

# Meperidine Exerts Agonist Activity at the $\alpha_{2B}$ -Adrenoceptor Subtype

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**Background:** The opioid agonist meperidine has actions, such as antishivering, that are more pronounced than those of other opioid agonists and that are not blocked with nonselective opioid antagonists. Agonists at the  $\alpha_2$  adrenoceptors, such as clonidine, are very effective antishivering drugs. Preliminary evidence also indicates that meperidine interacts with  $\alpha_2$  adrenoceptors. The authors therefore studied the ability of meperidine to bind and activate each of the  $\alpha_2$ -adrenoceptor subtypes in a transfected cell system.

**Methods:** The ability of meperidine to bind to and inhibit forskolin-stimulated cyclic adenosine monophosphate formation as mediated by the three  $\alpha_2$ -adrenoceptor subtypes transiently transfected into COS-7 cells has been tested. The ability of the opioid antagonist naloxone and the  $\alpha_2$ -adrenoceptor antagonists yohimbine and RX821002 to block the analgesic action of meperidine in the hot-plate test was also assessed. The ability of meperidine to fit into the  $\alpha_{2B}$  adrenoceptor was assessed using molecular modeling techniques.

**Results:** Meperidine bound to all  $\alpha_2$ -adrenoceptor subtypes, with  $\alpha_{2B}$  having the highest affinity ( $\alpha_{2B}$ ,  $8.6 \pm 0.3 \mu\text{M}$ ;  $\alpha_{2C}$ ,  $13.6 \pm 1.5 \mu\text{M}$ ,  $P < 0.05$ ;  $\alpha_{2A}$ ,  $38.6 \pm 0.7 \mu\text{M}$ ). Morphine was ineffective at binding to any of the receptor subtypes. Meperidine inhibited the production of forskolin-stimulated cyclic adenosine monophosphate mediated by all receptor subtypes but was most effective at the  $\alpha_{2B}$  adrenoceptor ( $\alpha_{2B}$ ,  $0.6 \mu\text{M}$ ;  $\alpha_{2A}$ ,  $1.3 \text{ mM}$ ;  $\alpha_{2C}$ ,  $0.3 \text{ mM}$ ), reaching the same level of inhibition (approximately 70%) as achieved with the  $\alpha_2$ -adrenoceptor agonist dexmedetomidine. The analgesic action of meperidine was blocked by naloxone but not by the  $\alpha_2$ -adrenoceptor antagonists yohimbine and RX821002. The modeling studies demonstrated that meperidine can fit into the  $\alpha_{2B}$ -adrenoceptor subtype.

**Conclusion:** Meperidine is a potent agonist at the  $\alpha_2$  adrenoceptors at its clinically relevant concentrations, especially at the  $\alpha_{2B}$ -adrenoceptor subtype. Activation of the  $\alpha_{2B}$  receptor does not contribute significantly to the analgesic action of meperidine. This raises the possibility that some of its actions, such as antishivering, are transduced by this mechanism.

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MEPERIDINE is a potent opioid commonly used as an analgesic in postoperative settings. This compound is also frequently used to control shivering after general anesthesia. However, the mechanism for this therapeutic action of meperidine is not clear because other opioid narcotics such as sufentanil are much less effective for antishivering purposes.<sup>1</sup> Further doubt about the role of opiate receptors for the antishivering effect of meperidine is raised by the finding that naloxone pretreatment does not inhibit the effects of meperidine on postanesthetic shivering.<sup>2</sup> The administration of  $\alpha_2$ -adrenoceptor agonists, such as clonidine, are even more effective than meperidine at reducing postanesthetic shivering.<sup>3,4</sup> Recently, we showed that meperidine is able to displace a radio-labeled  $\alpha_2$ -adrenoceptor ligand from its binding site in the central nervous system (unpublished observations). There are three  $\alpha_2$ -adrenoceptor subtypes ( $\alpha_{2A}$ ,  $\alpha_{2B}$ , and  $\alpha_{2C}$ ) that are ubiquitously distributed, and each may be uniquely responsible for some but not all of the actions of  $\alpha_2$  agonists. For example, the  $\alpha_{2B}$ -adrenoceptor subtype mediates the acute hypertensive response to  $\alpha_2$  agonists,<sup>5</sup> whereas the  $\alpha_{2A}$  adrenoceptor is responsible for the anesthetic and sympatholytic responses.<sup>6</sup> In this study we investigated whether meperidine binds to cells containing the different  $\alpha_2$ -receptor subtypes and, if so, whether it functions as an agonist or antagonist. Because the  $\alpha_2$  adrenoceptors also transduce analgesic responses, we studied whether these adrenoceptors are required for the analgesic action of meperidine. Finally, we sought to model the binding of meperidine at the putative binding pocket of the  $\alpha_{2B}$ -adrenoceptor subtype.

## Methods

All reagents were obtained from Sigma (St. Louis, MO) except bovine calf serum (Gemini Bio-Products, Calabasas, CA), gentamicin (Boehringer Mannheim, Indianapolis, IN), pCDNA-3 (Invitrogen, Carlsbad, CA), [<sup>3</sup>H]RX821002 (Amersham, Piscataway, NJ), RX821002 (RBI, Natick, MA), and meperidine (AstraZeneca, Wilmington, DE).

### Transfection of Cells

Genes encoding human  $\alpha_{2A}$  (or C10,<sup>7</sup>  $\alpha_{2B}$  [or C2])<sup>8</sup> and  $\alpha_{2C}$  (or C4)<sup>9</sup> adrenoceptors were cloned into the multiple cloning site of pCDNA-3 vectors. Each adrenoceptor was expressed transiently in COS-7 cells using diethylaminoethyl-dextran-mediated transfection.<sup>10</sup> COS-7 cells were maintained in Dulbecco modified Eagle medium

supplemented with 10% bovine calf serum and 25 mg/l gentamicin.

#### *Radiolabeled Ligand Binding of Transfected Cells*

Cells were collected 3 days after transfection and rinsed twice with cold phosphate-buffered saline, and then 7 ml ice-cold lysis buffer (10 mM Tris-HCl, 1 mM EDTA, pH 7.4) was added. Cells were scraped off the flask and collected. The flask was rinsed with an additional 3 ml lysis buffer, and the washings were pooled. Cells were homogenized by four 8-s bursts at full speed using a Polytron homogenizer. The nuclei were pelleted by centrifugation at 220g for 5 min at 4°C, and the supernatant was centrifuged at 30,000g for 30 min at 4°C. The pelleted membranes were resuspended in an appropriate volume of binding buffer (75 mM Tris-HCl, 12.5 mM MgCl<sub>2</sub>, 1 mM EDTA, 0.01% CHAPS, pH 7.4). Binding experiments were performed in 500- $\mu$ l volumes of buffer for 90 min at 25°C, using a nonselective  $\alpha_2$  ligand, [<sup>3</sup>H]RX821002 (1813 GBq). The bound radiolabeled ligand was separated from the free ligand by filtration through GF/C filters using a vacuum filtration manifold (Brandel Cell Harvester; Brandel, Gaithersburg, MD). Saturation binding isotherms were performed by incubating membranes with varying concentrations of the radioligand [<sup>3</sup>H]RX821002 (2.0, 13.0, and 3.2 nM for  $\alpha_{2A}$ ,  $\alpha_{2B}$ , and  $\alpha_{2C}$  adrenoceptors, respectively), and non-specific binding was determined by adding 10  $\mu$ M rauwolscine to radiolabeled binding studies. Competition experiments were conducted by incubating membranes with varying concentrations of competing ligand (meperidine and morphine) and a constant concentration of [<sup>3</sup>H]RX821002, and nonspecific binding was determined in the presence of 10  $\mu$ M rauwolscine. Equilibrium dissociation constants ( $K_d$  and  $K_i$  values) and EC<sub>50</sub> values were determined from saturation isotherms and competition curves. Saturation isotherm data were analyzed by a nonlinear least squares curve-fitting technique, and the competition data were analyzed according to the Cheng-Prusoff equation using GraphPAD Prism (GraphPAD Software Inc., San Diego, CA).

#### *Inhibition of Forskolin-stimulated Adenylyl Cyclase*

Cells were collected as for radiolabeled ligand binding of transfected cells. Membranes from cells (at least  $1.5 \times 10^6$  for each 24-well assay) were prepared fresh just before use by homogenizing in 600  $\mu$ l of ice-cold assay buffer (75 mM Tris, 25 mM MgCl<sub>2</sub>, 2 mM EDTA, pH 7.4) with 15 up-down strokes in a 2-ml glass homogenizer. Membrane preparation (20  $\mu$ l) was added to 20  $\mu$ l of "cyclase mixture" (2.7 mM mono(cyclohexylammonium) phosphoenolpyruvate, 53  $\mu$ M GTP3Na.3 H<sub>2</sub>O, 0.2 IU pyruvate kinase, 1.0 IU myokinase, 125  $\mu$ M adenosine triphosphate) and 10  $\mu$ l of "reaction mixture." To determine the inhibition of forskolin-stimulated adenylyl cyclase activity by meperidine and dexmedetomidine, the

reaction mixture was composed of 100  $\mu$ M forskolin and varying concentrations of meperidine or dexmedetomidine. Furthermore, to investigate the effect of rauwolscine on the inhibition of forskolin-stimulated adenylyl cyclase activity by meperidine, the reaction mixture was composed of 100  $\mu$ M forskolin, 0.5  $\mu$ M meperidine, and 20  $\mu$ M rauwolscine. After addition of the membrane preparation, the cells were shaken (220 rpm) at 37°C for 10 min. The reaction was terminated by adding 10  $\mu$ l ice-cold 0.8 M perchloric acid. The samples were left at room temperature for 20 min, after which 15  $\mu$ l of 2N KOH in 25 mM HEPES was added. Tubes were centrifuged for 5 min in a microcentrifuge, and the supernatant was removed and assayed for cyclic adenosine monophosphate (cAMP) by the scintillation proximity assay kit (Amersham Pharmacia Biotech, Piscataway, NJ). Reaction mixture-inhibited cAMP accumulation was expressed as a percent of forskolin-alone stimulation; these data were analyzed by a nonlinear least squares curve-fitting technique, and EC<sub>50</sub> values were determined using GraphPAD Prism (Graph-Pad Software Inc.).

#### *Analgesic Effects of Opioids*

**Experimental Animals.** All experimental protocols were reviewed and approved by the Subcommittee for Animal Studies (Veterans Affairs Palo Alto Health Care System, Palo Alto, CA) before the initiation of work. All protocols conform to the guidelines for the study of pain in awake animals as established by the International Association for the Study of Pain. Every effort was made to minimize animal suffering and to use only the number of animals necessary to produce reliable scientific data. In all studies presented, male C57BL/6 mice aged 12–16 weeks were used (Jackson Labs, Bar Harbor, ME). These mice were kept in cages (4–6 mice per cage) with a light-dark cycle of 12 h–12 h. Food and water were provided *ad libitum*. Animals were weighed on the day of experimentation for the calculation of drug dosages.

**Drug Administration.** Drugs were injected alone or in combination subcutaneously in a total volume of approximately 50  $\mu$ l. The animals were returned to their cages for 25 min after the injection of drugs and before hot-plate assay.

**Hot-plate Assay.** The hot-plate assay was performed as described by O'Callaghan and Holtzman,<sup>11</sup> with equipment from IITC (Woodland Hills, CA). The hot plate was set thermostatically at  $54 \pm 0.1^\circ\text{C}$ . Mice were placed in a rectangular clear enclosure on the hot plate, and the time to licking of a hind paw, the endpoint of the assay, was measured with a stopwatch. Animals were left on the hot plate no longer than 60 s to prevent injury.

#### *Molecular Modeling*

Initial transmembrane topology and secondary structure predictions were made by submitting the sequence of the human  $\alpha_{2B}$  adrenoceptor to the Predict Protein

Server at Columbia University (New York, NY, on August 19, 2000). The PHDhtm algorithm was used to make the topology predictions.<sup>12</sup> The secondary structure analysis of the rhodopsin template (chain A of protein ID 1F88 in the protein database of the Research Collaboratory for Structural Bioinformatics\*\*), and the production of an annotated template file was performed using the Kabsch and Sander<sup>13</sup> algorithm. The Seqfold algorithm,<sup>14</sup> available through the InsightII 2000 Modeling Environment (Molecular Simulations, Inc., San Diego, CA), was then used to align the sequence of the  $\alpha_{2A}$  adrenoceptor to that of bovine rhodopsin. This algorithm allows the alignment of a sequence of unknown 3-dimensional structure to a template of known 3-dimensional structure based on sequence similarity, as scored by the Gonnet matrix,<sup>15</sup> as well as fold similarity. Further refinement of the sequence alignment was made by importing it into the SwissPDB Viewer<sup>16</sup> and calculating the mean force potential of Sipp1<sup>17</sup> for several additional manual sequence threadings. Final alignment of the sequence of the  $\alpha_{2B}$  receptor to the rhodopsin template was refined based on the experimental data derived in this laboratory. The coordinates of the atoms within the  $\alpha_{2B}$  receptor were then assigned. Side chains were initially optimized using the rotamer refinement function of the SwissPDB Viewer.<sup>16</sup> Energy optimization of the structure was performed using the Hyperchem 5.1 software package (Hypercube Inc., Gainesville, FL) with the Amber 3.0 all-atom forcefield. This was performed initially only on the side chains and then on the entire structure without restraints. Optimization was initially performed *via* the steepest descents method followed by the method of Polak-Ribiere.

The initial meperidine molecule was constructed and optimized using the Builder module within InsightII 2000. A model of the  $\alpha_{2B}$  adrenoceptor was constructed based on homology modeling techniques. In an attempt to understand the selective binding of meperidine to the human  $\alpha_{2B}$ -adrenoceptor subtype, simulated docking experiments were performed using the Affinity module within the InsightII 2000 Modeling Environment (Molecular Simulations, Inc.).

#### Statistical and Data Analysis

Statistical significance was determined by using one-way analysis of variance followed by Bonferroni multiple comparison tests. The  $EC_{50}$  of the cAMP inhibition curves were calculated by nonlinear regression fitting to a sigmoidal dose-response model with variable slope.

## Results

Analysis of the equilibrium dissociation constants ( $K_D$ ) of [<sup>3</sup>H]RX821002 for human  $\alpha_2$ -adrenoceptor subtypes

\*\* Rutgers University Protein Data Bank (PDB). Available at: <http://www.rcsb.org/pdb/>. Accessed August 20, 2000.

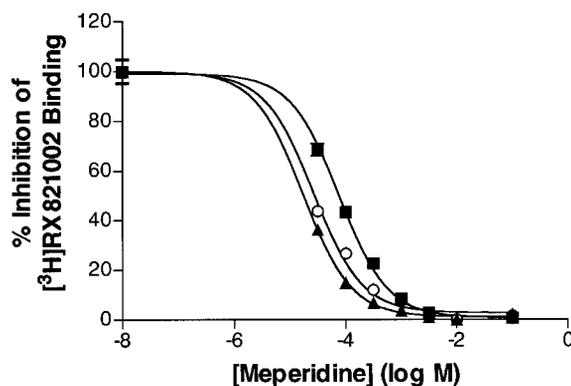


Fig. 1. Individual competition binding curves of meperidine against [<sup>3</sup>H]RX821002 for  $\alpha_{2A}$ -adrenoceptor (filled square),  $\alpha_{2B}$ -adrenoceptor (filled triangle), and  $\alpha_{2C}$ -adrenoceptor (open circles) subtypes.

showed that the ligand has lowest affinity for the human  $\alpha_{2B}$ -adrenoceptor subtype ( $\alpha_{2B}$ ,  $13.1 \pm 1.5$  nM;  $\alpha_{2A}$ ,  $2.2 \pm 0.3$  nM;  $\alpha_{2C}$ ,  $6.2 \pm 0.3$  nM; mean  $\pm$  SD,  $P < 0.01$ ,  $n = 4$ ). Meperidine competes for the binding of [<sup>3</sup>H]RX821002 for each of the human  $\alpha_2$ -adrenoceptor subtypes (fig. 1); morphine, another opioid ligand, does not displace the radiolabeled ligand off any of the human  $\alpha_2$ -adrenoceptor subtypes (fig. 2). Analysis of the inhibitory constant of meperidine showed that it has highest affinity for the human  $\alpha_{2B}$ -adrenoceptor subtype ( $\alpha_{2B}$ ,  $8.6 \pm 0.3$   $\mu$ M;  $\alpha_{2C}$ ,  $13.6 \pm 1.5$   $\mu$ M,  $P < 0.05$ ;  $\alpha_{2A}$ ,  $38.6 \pm 0.7$   $\mu$ M,  $P < 0.001$  from the  $\alpha_{2B}$ -receptor value; mean  $\pm$  SD,  $n = 4$ ).

Meperidine potently inhibits forskolin-stimulated adenylyl cyclase activities in cells transfected with the human  $\alpha_{2B}$ -adrenoceptor subtype over the same concentration range as its analgesic activity (0.63  $\mu$ M). The inhibitory action of meperidine on forskolin-stimulated adenylyl cyclase activity at the  $\alpha_{2B}$  receptor, as defined as the  $EC_{50}$  that achieved half maximal inhibition, is approximately 1,000-fold less than is required to reach the same level of inhibition with the other receptor subtypes ( $\alpha_{2A}$ , 1.3 mM;  $\alpha_{2C}$ , 0.3 mM; fig. 3). In fact,

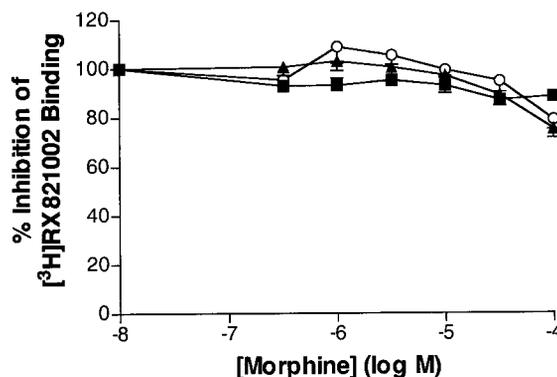


Fig. 2. Morphine does not completely displace [<sup>3</sup>H]RX821002 binding to either the  $\alpha_{2A}$ -adrenoceptor (filled square),  $\alpha_{2B}$ -adrenoceptor (filled triangle), and  $\alpha_{2C}$ -adrenoceptor (open circle) subtypes.

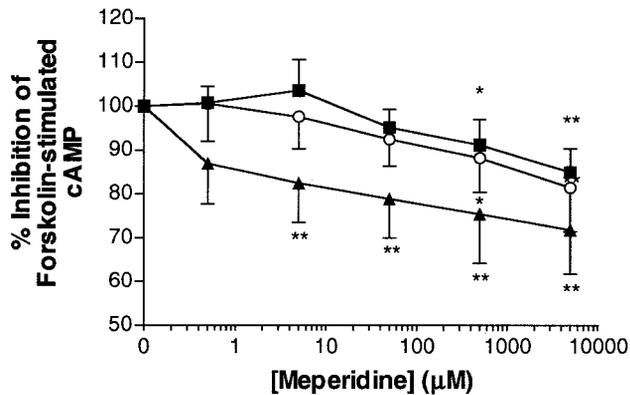


Fig. 3. Meperidine has agonist activity at inhibiting forskolin-stimulated cyclic adenosine monophosphate (cAMP) production at all receptor subtypes but is more potent on the  $\alpha_{2B}$ -adrenoceptor subtype (filled triangle) than the  $\alpha_{2A}$ -adrenoceptor (filled square) and  $\alpha_{2C}$ -adrenoceptor (open circle) subtypes. Mean  $\pm$  SD,  $n = 4$ . \*\* $P < 0.01$ ; \* $P < 0.05$ .

meperidine is as efficacious as dexmedetomidine, the highly selective and potent  $\alpha_2$  agonist, at inhibiting forskolin-stimulated adenylyl cyclase activities in cells transfected with the  $\alpha_{2B}$ -adrenoceptor subtype (fig. 4). That meperidine exerts its inhibitory action through  $\alpha_2$  adrenoceptors itself is evident from the fact that the inhibitory action of meperidine (50  $\mu\text{M}$ ) on  $\alpha_{2B}$ -mediated inhibition of forskolin-stimulated cAMP production is blocked by the  $\alpha_2$ -receptor antagonist rauwolscine (20  $\mu\text{M}$ ) (meperidine,  $73.8 \pm 5.1\%$ ; meperidine plus rauwolscine,  $101 \pm 2.5\%$  of control level; mean  $\pm$  SD,  $P < 0.01$ ,  $n = 4$ ).

The data in figure 5 demonstrate that mice treated with 50 mg/kg meperidine had prolonged latencies for hind-paw licking when compared with saline-injected animals. Preliminary experiments established that 50 mg/kg meperidine was an approximate  $\text{ED}_{50}$  for meperidine in this assay. This antinociceptive effect was completely blocked by the coadministration of 1 mg/kg naloxone,

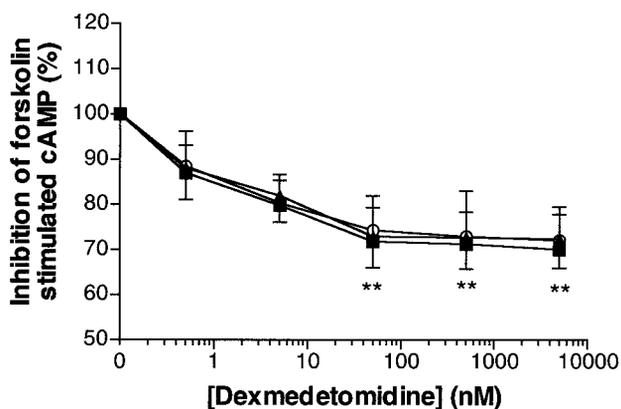


Fig. 4. Dexmedetomidine has equal agonist activity at inhibiting forskolin-stimulated cyclic adenosine monophosphate (cAMP) production at the  $\alpha_{2A}$ -adrenoceptor (filled square),  $\alpha_{2B}$ -adrenoceptor (filled triangle), and  $\alpha_{2C}$ -adrenoceptor (open circle) subtypes. Mean  $\pm$  SD,  $n = 4$ . \*\* $P < 0.01$  for all three receptor subtypes at concentrations greater than 50 nM.

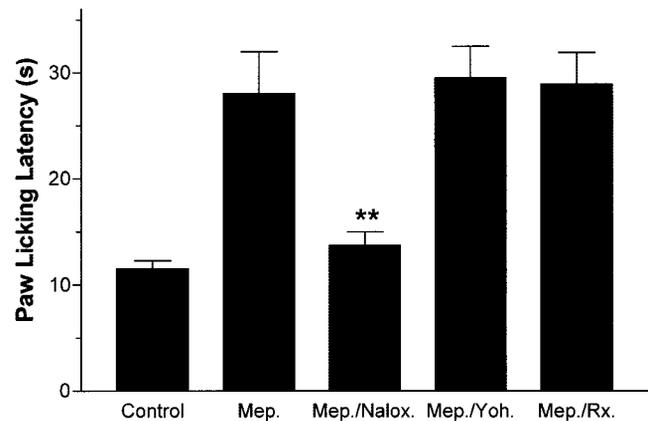


Fig. 5. The effects of various drugs in the hot-plate assay. Mice were injected subcutaneously with the indicated drugs 25 min before hot-plate assay. Mep = meperidine; Nalox = naloxone; Yoh = yohimbine; RX = RX 821002. \*\* $P < 0.01$ .

an opioid receptor antagonist. However, neither of two  $\alpha_2$ -adrenoceptor antagonists, namely, the nonimidazoline yohimbine (1 mg/kg) or the imidazoline RX 821002 (2 mg/kg), significantly reduced the meperidine-mediated analgesia. These doses of  $\alpha_2$ -adrenoceptor antagonists were previously shown to antagonize the antinociceptive effects of clonidine in mice when using the hot-plate assay.<sup>18</sup>

Assuming that the amino acid residues that could be affecting binding are within 0.4 nm of the ligand, there is a potential binding pocket made up of transmembrane domains 3, 4, 5, 6, and 7 (fig. 6). Further examination within this 4-Å radius reveals the amino acids in the  $\alpha_{2B}$  adrenoceptor, which differ from their counterparts in the  $\alpha_{2A}$  adrenoceptor. They are LEU 145, ILE 173, SER 177, and SER 390. The corresponding residues in the  $\alpha_{2A}$  adrenoceptor are PHE 166, VAL 197, CYS 201, and THR 393, respectively, which will impose different constraints on the ability of the ligand to dock (fig. 6). The corresponding residues in the  $\alpha_{2C}$  adrenoceptor are PHE 184, ILE 211, CYS 215, and ILE 400. Another possible residue that is in close proximity to the meperidine (but just outside of the arbitrary 4-Å limit) is GLY 394 on the  $\alpha_{2B}$  adrenoceptor; the corresponding residue on the other receptor subtypes is THR 397 in the  $\alpha_{2A}$  adrenoceptor and TYR 404 in the  $\alpha_{2C}$  adrenoceptor. Thus, there exist structural reasons why meperidine should have activity at one receptor subtype and not another.

## Discussion

The opiate receptor agonist meperidine competes directly with  $\alpha_2$ -adrenoceptor ligands for the human  $\alpha_2$ -adrenoceptor subtypes (fig. 1). Morphine, another  $\mu$ -opiate receptor agonist, has no affinity for these sites at clinically relevant concentrations (fig. 2). Meperidine has highest affinity for the  $\alpha_{2B}$ -adrenoceptor subtype, where

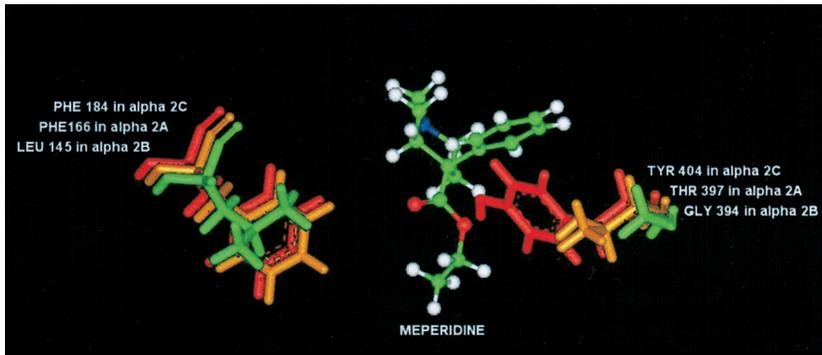


Fig. 6. Computer simulations demonstrate meperidine docking in proximity to the relevant residues for each of the  $\alpha_2$  adrenoceptors. The residues in the  $\alpha_{2A}$  (PHE and THR in orange) and  $\alpha_{2C}$  (PHE and TYR in red) adrenoceptors are very dissimilar to their respective, homologous residues in the  $\alpha_{2B}$  receptor (LEU, GLY in green). This suggests possible reasons for subtype-specific high affinity binding of meperidine to the  $\alpha_{2B}$  receptor.

it exerts comparable agonist efficacy to that seen with the highly selective  $\alpha_2$  agonist dexmedetomidine (fig. 4). This agonist action is blocked by an  $\alpha_2$  antagonist. The analgesic action of meperidine is not blocked by  $\alpha_2$  antagonists but is blocked by an opiate antagonist.

The  $\alpha_2$ -adrenoceptor agonists are either imidazoline, guanidinium, or phenylethanolamine compounds and are structurally dissimilar from the phenylpiperidine meperidine. However, there are examples of other piperidine compounds that exert their pharmacologic activity at the  $\alpha_2$  adrenoceptor.<sup>19</sup> Interestingly, another piperidine compound causes a diuretic action *via* renal  $\alpha_2$  adrenoceptors<sup>20</sup> and is considered to be mostly, if not exclusively, of the  $\alpha_{2B}$  variety. Furthermore, as is seen in the results of the molecular modeling, there appear to be structural differences that may grossly account for the differential, subtype-specific binding of meperidine. In particular, there are probably differences in the binding pocket size and relative affinity for meperidine in the  $\alpha_{2A}$  adrenoceptor and the  $\alpha_{2C}$  adrenoceptor because of the proximity of a phenylalanine in the binding pocket, where there is a much smaller ILE in the more favorable binding pocket of the  $\alpha_{2B}$  adrenoceptor. Likewise, there is a glycine in the  $\alpha_{2B}$  adrenoceptor, whereas there are the much larger and more polar THR in the  $\alpha_{2A}$  adrenoceptor and the TYR in the  $\alpha_{2C}$  adrenoceptor.

The fact that meperidine is a potent agonist at the  $\alpha_2$  adrenoceptors raises the possibility that some of its actions are transduced by this mechanism. In reviewing the pharmacologic actions of  $\alpha_2$ -adrenoceptor agonists and those of meperidine, there is correspondence for analgesia and antishivering actions. The free plasma concentration of meperidine corresponding to antishivering effect is approximately 1–3  $\mu\text{M}$ .<sup>21</sup> To reduce cAMP concentrations to 50% of maximal, a concentration of 1–3  $\mu\text{M}$  meperidine is certainly sufficient. It is unknown what proportion of  $\alpha_{2B}$  adrenoceptors must be occupied by meperidine to achieve an antishivering effect, but because 1  $\mu\text{M}$  meperidine has significant cyclase inhibitory power at the  $\alpha_{2B}$  receptor, it is feasible that 1–3  $\mu\text{M}$  would be sufficient. These clinically achieved meperidine concentrations would also be analgesic.<sup>22</sup> We have effectively ruled out the participation of  $\alpha_2$  adrenoceptors in the analgesic action of meperidine because nei-

ther the imidazoline (RX821002) nor the nonimidazoline (yohimbine)  $\alpha_2$ -adrenoceptor antagonists attenuated its antinociceptive action. As expected, the analgesic action of meperidine was reversed by a nonselective opiate receptor antagonist.

Of the many responses transduced by  $\alpha_2$  adrenoceptors, few are transduced by the  $\alpha_{2B}$ -adrenoceptor subtype.<sup>23</sup> Of the  $\alpha_2$ -adrenoceptor-mediated actions attributed to the  $\alpha_{2B}$  subtype, the most well substantiated is the direct vasoconstrictive response in the peripheral vasculature.<sup>5</sup> Concerning the analgesic action of the  $\alpha_2$  agonists, earlier pharmacologic evidence suggested that only the  $\alpha_{2A}$  subtype transduced the analgesic action<sup>24,25</sup>; however, evidence is now accumulating for a role for the other two subtypes. Prazosin-sensitive  $\alpha_2$ -adrenoceptor subtypes (either the  $\alpha_{2B}$  or  $\alpha_{2C}$ ) were shown to inhibit release of substance P in a spinal cord preparation.<sup>26</sup> In addition, ST-91, a non- $\alpha_{2A}$  subtype preferring  $\alpha_2$  agonist, was shown to induce antinociception in rats when it was administered intrathecally, an effect that was blocked by prazosin.<sup>27–30</sup> We recently showed that the antinociceptive action of nitrous oxide was reduced by 65% in the hot-plate assay and totally absent in the tail-flick assay in mice lacking the  $\alpha_{2B}$ -adrenoceptor subtype. These data therefore suggested that the  $\alpha_{2B}$ -adrenoceptor subtype has a role to play in nociception. However, there are no direct data implicating one or other  $\alpha_2$ -adrenoceptor subtype for the antishivering action of  $\alpha_2$  agonists.

Postanesthetic shivering occurs through a disorder in thermoregulation partly caused by differential recovery of excitatory and inhibitory centers. Thermoregulation is composed of multiple feedback systems and neurotransmitters together with the integration of information at each of several levels, including afferent impulses, hypothalamic centers, and skeletal muscles.<sup>31</sup> Based largely on responsiveness to pharmacologic probes, adrenergic,<sup>32</sup> serotonergic,<sup>3,33</sup> and cholinergic<sup>34</sup> pathways have each been causally implicated. Neuroanatomic studies have demonstrated that noradrenergic nuclei in the locus coeruleus are interconnected with hypothalamic thermoregulatory centers<sup>35–37</sup>; furthermore, there is evidence that  $\alpha$  adrenoceptors mediate the decrease in temperature after central administration of norepineph-

rine.<sup>38</sup> The distribution of the  $\alpha_{2B}$ -adrenoceptor subtype may shed light on whether meperidine transduces its antishivering action through the  $\alpha_{2B}$ -adrenoceptor subtype. In rat spinal cord and dorsal root ganglion, mRNA for the  $\alpha_{2A}$ -,  $\alpha_{2B}$ -, and  $\alpha_{2C}$ -adrenoceptor subtypes have been identified. Within the brain, the most prevalent sites for  $\alpha_{2B}$  mRNA are in the thalamus and the cerebellum.<sup>39</sup>

The antishivering action of meperidine has previously been attributed to its action on  $\kappa$ -opioid receptors. After administration of naloxone, an opioid receptor antagonist that blocks both  $\mu$  receptors and  $\kappa$  receptors at high doses and only  $\mu$  receptors when administered at low doses, a differential effect of meperidine was revealed. Only a high dose of naloxone was able to prevent the effect of meperidine on shivering; when administered in low doses, naloxone did not influence the action of meperidine, thus indicating that  $\kappa$  receptors and not  $\mu$  receptors may mediate its antishivering action.<sup>2</sup> However, other more  $\kappa$ -receptor-selective drugs, such as pentazocine, failed to inhibit postanesthetic shivering, thus suggesting that  $\kappa$ -opioid receptors are not implicated.<sup>40</sup> In addition, the effect of meperidine on shivering is more effective than that of pure  $\mu$ -opioid receptor agonists at equianalgesic doses.<sup>1,41</sup> Thus, an  $\alpha_2$ -adrenoceptor-mediated mechanism of action of meperidine on postanesthetic shivering as suggested by the data presented here is conceivable. Clinically,  $\alpha_2$ -adrenoceptor agonists such as clonidine have been successfully used both for prevention and treatment of postanesthetic shivering,<sup>3,42-45</sup> indicating that  $\alpha_2$  adrenoceptors mediate this action. Our current findings suggest that it is likely to be caused by the activation of the  $\alpha_{2B}$ -adrenoceptor subtype.

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