate symptoms of the 5-hydroxytryptophan-induced serotonin syndrome in α_{2C}-KO mice, suggesting an interaction with the 5-HT$_{1A}$ receptor (Sallinen et al., 1998a). In the isolation-induced aggression paradigm, α_{2C}-KO mice showed decreased attack latency, whereas α_{2C}-OE mice showed increased latency. There was, however, no significant difference in the overall number of attacks, and altered α_{2C}-adrenergic receptor expression did not affect preisolation aggressive behavior (Sallinen et al., 1998b). In a forced swimming test used to induce stress and assess behavioral despair, opposite effects in α_{2C}-KO and α_{2C}-OE mice indicate a possible association between the α_{2C}-subtype and stress-dependent depression. Thus, α_{2C}-adrenergic antagonists may have therapeutic value in the treatment of stress-related psychiatric disorders (Sallinen et al., 1999).

Many psychiatric disorders, including schizophrenia, manifest symptoms of exaggerated startle reactivity and/or reduced inhibition of startle by prepulses. Disrupted prepulse inhibition (PPI) of the startle reflex can be restored by antipsychotics, and the PPI model is used as an animal model in drug development. Compared with wild-type mice, α_{2C}-KO mice showed increased startle reactivity and reduced PPI of the startle response, whereas α_2-OE mice showed an increase in PPI that could be reversed by the α_2-adrenergic antagonist atipamezole (Sallinen et al., 1998b). In each of the behavioral paradigms, it is unclear whether the α_{2C}-subtype plays some direct role in mediating behavior or whether altered α_{2C}-receptor expression produces effects because of altered metabolism or downstream modulation of other neurotransmitter systems.

It has been demonstrated that α_2-adrenergic agonists improve memory processes in several models. In the T-maze delayed alternation task, dexmedetomidine dose dependently reduced nonperseverative errors and increased performance in both α_{2C}-KO and wild-type mice, indicating that the α_{2C}-subtype does not mediate the beneficial effects of α_2-adrenergic agonists on spatial working memory (Tanila et al., 1999). In the Morris water maze, α_{2C}-OE mice developed an ineffective search pattern, which was reversible by atipamezole, suggesting that α_{2C}-receptors may modulate the execution of complex navigation patterns (Björklund et al., 1999, 2000). In this same test, α_{2C}-OE mice showed a defective escape performance that could be improved by the α_2-adrenergic antagonist atipamezole. α_{2C}-OE mice, however, showed learning curves similar to wild-type mice, and atipamezole failed to affect the slope of the learning curve in either strain. Thus, α_{2C}-overexpression did not hinder performance by affecting memory. α_{2C}-OE mice showed no defects in open field or passive avoidance behaviors or in cortical electroencephalogram measurements, indicating that their defect in performance does not arise from defects in anxiety, stimulus-response learning, or general arousal (Björklund et al., 1998). These results suggest that the α_{2C}-subtype may play a role in modulating motor behavior and perhaps in memory processes.

Conclusions and Future Directions

The subtype involved in many α_2-adrenergic receptor-mediated physiological functions is now known (at least in the mouse), but there are still many unanswered questions con-

cerning their functional significance. For example, what is the role of each of the subtypes in development? Because the α_{2A}-subtype mediates most of the classical effects of α_2-adrenergic agonists, it is doubtful that an α_{2A}-selective agonist would have a substantially better clinical profile than the currently available agents. On the other hand, because the α_{2A}-subtype has not yet been shown to be important in cognitive functions, whereas the α_{2C}-subtype does appear to play a role in these functions, it may turn out that selective α_{2A}-agents may have fewer central nervous system side effects than nonselective agents. Drugs acting at α_{2B}- or α_{2C}-adrenergic receptors are likely to have fewer of the classical α_2-adrenergic side effects than α_{2A}-specific agents. However, because the functions of these subtypes are not as clear as those of the α_{2A}-subtype, the therapeutic value of α_{2B}- and α_{2C}-selective drugs is also unclear. It would appear likely, however, that α_{2C}-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 α_2-adrenergic receptors reveals some inconsistencies such as the role, if any, of the α_{2B}- or α_{2C}-subtypes in α_2-adrenergic-mediated spinal analgesia. The recent development of α_{2AC}-KO "double-knockout" mice may help answer these questions (Hein et al., 1999). α_{2AC}-KO mice (as well as α_{2AB}-KO mice if they can be produced), when tested against α_{2A}-KO mice, may show the involvement of the α_{2C}- (and α_{2B}-) subtype(s) in various functions that heretofore have been masked by the dominance of the α_{2A}-subtype.

Generation of mice with inducible gene knockouts would more closely resemble acute blockade of specific α_2-adrenergic receptor subtypes and avoid any compensatory adaptations that might occur during development. Thus, inducible KO mice might reveal physiological roles of α_2-adrenergic receptors that have been masked by compensatory changes in current α_2-adrenergic receptor KO mice. In addition, inducible knockout mice might allow such compensatory modifications 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 warranted. This is because the responses in α_{2A}-D79N mice are indistinguishable from wild-type mice treated with idazoxan, and idazoxan administration has no further effect in α_{2A}-D79N mice evaluated in the kindling paradigm (Janumpalli et al., 1998). Furthermore, transgenic studies using a limited promoter region of the α_{2A}-adrenergic receptor subtype indicate that we do not yet know how to achieve faithful reproduction of the expression profile of this subtype (Wang et al., 1996).

KO and transgenic mice are likely to be important tools in drug development for determining the physiological site of action for newly developed pharmacological agents. Such an approach has already been used to determine that the hypotensive effects of two putative imidazoline-1 receptor agonists, moxonidine and rilmenidine, are mediated predominantly by α_{2A}-adrenergic receptors in the mouse (Fairbanks and Wilcox, 1999; Zhu et al., 1999).

The ability to probe subtype-specific functions in mice by altering the same α_2-adrenergic receptor (α_{2A}-KO and α_{2A}-D79N mice; α_{2C}-KO and α_{2C}-OE) 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 α_2-adrenergic receptor subtypes.

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