Moxonidine, a Selective $\alpha_2$-Adrenergic and Imidazoline Receptor Agonist, Produces Spinal Antinociception in Mice

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ABSTRACT

$\alpha_2$-Adrenergic receptor (AR)-selective compounds produce antihypertensive and antinociceptive effects. Moxonidine alleviates hypertension in multiple species, including humans. This study demonstrates that intrathecally administered moxonidine produces antinociception in mice. Antinociception was detected via the (52.5°C) tail-flick and Substance P (SP) nociceptive tests. Moxonidine was intrathecally administered to ICR, mixed C57BL/6 $\times$ 129/Sv (wild type (WT)), or C57BL/6 $\times$ 129/Sv mice with dysfunctional $\alpha_2$ ARs (D79N-$\alpha_{2a}$). The $\alpha_2$ AR-selective antagonist SK&F 86466 and the mixed $I_1/\alpha_2$ AR-selective antagonist efaroxan were tested for inhibition of moxonidine-induced antinociception. Moxonidine prolonged tail-flick latencies in ICR (ED$_{50}$ = 0.5 nmol; 0.3–0.7), WT (0.17 nmol; 0.09–0.32), and D79N-$\alpha_{2a}$ (0.32 nmol; 0.074–1.6) mice. Moxonidine inhibited SP-elicited behavior in ICR (0.04 nmol; 0.03–0.07), WT (0.4 nmol; 0.3–0.5), and D79N-$\alpha_{2a}$ (1.1 nmol; 0.7–1.7) mice. Clonidine produced antinociception in WT but not D79N-$\alpha_{2a}$ mice. SK&F 86466 and efaroxan both antagonized moxonidine-induced inhibition of SP-elicited behavior in all mouse lines. SK&F 86466 antagonism of moxonidine-induced antinociception implicates the participation of $\alpha_2$ ARs. The comparable moxonidine potency between D79N-$\alpha_{2a}$ and WT mice suggests that receptors other than $\alpha_{2a}$ mediate moxonidine-induced antinociception. Conversely, absence of clonidine efficacy in D79N-$\alpha_{2a}$ mice implies that $\alpha_{2a}$ AR activation enables clonidine-induced antinociception. When clinically administered, moxonidine induces fewer side effects relative to clonidine; moxonidine-induced antinociception appears to involve a different $\alpha_2$ AR subtype than clonidine-induced antinociception. Therefore, moxonidine may prove to be an effective treatment for pain with an improved side effect profile.

Moxonidine belongs to the imidazoline class of compounds and acts centrally. The spinal antinociceptive actions of several adrenergics/imidazolines have been demonstrated in multiple species. Spinally administered clonidine and dexmedetomidine both produce antinociception in rat (Reddy et al., 1980), sheep (Eisenach and Dewan, 1990), mouse (Roerig et al., 1992; Stone et al., 1997), frog (Stevens and Brenner, 1996), and humans (Mendez et al., 1990). The spinal antinociceptive action of moxonidine, however, has not been previously reported.

Spinal administration of clonidine to humans for the treatment of pain has been used in clinical studies since the mid-1980s. Epidural administration of clonidine has since been approved for the treatment of severe cancer pain (Eisenach, 1996). Although clonidine is efficacious for the treatment of both hypertension and pain, treatment with clonidine is frequently accompanied by adverse side effects that include, but are not limited to, sedation and dry mouth (Davies et al., 1977; Wing et al., 1977; Thananopavarn et al., 1982; Eisenach et al., 1989a,b, 1995), rebound withdrawal symptoms (Hokfelt et al., 1970; Reid et al., 1977; Weber, 1980), hypotension (Eisenach et al., 1989a; Mendez et al., 1990), and tolerance (Meyer et al., 1977; Yaksh and Reddy, 1981; Takano and Yaksh, 1993). These reported limitations of the use of clonidine drive the continued search for improved $\alpha_2$-adrenergic receptor (AR) analgesics and, in particular, the exploration of $\alpha_2$ AR subtype-selective ligands that may invoke analgesia without also invoking undesirable side effects (Codd et al., 1995).

Moxonidine, a selective imidazoline $I_1/\alpha_2$ agonist, was developed (Armah and Stenzel, 1981) to be an effective antihypertensive agent acting centrally, predominantly on imidazoline receptors, with an improved side effect profile over clonidine (Pläñitz, 1984). Moxonidine has been tested in clinical trials for antihypertensive efficacy since the early 1980s. These studies demonstrate that moxonidine reduces hypertension in patients with mild to moderate hypertension with an efficacy comparable to that of clonidine (Pläñitz, 1984, 1986), the angiotensin-converting enzyme inhibitors (Ollivier and Christen, 1994) captopril (Kraft and Vetter, 1994) and enalapril (Küppers et al., 1997), nifedipine, and atenolol; all

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ABBREVIATIONS: ACE, angiotensin-converting enzyme; AR, adrenergic receptor; I$_1$, imidazoline; % MPE, percent maximum possible effect; NE, norepinephrine; 6-OHDA, 6-hydroxydopamine; SP, Substance P; WT, wild-type.
these studies suggest that moxonidine is well tolerated. Furthermore, several studies (Pläñitz, 1984, 1986) report that, at low but effective doses, moxonidine does not invoke the side effects of sedation or dry mouth. At least one study reports that some patients experienced dry mouth and sedation after moxonidine administration (Pläñitz, 1984, 1986); however, the incidence of these moxonidine-induced side effects was significantly below (6 of 20, 30%) that reported from patients taking clonidine (17 of 20, 85%). Complementarily, several reports indicate that rebound withdrawal symptoms are not observed after the cessation of moxonidine treatment (Kraft and Vetter, 1994; Webster and Koch, 1996; Ziegler et al., 1996). The hypotensive effect of clonidine (Mendez et al., 1996) (not a null mutation). These experiments suggest that moxonidine produces analgesia in normotensive patients with reduced risk of hypotensive side effects.

To our knowledge, the present experiments comprise the first report of an antinociceptive action of moxonidine. We tested moxonidine for potential antinoceptive action in the tail-flick thermal nociceptive test and the SP nociceptive behavioral test in ICR mice, mixed C57BL/6 × 129/Sv (WT) mice, and C57BL/6 × 129/Sv mice with a mutation (D79N) in the α2a receptor subtype that renders that receptor dysfunctional (MacMillan et al., 1996) (not a null mutation). These experiments revealed that moxonidine produces antinoceptive in both strains of mice and in the D79N-α2a mutant line in both tests of antinociception. We also tested the ability of the α2-AR-selective antagonist SK&F 86466 to inhibit moxonidine-induced antinoceptive in both strains of mice and in the line of D79N-α2a mutated mice. Moxonidine is reported to have a 30-fold lower affinity for α2ARs compared with that of clonidine (Arma et al., 1988; Ferry et al., 1988) and is thought to preferentially activate the putative I1 receptor (Ernsberger et al., 1993). Therefore, we also used an antagonist strategy to attempt to identify the relative I1/α2AR contributions to moxonidine-mediated antinoceptive. Finally, we tested for potential involvement of descending noradrenergic mechanisms in the spinal action of moxonidine using the selective noradrenergic neurotoxin 6-hydroxydopamine (6-OHDA) in an effort to destroy descending noradrenergic nerve terminals.

Materials and Methods

Animals. Experimental subjects were 20- to 25-g male ICR mice (Harlan, Madison, WI) or 15- to 20-g male and female mice (gender-matched) with a mixed C57BL/6 × 129/Sv genetic background (designated WT for wild type). We also used male and female mice with this same background (C57BL/6 × 129/Sv) with a “hit and run” gene-targeted mutation (D79N) that renders the α2-AR dysfunctional (MacMillan et al., 1996; Stone et al., 1997). These experiments were approved by the Institutional Animal Care and Use Committee. Subjects were housed in groups of 5 to 10 in 25 × 48 × 15-cm plastic cages in a temperature- and humidity-controlled environment. Subjects were maintained on a 12-h light/dark cycle and had free access to food and water. In the experiment using the noradrenergic neurotoxin 6-OHDA, the mice that were tested 3 days after treatment (saline or 6-OHDA) were randomized and retested 9 days later on day 14 after treatment. In all other experiments, each animal was used only once.

Chemicals. Moxonidine [4-chloro-5-(2-imidazolin-2-ylamino)-6-methoxy-2-methylpyrimidine] chloride was a generous gift of Solvay Pharma GmbH (Hannover, Germany). Norepinephrine (NE) was purchased from Sigma Chemical (St. Louis, MO) and was prepared fresh for each experiment in acidified saline. Smith Kline & French (King of Prussia, PA) donated the SK&F 86466 [6-chloro-2,3,4,5-tetrahydro-3-methyl-1H-3-benzazepine]. Zeneva (Wilmington, DE) donated the dexmedetomidine [1-(+)S]-4-[1-(2,3-dimethylphenyl)ethyl][1H-imidazole]. Efaroxan [2-(2-ethyl-2,3-dihydrobenzofuranyl)-2-imidazoline] hydrochloride was purchased from Research Biochemicals International (Natick, MA). SP was purchased from Sigma Chemical Co. (St. Louis, MO). Clonidine HCl (2-(2,6-dichloroaniline)-2-imidazoline) was from Boehringer-Ingelheim Ltd. Moxonidine was dissolved in 1% acetic acid and diluted with acidified saline (pH 3.2–4.0, 0.01 N acetic acid). NE and SP were dissolved in acidified saline. All other drugs were dissolved in 0.9% saline. All drugs were administered intrathecally in a 5-μl volume in conscious mice according to the method of Hylden and Wilcox (1980) as modified by Wiggard and Wilcox (1987).

Thermal Nociception. Thermal nociceptive responsiveness was determined using the warm water (52.5°C) immersion tail-flick test. The latency to the first rapid tail-flick represented the behavioral endpoint (Janssen et al., 1963). Baseline measurements of tail-flick latencies were collected on all mice before testing. The mean baseline tail-flick latency of the ICR mice was 3.6 s (S.D. = 0.69 s, n = 31). ICR mice that failed to respond within 5 s to baseline tests were excluded from analysis. The mean baseline tail-flick latency of the WT and D79N-α2a mutant mice was 6.4 s (S.D. = 2.6 s, n = 250). The baseline tail-flick latency did not differ (unpaired t-test: p > .05) between the WT and D79N-α2a mice (WT: mean = 6.5 s, S.D. = 2.5 s, S.E.M. = 0.27 s, n = 133 mice; D79N-α2a mice: mean = 6.2 s, S.D. = 2.7 s, S.E.M. = 0.24 s, n = 85 mice). WT and D79N-α2a mice that failed to respond within 11.5 s (a value more than 2 of S.D. from the mean) were eliminated from analysis (5.5%) (Horan et al., 1991). The percent of maximum possible antinoceptive effect (%) of injected (drug or saline control) was determined according to the following formula:

% MPE = [(Postdrug Latency − Predrug latency)/Cutoff − Predrug latency] × 100

In all three lines of mice, a maximum score of 100% was assigned to those animals not responding before a 12-s (ICR) or 15-s (WT/ D79N-α2a) cutoff to avoid tissue injury. In each case in which the animal did not respond before the cutoff, the tail was examined for loss of motor control. Only animals capable of movement in their tails were included in analysis.

SP Nociceptive Test. Nociceptive responsiveness was also tested in the SP nociceptive test. The SP assay is a sensitive indicator of milder analgesics (Hylden and Wilcox, 1981). A constant dose of SP
In some experiments, antagonists were coadministered with the moxonidine-SP combinations. In the case of the SK&F 86466 compound and efaroxan, dose-antagonism curves were determined for their respective abilities to antagonize the inhibition of SP behavior by an 80% effective dose of moxonidine. The ED$_{50}$ value was calculated from the regression line of the dose-inhibition curve. This measure was expressed by the equation:

$$% \text{Antagonism} = \left( \frac{\text{control} - \text{experimental}}{\text{control}} \right) \times 100$$

Each dose selected from the calculated ED$_{50}$ value was tested experimentally on the day of the percent antagonism experiment. In the event that the experimental dose did not produce an exact 80% inhibition, the equation was adjusted to reflect exact percent inhibition that the antagonist was acting against (e.g., 70 or 90 for 70% or 90%, respectively).

**Antagonism of Moxonidine**

Dose-response curves to moxonidine and dexmedetomidine were generated in ICR mice in the SP nociceptive test. ED$_{50}$ doses were calculated according to the method of Tallarida and Murray (1987). IC$_R$, WT, and D78N-$a_2$ mutant mice were coadministered with either SK&F 86466 (0.01–10 nmol i.t.) or efaroxan (0.001–10 nmol i.t.) and an ED$_{50}$ dose of either moxonidine (ICR: efaroxan, 0.2 nmol; SK&F 86466, 0.3 nmol; WT: 1.5 nmol; D78N: 8.6 nmol i.t.) or dexmedetomidine (ICR: efaroxan, 0.2 nmol; SK&F 86466, 0.8 nmol) in the first minute after injection. The dose of SP required to produce this number of behaviors was confirmed with each new experiment. Co-administration of opioid or adrenergic analgesics dose-dependently inhibits those behaviors (Hylden and Wilcox, 1983). To test the ability of moxonidine, dexmedetomidine, clonidine, and NE to inhibit SP-induced behavior, the drugs were coadministered with SP and inhibition was expressed as a percent of the mean response of the control group (determined with each new experiment) according to the following equation:

$$\% \text{Inhibition} = \left( \frac{\text{control} - \text{experimental}}{\text{control}} \right) \times 100$$

In experiments on the day of the percent antagonism experiment. In the event that the experimental dose did not produce an exact 80% inhibition, the equation was adjusted to reflect exact percent inhibition that the antagonist was acting against (e.g., 70 or 90 for 70% or 90%, respectively).

**Statistical Analysis.** Data describing antinociception are expressed as % MPE or percent inhibition with S.E.M. In experiments in which full dose-response curves were generated, a minimum of three doses was used for each drug or combination of drugs. Statistical comparisons of potencies are based on the confidence limits of the ED$_{50}$ values. A shift in a dose-response curve is considered significant when the calculated ED$_{50}$ value of one curve falls outside the confidence limits of the ED$_{50}$ value of the curve to which it is being compared. The ED$_{50}$ values and confidence limits were calculated according to the method of Tallarida and Murray (1987).

**6-OHDA Treatment.** ICR mice were injected intrathecally with the noradrenergic neurotoxin 6-OHDA (5 µg i.t.) (Fig. 6). This dose of 6-OHDA was selected because it has been demonstrated to be effective when administered intrathecally in mice; Fasmer and colleagues (1986) demonstrated that uptake of ³H-NE was dramatically reduced in mouse spinal cord 14 days after the i.t. injection of 5 µg of 6-OHDA. At 3 and 14 days after treatment, saline-treated mice and 6-OHDA-treated mice were injected with different doses of moxonidine or NE. Dose-inhibition curves were collected and ID$_{50}$ values calculated for each drug in each treatment group. The 14-day time point represents the same mice used for the 3-day time point. Mice from the first group were randomized for the subsequent treatment (NE or moxonidine) to reduce potential confound from previous drug exposure. The time points of 3 and 14 days treatment were selected to be comparable to the testing days used by Fasmer et al. (1986); this group observed differences in the responses to nociceptive stimuli of 6-OHDA-treated and control mice on day 3 but not on day 14 after treatment.

**Results**

**Moxonidine-Induced Antinociception**

Moxonidine Produces Antinociception in ICR Mice in Tail-Flick Test and SP Nociceptive Test. We deter-
minded dose-response curves (Fig. 1) for the effects of moxonidine in the tail-flick test [0.1, 0.3, and 1 nmol, i.t; ED$_{50}$ = 0.5 nmol (0.3–0.7)] and in the SP nociceptive behavioral test in [(0.01, 0.03, 0.1, and 0.3 nmol, i.t; ED$_{50}$ = 0.04 nmol (0.03–0.07)]. The lower ED$_{50}$ value of moxonidine in the SP test versus the tail-flick test is consistent with comparisons made between the tests for other antinociceptive compounds. We have observed that both opioid and adrenergic agonists produce antinociception in the SP nociceptive test with greater potency compared with their respective potencies in the tail-flick assay (Hylden and Wilcox, 1983). Furthermore, the ED$_{50}$ values of moxonidine in both tests are comparable to those observed with spinaly administered morphine or clonidine (see Discussion).

**Antagonism of Moxonidine-Induced Antinociception**

SKF-86466 and Efaroxan Dose-Dependently Antagonize Moxonidine-Induced Antinociception in SP Nociceptive Test. We compared the abilities of the $\alpha_2$AR-selective antagonist SK&F 86466 (Hieble et al., 1986) and the $\alpha_2$AR-selective mixed antagonist efaroxan (Haxhiu et al., 1994) to antagonize the effects of moxonidine and dexmedetomidine in the SP test in ICR mice.

SK&F 86466 and Efaroxan Antagonist of Dexametomidine in ICR Mice. As a positive control, we antagonized dexmedetomidine antinociception with both SK&F 86466 and efaroxan. On each experimental day, we generated a dose-response curve to dexmedetomidine in ICR mice and the calculated the ED$_{50}$ value from the regression line. For the SK&F 86466 antagonism experiment, the ED$_{50}$ value of dexmedetomidine was calculated to be 0.0013 nmol and the ED$_{50}$ dose was calculated to be 0.8 nmol. This constant dose of dexmedetomidine was administered with varying doses of SK&F 86466 (0.1, 0.3, 1, 3, 6, and 8 nmol i.t.) to generate a dose-inhibition curve (Fig. 2A). A high dose of SK&F 86466 (8 nmol i.t.) alone did not affect the number of SP-elicited behaviors (data not shown). Similarly, we observed that a single dose of the nonimidazoline $\alpha_2$AR-selective antagonist yohimbine (2.5 nmol) effectively antagonized high efficacy doses of moxonidine (3 nmol, data not shown) in the tail-flick test. For the efaroxan antagonism experiment, the ED$_{50}$ value of dexmedetomidine was calculated to be 0.003 nmol (0.001–0.1) and the ED$_{50}$ dose was calculated to be 0.2 nmol. This constant dose of dexmedetomidine was administered with varying doses of efaroxan (0.01, 0.1, 0.3, and 1 nmol i.t.) to generate a dose-inhibition curve (Fig. 2A).

A high dose of efaroxan (1 nmol i.t.) did not affect the number of SP-elicited behaviors (data not shown). The ID$_{50}$ value of SK&F 86466 antagonism of dexmedetomidine was 1.5 nmol (0.87–2.6), which is 15-fold greater than the ID$_{50}$ value of efaroxan (0.1 nmol, 0.028–0.38) antagonism of dexmedetomidine.

SK&F 86466 and Efaroxan Antagonism of Moxonidine in ICR Mice in SP Test. On each experimental day, we generated a dose-response curve to moxonidine in ICR mice and calculated the ED$_{50}$ value from the regression line. For the SK&F 86466 antagonism experiment, the ED$_{50}$ value of moxonidine was 0.04 nmol (0.03–0.07) and the ED$_{50}$ dose was calculated to be 0.3 nmol. This constant dose of moxonidine was administered with varying doses of SK&F 86466 (0.01, 0.1, and 1 nmol i.t.) to generate a dose-antagonism curve (Fig. 2B). Similarly, we observed that a single dose of the yohimbine (0.3 nmol) effectively antagonized high-efficacy doses of moxonidine (0.3 nmol, data not shown) in the SP test.

For the efaroxan antagonism experiment, the ED$_{50}$ value of moxonidine was 0.06 nmol (0.04–0.08) and the ED$_{50}$ dose was calculated to be 0.2 nmol. This constant dose of moxonidine was administered with varying doses of efaroxan (0.001, 0.01, 0.1, and 1 nmol i.t.) to generate a dose-inhibition curve (Fig. 2B). The ID$_{50}$ value of efaroxan antagonism of moxonidine was 0.05 nmol (0.014–0.18), which did not differ significantly from the ID$_{50}$ value of SK&F 86466 (0.15 nmol, 0.079–0.29) antagonism of moxonidine.

**Adrenergic Receptor Subtype Participation in Moxonidine-Induced Antinociception**

Moxonidine Produces Antinociception in WT Mice and D79N-$\alpha_2$AR Mutant Mice in Tail-Flick and SP Nociceptive Tests. The ability of SK&F 86466 to antagonize moxonidine in ICR mice strongly suggested the participation of an $\alpha_2$AR in moxonidine-induced antinociception. Other studies suggest that the $\alpha_2$AR subtype is the primary mediator of $\alpha_2$AR agonist-mediated antinociception (Stone et al., 1997). To examine the participation of the $\alpha_2$AR subtype in moxonidine-mediated antinociception, we tested moxonidine for its ability to produce antinociception in mice with a mutation (D79N) in the $\alpha_2$AR. This mutation results in a functional disruption of the $\alpha_2$AR and an 80% knock-down in receptor binding in membrane preparations from brain (MacMillan et al., 1996). To begin, we tested for moxonidine-induced antinociception in the D79N-$\alpha_2$AR mutant mouse and WT counterpart line and compared potency to that of clonidine. Moxonidine dose-dependently (0.01, 0.03, 0.06, 0.1, and 3 nmol i.t.) produced potent antinociception in WT mice in the tail-flick test [Fig. 3A, ED$_{50}$ = 0.17 nmol (0.09–0.32)]. Clonidine also dose-dependently [1, 10, 60, and 100 nmol i.t. ED$_{50}$ = 28 nmol (11–72)] produced potent antinociception in WT mice in the tail-flick test (Fig. 3B). We also tested both moxonidine and clonidine for the inhibition of SP-induced behavior. Moxonidine [0.1, 0.3, and 1 nmol i.t. ED$_{50}$ = 0.4 (0.3–0.5)] (Fig. 4A), clonidine [0.1, 1, and 10 nmol i.t. ED$_{50}$ =...
2.7 (1.3–5.4) (Fig. 4B), and NE (0.001, 0.03, 0.1, 0.3, 1 pmol i.t. ED$_{50}$ = 0.087 pmol (0.016–0.48), Fig. 4C) all dose-dependently inhibited the SP-induced nociceptive behavior. We then evaluated moxonidine antinociception in D79N-$\alpha_2$AR mice in the tail-flick test [0.01, 0.1, 1, and 3 nmol, ED$_{50}$ = 0.32 (0.074–1.6)] and in the SP nociceptive test [0.1, 1, and 10 nmol, ED$_{50}$ = 1.1 (0.7–1.7)]. In both tests, moxonidine potency was approximately 2-fold lower in the $\alpha_2$ mutant mice than in their WT counterparts. In contrast, clonidine did not produce antinociception in the D79N-$\alpha_2$AR mice in either the tail-flick (Fig. 3B) or the SP test (Fig. 4B) at doses up to 100 (SP test) or 300 (tail-flick test) nmol. In contrast to both moxonidine and clonidine, the nonselective catecholamine $\alpha_2$AR agonist NE produced antinociception in the D79N-$\alpha_2$AR mice [0.3, 100, and 300 pmol i.t. ED$_{50}$ = 68 pmol (8.1–564), Fig. 4C] but with 780-fold decreased potency relative to their WT counterparts.

SKF-86466 and Efaroxan Dose-Dependently Antagonize Moxonidine-Induced Antinociception in SP Nociceptive Test. The ability of moxonidine to produce antinociception in both WT and D79N-$\alpha_2$AR mice suggested the participation of some $\alpha_2$AR, although perhaps not $\alpha_2$AR. However, moxonidine is also thought to act at the imidazoline receptor, which could explain its effectiveness in the D79N-$\alpha_2$AR mutant mice. To address this issue, we tested the ability of the $\alpha_2$AR-selective antagonist SK&F 86466 as well as the mixed $\alpha_2$/$\alpha_2$AR-selective antagonist efaroxan to antagonize the effects of moxonidine and dexmedetomidine WT and D79N-$\alpha_2$AR mice in the SP test.

WT Mice. ED$_{50}$ doses of moxonidine were estimated from previous dose-response curves and confirmed by administering that dose (1.46 nmol) against SP. This constant dose of moxonidine was administered with varying doses of SK&F 86466 (0.1, 0.2, 0.5, and 1 nmol i.t.) to generate a dose-inhibition curve [ID$_{50}$ = 0.4 nmol (0.3–0.5)] (Fig. 5A). Similarly, we observed that a single dose of the yohimbine (0.3 nmol) effectively antagonized high-efficacy doses of moxonidine in WT mice (0.1 nmol, data not shown). Efaroxan

![SUBSTANCE P TEST](image-url)

**Fig. 4.** Moxonidine produces spinal antinociception in the SP test in WT and D79N-$\alpha_2$AR mutant mice. A, SP test: moxonidine. Dose-response curves to moxonidine were generated in WT [ED$_{50}$ = 0.4 (0.3–0.5), ■] and D79N-$\alpha_2$AR [ED$_{50}$ = 1.1 (0.7–1.7), ●] mice. B, SP test: clonidine. Varying doses of clonidine were administered intrathecally to WT [ED$_{50}$ = 2.7 (1.3–5.4), ■] and D79N-$\alpha_2$AR (ED$_{50}$ not calculable, ●) mice. C, SP test: norepinephrine. Varying doses of NE were administered intrathecally to WT [ED$_{50}$ = 0.087 pmol (0.016–0.48), ■] and D79N-$\alpha_2$AR [ED$_{50}$ = 68 pmol (8.1–564)] mice.
Moxonidine produced potent antinociception in the tail-flick test and the SP nociceptive test in two different strains of mice: ICR and C57BL/6 × 129/Sv (WT). Two α2-AR antagonists [efaroxan (I1/2a) and SK&F 86466 (a2)] dose-dependently antagonized moxonidine-induced antinociception, confirming the requirement for α2-AR. Moxonidine potency was decreased 2-fold in the D79N-α2a mutant mice compared with their WT counterparts. This stands in sharp contrast to the complete lack of a spinal antinociceptive effect of clonidine in the D79N-α2a mutant mice and substantially decreased potency for NE. Taken together, these data suggest that moxonidine-induced antinociception is governed primarily by an α2AR subtype, but not likely the α2a-AR subtype.

**Moxonidine-Induced Antinociception.** The ability of α2-adrenergic agonists to prolong tail-flick latencies is well established (Reddy et al., 1980; Yasuoka and Yaksh, 1983; Milne et al., 1985; Solomon et al., 1989; Ossipov et al., 1990). Spinal administration of moxonidine dose-dependently increased tail-flick latency in two strains of mice: ICR (Fig. 1) and C57BL/6 × 129/Sv (Fig. 3A). Moxonidine and morphine have similar tail-flick ED50 values in both ICR mice (Fairbanks and Wilcox, 1997) and C57BL/6 × 129/Sv (WT) mice (Stone et al., 1997): in ICR mice, moxonidine (0.5 nmol, 0.3–0.7), morphine (1.2 nmol, 0.7–2.4); and in WT mice, moxonidine (0.17 nmol, 0.09–0.32), morphine (0.52 nmol, 0.36–0.74). Morphine remains the gold standard with which other analgesics are compared. The observation that moxonidine prolongs tail-flick latencies with potency comparable to that of morphine illustrates the relevance of moxonidine-induced antinociception. Like morphine, clonidine represents the gold standard for α2-AR-mediated analgesia. Interestingly, we observed that the potency of moxonidine in the tail-flick test is 100-fold greater than that of clonidine [Fairbanks and Wilcox, 1999; ED50 = 49 nmol (29–82) in ICR mice Fig. 1A] and 164-fold greater than that of clonidine in WT mice (Fig. 3A). That moxonidine is more potent than clonidine further attests to the relevance of moxonidine-induced antinociception.

These potency relationships observed in the tail-flick test generalize to a chemical test of nociception, the SP test. This test sensitively and reliably detects antinociception induced by both opioid and adrenergic agonists: morphine, [d-Ala2,N-MePhe4,Gly-ol5]-enkephalin, deltorphin II, UK-14,304, and dexmedetomidine (Hylden and Wilcox, 1983; Stone et al., 1997); all inhibit SP-induced nociceptive behavior with greater potency than they prolong tail-flick latencies (52.5°C). Like these other antinociceptive compounds, intrathecally administered moxonidine dose-dependently inhibited SP-elicted behavior in both strains of mice [ICR (Fig. 1) and C57BL/6 × 129/Sv (WT) (Fig. 4A)] with comparable potency. In the SP test in ICR mice, the ED50 value of moxonidine (0.04 nmol, 0.03–0.07) is lower than that of morphine (0.5 nmol, 0.4–0.6) but similar to that of clonidine (0.015 nmol, 0.004–0.05) (Hylden and Wilcox, 1981). In the SP test in WT mice, the ED50 value of moxonidine (0.23 nmol, 0.18–0.31) is also comparable to that of morphine (0.13 nmol, 0.05–0.3) (Stone et al., 1997) and lower than that of clonidine (2.7 nmol, 1.3–5.4). Therefore, moxonidine is either more potent than or as potent as the prototypic opioid and adrenerg-
ergic analgesics, morphine and clonidine, in both tests and both mouse strains tested here. However, predictive extrapolation from effective intrathecal doses in the rodent models reported here to analgesically effective human doses awaits further testing in other larger species. Further studies are also needed to evaluate potential hemodynamic changes evoked by spinal administration of moxonidine because systematically administered moxonidine has been reported to decrease blood pressure in WT mice (Eglen et al., 1998).

It is noteworthy that baseline nociceptive responsiveness differed to some degree in these two strains: the mean tail-flick latency in the ICR mice (3.6 ± 0.69 s, n = 31) was shorter than that of the C57BL/6 × 129/Sv strain (6.4 ± 2.6 s, n = 250). On the other hand, the C57BL/6 × 129/Sv strain appeared to be more sensitive to SP, requiring a smaller dose of SP (10 ng) than ICR mice (15 ng) to elicit 40 to 60 behaviors. Recent comparisons of nociceptive sensitivities across multiple inbred mouse lines in multiple pain tests reveal that a particular strain may show low sensitivity in one test (e.g., C57BL/6, carrageenan-induced thermal hypersensitivity) and high sensitivity in another (e.g., C57BL/6, formalin test, late phase) (Mogil, 1999). Although we did not systematically compare the nociceptive sensitivities between ICR and WT lines, we did observe that moxonidine was more potent in WT than in the ICR mice in the tail-flick test, whereas the opposite was true in the SP test. In the absence of further experiments, the relevance of these potency differences remains unclear; however, it is noteworthy that moxonidine antinociception is not specific to a particular mouse strain.

Receptor Subtype Participation in Moxonidine-Induced Antinociception. Elucidation of the specific receptors that mediate antinociceptive drug action remains an area of intense investigation. Pharmacological antagonism has proved to be effective in determining the opioid receptor subtypes (μ, δ, or κ) activated by various opioid agonists. Unfortunately, α2AR antagonists are insufficiently selective between the subtypes α2aR, α2bR, and α2cR to make similar distinctions. Generation of antisense oligonucleotides and mutant mouse lines with absent or disrupted α2aR, α2bR, and α2cR permitted studies to determine the selective function of these subtypes. These studies have concluded that the α2aR mediates opioid-adrenergic synergy (Stone et al., 1997), epileptogenesis (Janumppalli et al., 1998), and sedation (Mizobe et al., 1996; Hunter et al., 1997; Lakhiani et al., 1997). Moxonidine is an α2aR agonist; however, both moxonidine and clonidine are also thought to act at a nonadrenergic receptor, the imidazoline receptor, to produce an antinociceptive effect. The observation that efaroxan antagonized moxonidine with comparable potency to SK&F 86466 suggests that the two compounds act at the same site, specifically an α2AR receptor. These data are consistent with those of another study by Monroe et al. (1995), who tested for imidazoline receptor mediation of clonidine-induced spinal antinociception. Similar to the efaroxan/SK&F 86466 strategy, this group (Monroe et al., 1995) tested idazoxan (imidazoline) and yohimbine (nonimidazoline) for antagonism of clonidine-mediated antinociception. They showed that these two antagonists completely and equipotently blocked clonidine-induced antinociception. From these results, they concluded that the imidazoline receptor does not participate in the antinociception induced by spinal administration of clonidine.

The α2aR receptor subtype has been suggested to be the primary mediator of α2AR-mediated spinal analgesia (Stone et al., 1997). We tested moxonidine for antinociceptive efficacy in a line of mice expressing a point mutation (D79N) in the α2aR receptor, which uncouples the receptor from K⁺ and Ca²⁺ channels and reduces α2a receptor binding (MacMillan et al., 1996). Taken together, these observations support the assertion that this mouse line can be considered a functional knockout of the α2aR receptor. The antinociceptive potency of three α2AR agonists is substantially diminished in these animals (Stone et al., 1997) (Figs. 3 and 4). Dexmedetomidine inhibits SP-elicited behavior with a 2500-fold decreased potency in the D79N-α2a mice compared with their WT counterparts (Stone et al., 1997). Similarly, the antinociceptive potency of UK-14,304 is decreased 250-fold (Stone et al., 1997) and NE antinociceptive potency is decreased 780-fold in the D79N-α2a mice (Fig. 4C). Clonidine efficacy is absent (Fig. 3B, 4B) in these animals in both the SP (Fig. 4B) and tail-flick tests (Fig. 3B). In contrast, moxonidine is only 2-fold less potent in D79N-α2a animals compared with their WT controls in both the SP (Fig. 4A) and tail-flick (Fig. 3A) tests. The relevance of this small shift is questionable in light of the large decreases in potency observed in D79N-α2a mice with the other agonists; therefore, we assert that this small decrease in potency observed in the D79N-α2a mice implicates the participation of receptors other than α2aR subtype in the mediation of moxonidine-induced antinociception. To indirectly test for participation by the α2aR or α2cR subtype and the α1 receptor, we combined the strategies of pharma-
cological antagonism and genetically mutated mice by antagonizing moxonidine antinociception in the D79N-α2a mice with SK&F 84666 and efaroxan. SK&F 84666 effectively antagonized moxonidine-induced inhibition of SP-induced behavior in ICR, WT, and D79N-α2a mice (Figs. 2 and 5, A and B). The observation that SK&F 84666 antagonized the antinociceptive action in the D79N-α2a mice strongly implicates a role for some α2AR in this effect, although probably not the α2a-AR subtype. In the D79N-α2a mice, SK&F 84666 antagonized moxonidine-induced antinociception with significantly greater (4.5-fold) potency than did efaroxan. Furthermore, efaroxan antagonized moxonidine antinociception with 19-fold greater potency in the WT mice compared with the D79N-α2a mutant mice. This result may indicate a functional selectivity of efaroxan for the α2a-AR. Given that efaroxan is a mixed I1/α2-AR antagonist, the participation of imidazoline receptors in moxonidine-induced analgesia cannot be ruled out, but the antinociceptive effect of moxonidine clearly requires α2AR. The principal finding from the present experiments is that the ability of SK&F 84666 to antagonize moxonidine-induced antinociception in the D79N-α2a mutant mice solidly implicates participation of α2 adrenergic subtypes, either α2a or α2b. Further studies are required to rule the contribution of those α2AR subtypes to moxonidine-induced antinociception.

Lack of Participation of Autoreceptors on Descending Noradrenergic Terminals in Moxonidine-Induced Antinociception. It is conceivable that SK&F 84666-sensitive moxonidine-induced antinociception could require the participation of autoreceptors on descending noradrenergic terminals. In other words, moxonidine-induced antinociception could be mediated by action at autoreceptors on descending noradrenergic terminals, resulting in the release of NE to act on postsynaptic α2-AR. In support of the participation of autoreceptors and subsequent NE release, Klimscha et al. (1997) have demonstrated that intrathecally administered clonidine increased the concentration of NE in spinal cord dorsal horn microdialysate in sheep. Furthermore, Armah and colleagues (Ziegler et al., 1996) found that the hypertensive effects of moxonidine in rabbits were substantially diminished with 6-OHDA pretreatment, implicating the participation of catecholamine stores in the activity of moxonidine. The present experiments revealed that NE potency increased in animals treated with 6-OHDA compared with saline-treated control animals (Fig. 6). This apparent supersensitivity after a catecholaminergic lesion suggests increased affinity of postsynaptic α2ARs (Post et al., 1987; Swerdlow and Koob, 1989), indicating that the 6-OHDA treatment successfully destroyed descending noradrenergic terminals. If moxonidine-induced antinociception requires postsynaptic activation of α2ARs by release of NE from descending terminal, the potency of moxonidine should increase in parallel with that of the exogenously applied NE. However, moxonidine potency did not differ between 6-OHDA-treated and saline-treated animals at either 3 or 14 days after treatment (Fig. 6). We interpret this result to indicate that moxonidine-induced antinociception does not require the release of NE. This is in contrast to supersensitization observed with clonidine-mediated antinociception after 6-OHDA lesioning (Post et al., 1987). These results combined with the observation that (in these murine models of nociception) both clonidine and NE demonstrate selectivity for the α2AR suggests that the α2a-AR receptor is the subtype that becomes supersensitive after 6-OHDA administration. This selectivity could explain why moxonidine-induced antinociception does not display supersensitization after 6-OHDA lesioning. Further studies would be needed to confirm this proposal.

Conclusion. To our knowledge, this study is the first to report an antinociceptive application for the first of a new generation of antihypertensive compounds, moxonidine. Based on the scientific experience with other adrenergic agonists such as clonidine and dexmedetomidine, we anticipate that the antinociceptive effect of moxonidine will generalize to other species. Unlike antinociception induced by clonidine, dexmedetomidine, UK-14,304, and NE, moxonidine-induced antinociception requires α2AR activation independent of the α2aAR subtype. Others have suggested that differential receptor selectivities (imidazoline receptor versus α2AR) account for the improved side effect liabilities of moxonidine versus clonidine when given systemically for the treatment of hypertension. We speculate that our observed differences in α2AR subtype requirements for the effects of moxonidine and clonidine could also result in differential side effect profiles in spinal administration of moxonidine for the treatment of pain.

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