Novel Molecular Targets of Dezocine and Their Clinical Implications

Renyu Liu, M.D., Ph.D., Xi-Ping Huang, Ph.D., Alexei Yeliseev, Ph.D., Jin Xi, M.S.,
Bryan L. Roth, M.D., Ph.D.

ABSTRACT

Background: Although dezocine is a partial μ-opioid receptor agonist, it is not a controlled substance. Thus, the characterization of the molecular targets of dezocine is critical for scientific and clinical implications. The goal of this study is to characterize molecular targets for dezocine and determine their implications.

Methods: A binding screen for dezocine was performed on 44 available receptors and transporter proteins. Functional assays for the novel targets were performed along with computation calculations to locate the binding site. A G protein activation study was performed for the human κ opioid receptor to determine whether dezocine is a κ-antagonist. Data are presented as mean ± standard error.

Results: The affinities for dezocine were 3.7 ± 0.7 nM for the μ receptor, 527 ± 70 nM for the δ-receptor, and 31.9 ± 1.9 nM for the κ-receptor. Dezocine failed to induce G protein activation with κ-opioid receptor and concentration dependently inhibited κ-agonist (salvinorin A and nalbuphine)–induced receptor activation, indicating that dezocine is a κ-antagonist. Two novel molecular targets (norepinephrine transporter and serotonin transporter) were identified. Dezocine concentration-dependently inhibited norepinephrine and serotonin reuptake *in vitro*. The half maximal inhibitory concentrations (expressed as pIC50) were 5.68 ± 0.11 for norepinephrine transporter and 5.86 ± 0.17 for serotonin transporter. Dezocine occupied the binding site for known norepinephrine transporter and serotonin transporter inhibitors.

Conclusions: The unique molecular pharmacological profile of dezocine as a partial μ-receptor agonist, a κ-receptor antagonist, and a norepinephrine and serotonin reuptake inhibitor (*via* norepinephrine transporter and serotonin transporter) was revealed. These discoveries reveal potentially important novel clinical implications and drug interactions of dezocine.
(Anesthesiology 2014; 120:714-23)

DEZOCINE is an opioid medication that is structurally similar to pentazocine, a mixed opioid receptor partial agonist/antagonist developed in 1970s by the American Home Products Corporation.[1] Dezocine was approved by the Food and Drug Administration for perioperative care management but was discontinued with the closure of its parent company. PubMed lists only 74 articles related to dezocine,* with the first article published in 1978 in *Anesthesia & Analgesia*, demonstrating its use for postoperative pain management.[1] Although no longer used clinically in Western countries, dezocine is gaining popularity in China as an alternative medication for perioperative pain management.[2]

Dezocine is an opioid μ-receptor partial agonist/antagonist.[1] Because of its partial μ-agonism, it exhibits a "ceiling effect" for respiratory depression (a notorious and fatal side effect caused by commonly used clinical opiates). Though initially identified as a κ-receptor agonist, a later study suggests that dezocine is a κ-receptor antagonist.[3] Further study is needed to resolve this discrepancy. Because dezocine is a partial μ-antagonist, in theory, a concerted use of dezocine together with a μ-receptor agonist like morphine should decrease the analgesia effect of

> ### What We Already Know about This Topic
>
> - Dezocine is a partial μ opioid receptor agonist
> - Dezocine was thought to be a κ opioid receptor agonist, but recent data suggest it could be a κ opioid receptor antagonist
> - It is not listed as a controlled substance in the United States
>
> ### What This Article Tells Us That Is New
>
> - Dezocine is a κ opioid receptor antagonist
> - Dezocine also interacts with the norepinephrine transporter and the serotonin transporter and inhibits norepinephrine and serotonin reuptake *in vitro* in a concentration-dependent manner
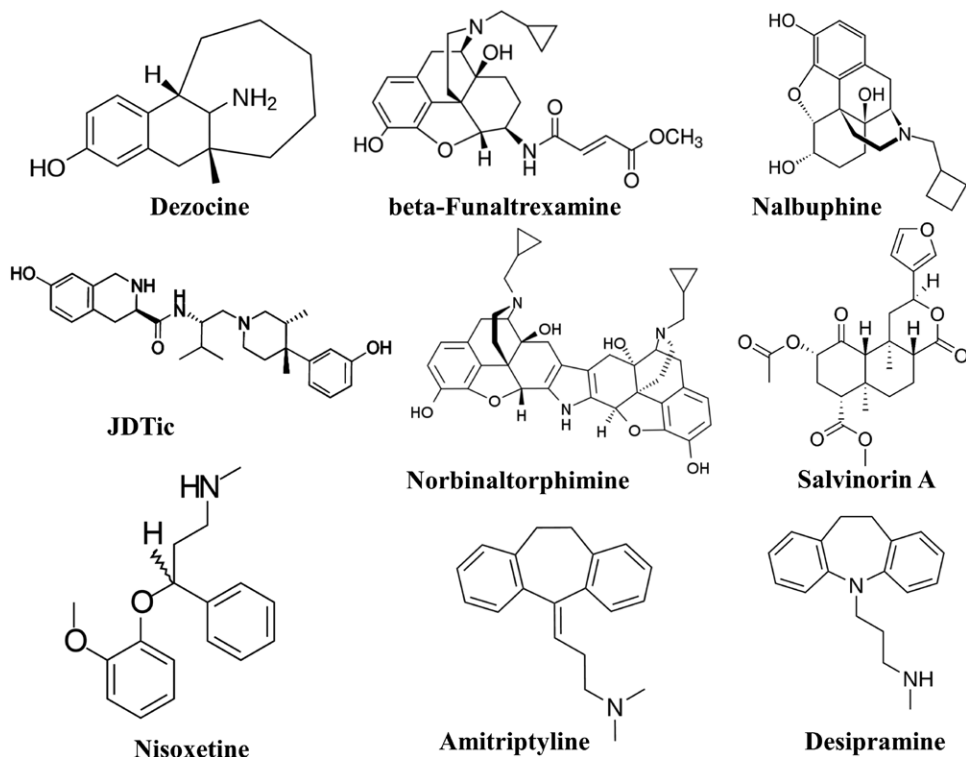
morphine significantly. However, it is reported that the combination of morphine and dezocine increases the analgesic effects significantly,[4] indicating that dezocine may induce analgesia through an additional mechanism(s).

Opiate receptors belong to the G protein coupled receptor family. It is highly possible that, in addition to opiate receptors, opioids could interact with other G protein coupled

* Available at: www.pubmed.com. Accessed April 18, 2013.

Fig. 1. The structures of the ligands used in this study to probe the pharmacological properties of dezocine are listed. All structures, except salvinorin A and JDTic, were obtained from public domain without further modification or verification by a chemist. Salvinorin A (http://commons.wikimedia.org/wiki/File:Salvinorin-A_structure.png) and JDTic (http://commons.wikimedia.org/wiki/File:JDTic_cas_361444-66–8.svg) are obtained from Wikimedia Commons and are licensed under the Creative Commons Attribution-Share Alike 3.0 Unported license.

receptors. In this study, we hypothesized that dezocine might also act at other membrane receptors, and we therefore screened a large group of available recombinant G protein coupled receptors and transporter proteins in an attempt to identify novel pharmacological targets for dezocine. We further investigated whether dezocine is a κ-receptor agonist or antagonist. The molecular interactions with the target proteins were analyzed using available crystal structures and molecular models for docking calculations.

Materials and Methods

All chemicals (except for those specified otherwise) were obtained from Sigma-Aldrich (St. Louis, MO) and were reagent grade or higher. Dezocine was obtained from Yangtz River pharmaceutical group (Taizhou, Jiangsu, China), with 99.9% purity. All chemicals were used without further purification. The chemical structures of the ligands that have the same targets as dezocine and have been used for comparison purposes in this study are listed in figure 1.

Radioligand Binding Assays and Affinity Determination

A primary binding screen for dezocine was performed on 44 available receptors (mostly G protein coupled receptors;

† Available at: http://pdsp.med.unc.edu/PDSP%20Protocols%20II%202013-03-28.pdf, pages 18–24. Accessed October 15, 2013.

table 1). Evidence for interaction was based on the inhibition of the reference ligand-binding signal. Dezocine was diluted in standard binding buffer (50 mM Tris-HCl, 10 mM MgCl$_2$, 0.1 mM EDTA, pH 7.4) to a final concentration of 10 µM. In brief, 50 µl aliquots of radioactive ligand (5 nM) were added to wells of a 96-well plate, which contained 25 µl of the reference or test ligands. We used transfected cell lines expressing mainly human (unless otherwise specified) recombinant receptors, monoamine transporters, or ion channels for crude membrane preparation. Detailed information about our membrane preparation can be obtained from the protocol online.† Crude membrane fractions containing the receptors were resuspended in standard binding buffer and 50 µl aliquots added to each well. The reactions were incubated at room temperature for 1.5 h to allow for radioligand binding equilibration. Bound radioactivity was harvested by rapid filtration through a 0.3% polyethyleneimine-treated, 96-well filter mats by using a 96-well Filtermate harvester (PerkinElmer, Waltham, MA). The dried filters were treated with melted scintillant, and a Microbeta scintillation counter (PerkinElmer) was used to measure the radioactivity retained on the filter.

The secondary binding assay was performed only when the inhibition in the primary screen was more than 50%. This assay was used to determine the binding affinity for the

Table 1. Receptor and Ion Channels Screened in This Study

Receptor Name	Receptor Subtypes
Serotonin receptors	5-HT1A, 5-HT1B, 5-HT1D, 5-HT1E, 5-HT2A, 5-HT2B,5-HT2C, 5-HT3, 5-HT5A, 5-HT6, 5-HT7
Adrenoceptor	Alpha 1A, alpha 1B, alpha 1D, alpha 2A, alpha 2B, alpha 2C, Beta 1, Beta 2, Beta 3
Dopamine receptors	D1, D2, D3, D4, D5
GABA receptors	GABA-A, rat benzodiazepine site
Muscarinic receptors	M1, M2, M3, M4, M5
Sigma receptors	Sigma 1, Sigma 2
Histamine receptors	H1, H2, H3, H4
Biogenic amine transporters	Serotonin transporter; norepinephrine transporter; dopamine transporter
Opioid receptors	μ-receptor; κ-receptor; δ-receptor

All the proteins are from humans except for Sigma 2 and benzodiazepine site, which are from rat.

Alpha = alpha adrenergic receptor; Beta = beta adrenergic receptor; D = dopamine receptor; GABA = γ-aminobutyric acid receptor; H = histamine receptor; M = muscarinic receptor; Sigma = sigma receptor; 5-HT = serotonin receptor.

identified receptor. Dezocine was prepared in standard binding buffer and serially diluted to the desired concentrations.

Radioactive ligand (5 nM) aliquots of 50 μl were added to wells of a 96-well plate, which contained 25 μl aliquots of dezocine; 50 μl of a crude membrane fraction of cells expressing the respective receptors were applied to each well. Reaction incubation, harvesting, and radioactivity measurement procedures from the primary assay were repeated. Affinity is expressed as Ki or pKi (–logKi).

G Protein Activation by κ-receptor Treated with Agonists, Partial Agonists, and Antagonists

Membrane preparations of recombinant human κ-opioid receptor expressed in the mammalian cell line Chem-5 were obtained from Millipore (Billerica, MA). The effects of specific κ-opioid receptor ligands on the activation of the recombinant receptor were investigated by measuring G protein activation *in vitro*. Nalbuphine and salvinorin A (full agonist) and nor-binaltorphimine (antagonist) were used as controls.

The assay reports the initial rates of activation of heterotrimeric G proteins ($G\alpha_{i1}\beta_1\gamma_2$) on an agonist-bound receptor by measuring the accumulation of [35S]-(guanosine 5'-O-[gamma-thio]triphosphate, nonhydrollyzable analog of guanosine triphosphate) bound to the activated $G\alpha_{i1}$ subunit. Myristoylated $G\alpha_{i1}$ was expressed in *Escherichia coli* and purified as previously described.[5] Recombinant human $\beta_1\gamma_2$ subunits of G protein were expressed in baculovirus-infected Sf9 cells and purified as previously described.[6] The G protein activation assay was conducted as follows (final concentrations in 50 μl reaction mixture are given in parentheses): the membrane sample was diluted into ice-cold 10 mM 3-(N-morpholino) propanesulfonic acid buffer to reach a protein concentration of 40 ng/μl. Ten microliter of the diluted dispersion was dispensed into presiliconized glass tubes and mixed with the ligand in 3-(N-morpholino)propanesulfonic acid buffer containing 0.1% (w/v) bovine serum albumin. Upon addition of a mixture of $G_{\alpha i1}$ (100 nM) and $G_{\beta1\gamma2}$ (500 nM), the tubes were incubated on ice for 30 min. The reaction was started by addition of 3-(N-morpholino)propanesulfonic acid buffer pH = 7.5 (50 mM), EDTA (1 mM), $MgCl_2$ (3 mM), guanosine diphosphate (4 μM), bovine serum albumin (0.3% w/v), NaCl (100 mM), dithiothreitol (1 mM), and [35S]-GTPγS (5 nM, 1,250 Ci/mmol) followed by rapid transfer of the tubes to a water bath at 30°C. The incubation continued for 45 min. The reaction was terminated by addition of 2 ml of ice-cold stop solution, TNMg (20 mM Tris-HCl pH = 8.0, 100 mM NaCl, and 25 mM $MgCl_2$). The reaction mixture was rapidly filtered through nitrocellulose filters (Millipore). Filters were washed four times with 2 ml each of cold TNMg buffer, dried, placed in scintillation vials filled with ScintiSafe Econo F scintillation liquid (Fisher, Waltham, MA), and the radioactivity counted. Duplicate samples corresponding to every ligand concentration point were counted.

To test whether dezocine could antagonize the full agonists, the κ-receptor was preactivated with either nalbuphine (250 nM) or salvinorin A (20 nM), a highly selective nonopioid κ-receptor agonist with strong affinity.[7] The κ-receptor was then treated with increasing concentrations of dezocine.

Norepinephrine Transporter and Serotonin Transporter Reuptake Assay

The potency of dezocine as an inhibitor of norepinephrine and serotonin uptake on human cloned norepinephrine transporter (NET) and serotonin transporter (SERT), stably expressed in human embryonic kidney 293 cells, was determined using the neurotransmitter assay kit from the Molecular Devices (Sunnyvale, CA) as described previously.[8] In brief, human embryonic kidney 293 cells were plated in poly-L-lys coated 384-well, black, clear-bottom cell culture plates in Dulbecco's modified eagle medium + 1% dialyzed fetal bovine serum, at a density of 15,000 cells per well in a total volume of 40 μl. The cells were incubated for a minimum of 6 h before use in the assays. The medium was removed and 20 μl of assay buffer (20 mM HEPES, ×1 Hank's balanced salt solution, pH 7.40) was added, followed by 5 μl of ×5 drug solutions. The plate was incubated at 37°C for 30 min. After the incubation, 25 μl of dye solution was added and fluorescence intensity was measured after 30 min at 37°C, using FlexStation II (bottom read mode, excitation at 440 nm, emission at 520 nm with 510 nm cutoff) from the

Molecular Devices. Results (relative fluorescence unit) were exported and plotted against drug concentrations in Prism 5.02 (GraphPad Software, Inc., La Jolla, CA) for nonlinear regression to obtain inhibitory potency. The half-maximal inhibitory concentration was determined and expressed as pIC50 (pIC50 = –log (IC50)).

Docking Calculations

Docking calculations were carried out using Docking-Server,[9]‡ as previously described, to locate and visualize the binding site.[10] The coordinates of the crystal structure were obtained from the protein data bank§ with access code 4DKL for murine μ-receptor[11] and 4DJH for κ-receptor.[12] The coordinates for the SERT were taken from the recently published model[13] based on the LeuT 3F3A crystal structure.[14] The coordinates for the NET were obtained from the recently published model[15] based on the crystal structure (protein data bank ID code 2A65)[16] of LeuT from *Aquifex aeolicus.* Dezocine docking calculation on a LeuT crystal coupled with desipramine (protein data bank ID code 2QJU)[17] was also performed to identify the potential overlap of the binding sites. Semiempirical charges calculated by MOPAC2009 were added to the ligand atoms.[18]‖ Essential hydrogen atoms, Kollman united atom type charges, and solvation parameters were added to the receptor by using AutoDock tools provided by the server. Grid maps of $30 \times 30 \times 30$ Å grid points with 0.375 Å spacing centered at the known ligand binding site were generated using the Autogrid program.[19,20] All the ligand searches were performed using the Solis and Wets[21] local search method with a Lamarckian genetic algorithm. Initial position, orientation, and torsions of the ligand molecules were set randomly. The three-dimensional coordinates of the tested compound were obtained from the PubChem database.# PyMOL (Version 1.5.0.4; Schrodinger LLC, New York, NY) was used to render the graphics for presentation.

Data Analysis

The data are presented as mean ± standard error from three repeats. The results were analyzed using GraphPad Prism (version 5.02 Windows version). EC50s are determined using the following model as defined in GraphPad: $Y = Bottom + (Top - Bottom)/(1 + 10^{((LogEC50-X) \times HillSlope)})$. Top and Bottom are plateaus in the units of the Y axis. EC50 is the concentration of a ligand that gives a response half way between Bottom and Top. HillSlope describes the steepness of the family of curves.

‡ Available at: http://www.dockingserver.com. Accessed October 15, 2013.

§ Available at: http://www.rcsb.org/. Accessed October 15, 2013.

‖ Available at: http://openmopac.net/MOPAC2009.html. Accessed October 15, 2013.

Available at: http://pubchem.ncbi.nlm.nih.gov/. Accessed October 15, 2013.

Results

Interaction with Opioid Receptors

Although dezocine binds to all three major subtypes of opioid receptors (table 2), it only weakly interacts with the δ-receptor. We determined affinities for dezocine as 3.7 ± 0.7 nM for the human μ-receptor, 527 ± 70 nM for the human δ-receptor, and 31.9 ± 1.9 nM for the human κ-receptor (table 3). As indicated in figure 2, dezocine docks to the known binding site for opioid ligands in both the μ and κ-receptor. Hydrogen bonding with ASP 147 (149 in human μ) contributes to the strong affinity of dezocine to the μ-receptor. TYR326 also has polar interaction with dezocine in the μ-receptor as demonstrated in figure 1A. In the case of the κ-receptor, dezocine hydrogen bonds with ASP138, as predicted by docking calculations (fig. 2B).

Kappa Receptor Antagonism

Consistent with published data, nalbuphine behaved as a full κ-receptor agonist and fully activated the G protein in the presence of membranes containing κ-receptor, as indicated in figure 3A. There was no significant G protein activation with dezocine in the presence of κ-receptor, indicating that dezocine acted as an antagonist (fig. 3A). To confirm this, the G protein was preactivated with a full agonist (nalbuphine or salvinorin A), and then increasing amounts of dezocine were added. As indicated in figure 3B, dezocine inhibited the agonist effect concentration-dependently with a total blockage at high concentration. This finding correlated with the lack of G protein activation shown in figure 3A. The IC$_{50}$ (T = 30°C) values of inhibition were in a high nanomolar range (approximately 350 nM for competition with nalbuphine or approximately 800 nM for competition with salvinorin A), suggesting that dezocine binds to the receptor at the same site as these two full agonists. Interestingly, based on this G protein activation study, nor-binaltorphimine acted as an inverse κ-agonist.

Amine Transporter Proteins as Novel Targets of Dezocine

As indicated in table 2, in addition to binding to the opioid receptor, dezocine also inhibits the NET with pK$_i$ of 6.00 ± 0.10 and the SERT with pK$_i$ of 6.96 ± 0.08. These interactions were further confirmed by norepinephrine and serotonin reuptake studies. The pIC$_{50}$s at NET were 7.57 ± 0.23 for nisoxetine (positive control) and 5.68 ± 0.11 for dezocine (fig. 4A). The pIC$_{50}$s of SERT were 5.99 ± 0.07 for nisoxetine and 5.86 ± 0.17 for dezocine (fig. 4B).

Binding Site Location in NET and SERT

Consistent with the competitive binding assay, dezocine is predicted to share the same binding site with nisoxetine in the NET, as indicated in figure 5. Dezocine is located in close proximity to TRP103, TYR127, GLU281, and LEU368 and might form hydrogen bonds with these residues. On the basis of the docking prediction shown in figure 6A, dezocine binds to the preformed ligand-binding pocket in the model of human SERT. This pocket has been demonstrated

Table 2. Primary Binding for the Receptors

Receptors	Dezocine (% Inhibition)	Naloxone (% Inhibition)
5-HT1A	43.5	5.8
5-HT1B	−14.1	−1
5-HT1D	−0.4	−1.6
5-HT1E	−2.4	−5.7
5-HT2A	−10	15.2
5-HT2B	−11.9	49.6
5-HT2C	19.8	21.1
5-HT3	11.8	0
5-ht5a	3.4	28.9
5-HT6	10.9	27.6
5-HT7	20.9	60.3
Alpha 1A	4.9	4
Alpha 1B	14.7	−2
Alpha 1D	8.9	19.7
Alpha 2A	40.9	12.9
Alpha 2B	21.2	14.7
Alpha 2C	−7.9	22.7
Beta 1	10	14.7
Beta 2	−4.5	−2.6
Beta 3	−15	−3.1
BZP rat brain site	11.5	22.2
D1	11.5	5.5
D2	−3.8	−0.8
D3	−9.4	1.9
D4	−5.1	−5.8
D5	−6.3	2.1
DAT	15.8	−12.6
DOR	58.5	99.3
GABAA	−10.1	−1.1
H1	20.7	10.8
H2	33.6	57.4
H3	−1.6	8.9
KOR	91.3	100.5
M1	−6.8	24
M2	31.5	−11.4
M3	−3.4	20.1
M4	2.5	11
M5	19.4	32.1
MOR	89.6	99.4
NET	86.4	43.8
SERT	97.7	44
Sigma 1	21.7	9.6
Sigma 2	6.3	26.4

All the proteins are from humans except for Sigma2 and benzodiazepine site, which are from rats.

Alpha = alpha adrenergic receptor; Beta = beta adrenergic receptor; BZP = benzodiazepine; D = dopamine receptor; DAT = dopamine transporter; DOR = δ-opioid receptor; GABA = γ-aminobutyric acid receptor; H = histamine receptor; KOR = κ-opioid receptor; M = muscarinic receptor; MOR = μ-opioid receptor; NET = norepinephrine transporter; SERT = serotonin transporter; Sigma = sigma receptor; 5-HT = serotonin receptor.

to be the binding site for many selective serotonin reuptake inhibitors including fluoxetine, citalopram, sertraline, fluvoxamine, and tricyclic antidepressants such as amitriptyline, desipramine, and imipramine. Mutation of the residues lining this pocket (Y95, D98, I172, Y176, F335, F341, and S438) changed the binding capability of these ligands

Table 3. Affinities with Major Opioid Receptors for Dezocine

Ki (nM)	MOR	DOR	KOR
Dezocine	3.7 ± 0.7	527 ± 70	31.9 ± 1.9
Naloxone	6.12 ± 0.4	81.4 ± 2.66	2.55 ± 0.14
Morphine	2.8 ± 0.2	648.8 ± 59.7	55.96 ± 6.99

DOR = δ-opioid receptor; KOR = κ-opioid receptor; MOR = μ-opioid receptor.

significantly.[13] Dezocine shares the same binding site for desipramine found in the crystal structure of LeuT, as indicated in figure 6B. Both findings indicate that dezocine may share the same site as selective serotonin reuptake inhibitors or tricyclic antidepressants.

Discussion

This study details the discovery of the pharmacological interactions of dezocine with the human NET and SERT proteins as well as the molecular characterization of these interactions. This study also confirms the interaction of dezocine with three opioid receptors with different affinities and verifies that dezocine acts as an antagonist of the κ-receptor rather than as an agonist. Collectively, these findings have significant implications, as they help elucidate the mechanisms underlying dezocine's pharmacological effects and present evidence supporting the compound's potential novel clinical applications.

Interaction with Opioid Receptors

Consistent with published data,[22] dezocine exhibits strong affinity for both μ and κ-receptors, but relatively weak interactions with the δ-receptor. The determined affinities for all three receptors are comparable with those published previously.[22] It is predicted that dezocine shares the same binding site with the known ligands in the crystal structures. The predicted hydrogen bonding and polar interactions of dezocine in the binding sites of μ and κ-receptors could be used to explain why the addition of sodium ion in the binding buffer can decrease the affinities significantly.[22]

Dezocine is a well-known partial μ agonist[23–25] that has been used for perioperative pain management because of its analgesic effects.[23,25] Similar to other opioids, dezocine could decrease anesthetic requirement by up to 50%.[26] In a double-blinded study comparing the analgesic effects of dezocine and meperidine in patients for postoperative pain management (n = 187), 10 mg of dezocine produced the same analgesic activity as 50 mg of meperidine.[1] Due to its partial agonism on the μ-opioid receptor, common side effects observed in opioids with full agonism are significantly reduced.[27] Most importantly, dezocine is not a scheduled medication classified by the World Health Organization and no addiction related to its use has been reported.[27] Because addiction is one of the most significant side effects of opioids, it is critical to understand why dezocine is not addictive. This compound could serve as a key
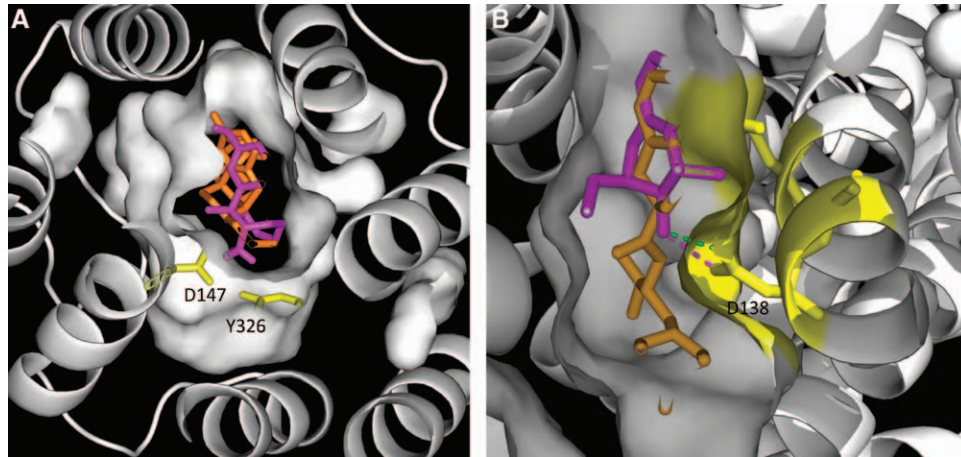
Fig. 2. (*A*) Dezocine (*magenta*) overlaps with beta-funaltrexamine (*orange*) a μ-receptor antagonist and the ligand found in the crystal structure of the μ-opioid receptor (4DKL),[13] in the binding pocket. Polar interaction with ASP147 and TYR326 is predicted. (*B*) Dezocine (*magenta*) overlaps with JDTic (*orange*), a κ-receptor antagonist and the ligand found in the crystal structure of the κ-opioid receptor (4DJH),[14] in the binding pocket. Some of interacting residues (ASP138, TYR139, and MET142) are in *yellow*. A nitrogen in dezocine might hydrogen bond with oxygen atoms of ASP138 (distance in *magenta*, 2.6 Å, or in *green*, 2.8 Å).
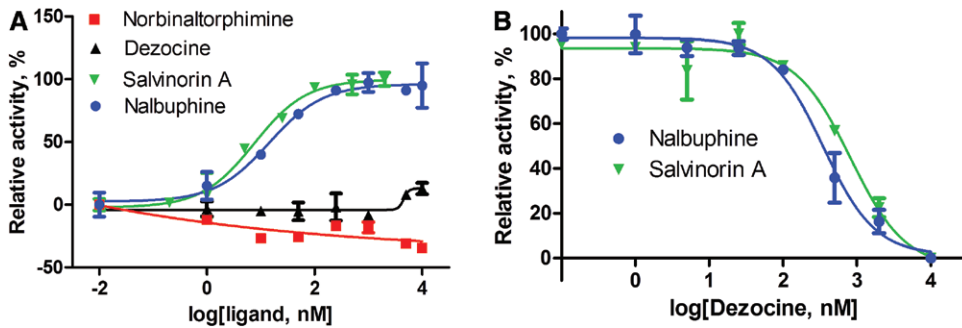

Fig. 3. (*A*) Nalbuphine and salvinorin A, full agonists of κ-opioid receptors, concentration-dependently activate the G protein in the presence of κ-receptor. Dezocine fails to induce any G protein activation, indicating receptor antagonism. On the basis of the activity of the G protein in its presence, nor-binaltorphimine is an inverse agonist of κ-opioid receptor. (*B*) G protein was preactivated with a full agonist (nalbuphine, 250 nM or salvinorin A, 20 nM) and then increasing amounts of dezocine were added. Dezocine inhibited the agonist effects of nalbuphine and salvinorin A concentration-dependently with a total blockage at high concentration, confirming the κ-receptor antagonism effect of dezocine. The relationship is plotted using the following model: $Y = Bottom + (Top - Bottom)/(1 + 10^{((LogEC50-X) \times HillSlope)})$.
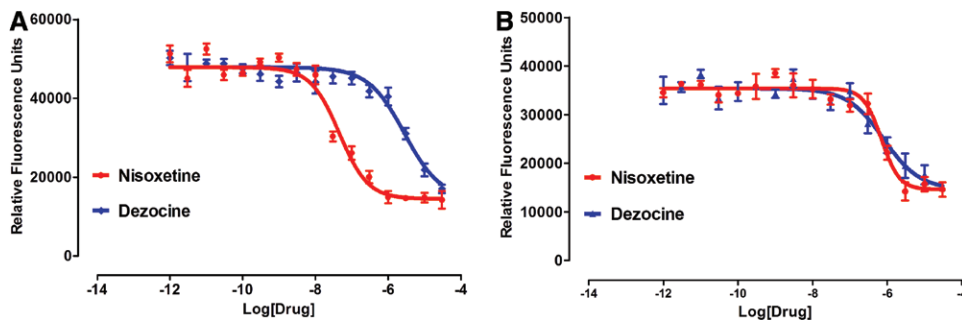
Fig. 4. (*A*) Norepinephrine reuptake is dose dependently inhibited by both dezocine and nisoxetine. However, inhibition is weaker in the case of dezocine. (*B*) Serotonin reuptake is concentration dependently inhibited in the presence of dezocine and nisoxetine with comparable potency. The relationship is plotted using the following model: $Y = Bottom + (Top - Bottom)/(1 + 10^{((LogEC50-X) \times HillSlope)})$.

molecular probe to elucidate the mechanism(s) of addiction by comparing the molecular targets with other known addictive opioid molecules.

Dezocine is structurally related to the κ-agonist pentazocine. Due to this similarity, dezocine was initially considered a κ-agonist with the potential to generate the psychotomimetic
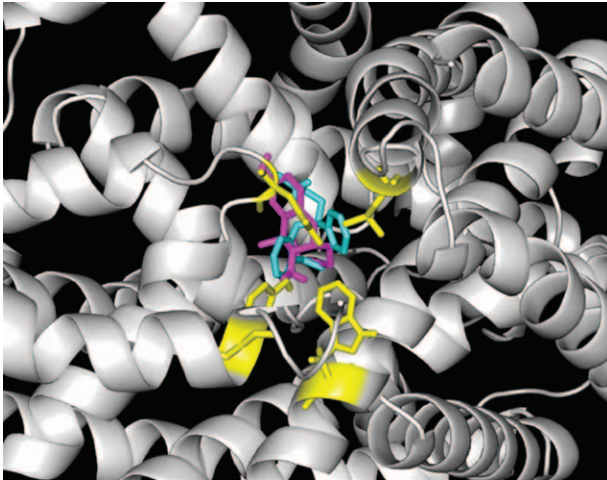
Fig. 5. Docking result of dezocine and nisoxetine in the model of norepinephrine transporter. Dezocine (*magenta*) shares the same binding site of nisoxetine (*cyan*), a norepinephrine transporter inhibitor, as indicated by the close overlap. Dezocine is located in close proximity to TRP103, TYR127, GLU281, and LEU368, which are all in *yellow*.

effects observed with pentazocine. However, such effects were not observed in analgesic dosages for dezocine used in clinical trials.[1] In fact, a recent study suggested that dezocine is a κ-antagonist.[3] We confirmed this finding by demonstrating the compound's inability to activate the receptor and its capacity to antagonize the agonist effects of salvinorin A, a potent and highly selective κ-receptor agonist, and nalbuphine, a nonselective κ-receptor agonist. Further study is needed to demonstrate whether this κ-receptor antagonism is connected to the compound's lack of addictive properties.[28] Buprenorphine, a partial μ-agonist and κ-antagonist,

has been successfully used for addiction treatment for many years with the outcome equivalent to methadone therapy.[29] However, buprenorphine itself is an addictive Schedule III medication[30] and its chronic use creates significant difficulty for optimal perioperative pain management due to its high affinity to the receptor and its long half-life.[31–33] On the basis of our current findings, similar to buprenorphine, dezocine is also a partial μ-agonist and a κ-antagonist. Further study is warranted to demonstrate whether dezocine, a nonaddictive compound, could be used as a potential alternative medication for addiction management. Its shorter half-life allows for easier titration to an optimal effect as well as rapid removal when full agonism is required during the perioperative period.

Although nor-binaltorphimine was traditionally considered a κ-antagonist, this study's finding is consistent with the report that nor-binaltorphimine is an inverse κ-agonist, a property that may be related to its antidepressive effects.[34]

Interaction with NET and Its Related Clinical Implications

Although dezocine is a μ-opioid receptor partial antagonist, theoretically, it could antagonize the antinociceptive effects of morphine. However, when used in combination with morphine, dezocine concentration-dependently enhances the analgesic effects of morphine.[35] The combination of morphine and dezocine also reduces the anesthetic requirement.[26] These suggest the existence of alternative targets for dezocine (besides the opioid receptors) that could have additive effects for other opioids. Our findings support these earlier pharmacological observations. Dezocine interacts with NET and inhibits the norepinephrine reuptake. The competitive binding assay and the computational docking calculation suggest that dezocine interacts with NET
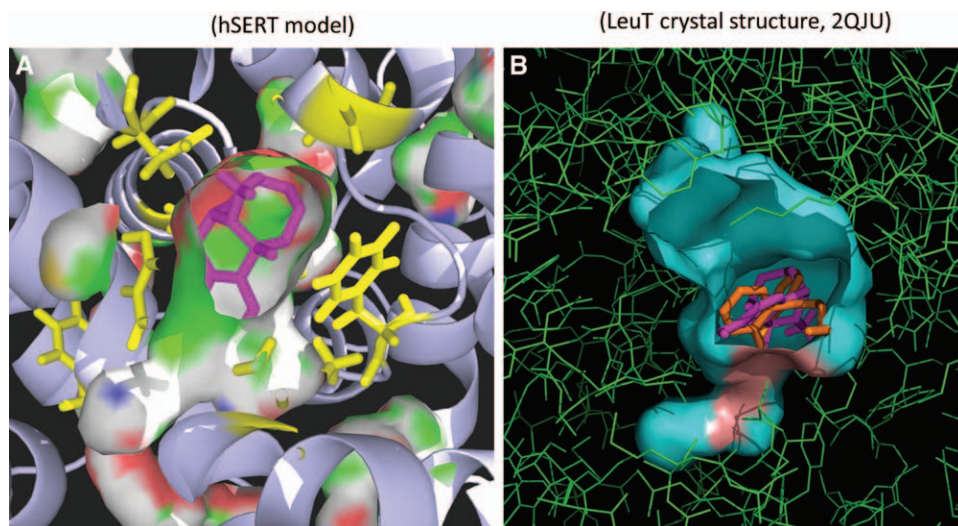
Fig. 6. (*A*) Dezocine (*magenta*) sits in the preformed ligand binding pocket for selective serotonin reuptake inhibitors in the model of human serotonin transporter. The key interacting residues lining the pocket (Y95, D98, I172, Y176, F335, F341, and S438) are in *yellow*. This binding pocket has been demonstrated to be the binding site for many important clinical drugs such as fluoxetine, sertraline, and amitriptyline. (*B*) Dezocine (*magenta*) shares the same binding pocket and overlaps well with desiprimine (*orange*), the ligand in the LeuT crystal structure (2QJU).

directly at the binding site for the intrinsic NET ligand. Interestingly, a recent study indicates that pentazocine, a compound structurally similar to dezocine, inhibits NET function by reducing the amount of NET in cell surface membranes through an opioid receptor–independent pathway.[36] It is unclear whether dezocine could have similar properties as pentazocine with regard to indirect NET interaction. NET plays an important role in pain pathways and norepinephrine reuptake inhibition could be used for treating pain,[37] especially in neuropathic pain.[38] Although its direct interaction with NET is relatively weak, it is still worthwhile to investigate whether dezocine could be used to target neuropathic pain in addition to its opioid receptor–mediated analgesia.

Interaction with SERT and Its Related Clinical Implications

One of the striking findings of this study is that dezocine interacts with SERT at its ligand binding site and that serotonin reuptake can be inhibited concentration-dependently by dezocine. It is important to note that because dezocine shares a binding site with some clinical antidepressant drugs such as fluoxetine, sertraline, and amitriptyline, cross interactions may occur. Thus precaution may be needed. The κ-opioid receptor, NET and SERT are all important targets for depression treatment.[39–42] Because dezocine interacts with all three targets, it is critical to investigate whether dezocine could be an alternative medication to treat depression and prevent opioid-induced depression. Opioids are the main class of medications used to manage moderate and severe pain; however, in addition to addiction, depression is another common side effect of chronic opioid usage.[43] Patients with chronic pain frequently report depression, a condition associated with high pain intensity and greater prevalence of chronic opioid therapy.[44] Identifying and treating depression symptoms in chronic pain patients are essential for reducing comorbidity and disability.[45] Eighty percent of patients with major depression without psychotic features have painful physical symptoms.[46] Consequently, there is a significant medical impetus for developing medications that simultaneously and effectively target pain and depression. *In vivo* studies are warranted to demonstrate whether dezocine is a medication of choice targeting both pain and depression due to its unique molecular targets of κ-receptor antagonism, NET and SERT inhibition. Potential drug interaction could occur when using similar drugs with shared binding site(s).

Limitation

The major limitation of this study is that the profiling was limited to 44 receptors available to us and it is possible that dezocine could interact with additional targets. No *in vivo* evidence is provided in this study.

Conclusions

This study explored the interaction of dezocine with three major opioid receptors, demonstrated that dezocine is a

κ-antagonist, and suggested its potential use for addition treatment. Through molecular target profiling, we discovered two novel molecular targets of dezocine *in vitro*: NET and SERT. The binding sites were characterized using available structural models and docking experiments. Dezocine concentration-dependently inhibited norepinephrine and serotonin reuptake *in vitro*. These findings suggest the potential use of dezocine as a novel medication for the simultaneous treatment of pain and depression. Potential drug interactions should be considered when administering drugs with similar pharmacological targets. Further studies are warranted.

Competing Interests

The authors declare no competing interests.

Correspondence

Address correspondence to Dr. Liu: Department of Anesthesiology and Critical Care, Perelman School of Medicine at the University of Pennsylvania, 336 John Morgan Building, 3620 Hamilton Walk, Philadelphia, Pennsylvania 19104. liur@uphs.upenn.edu. Information on purchasing reprints may be found at www.anesthesiology.org or on the masthead page at the beginning of this issue. ANESTHESIOLOGY's articles are made freely accessible to all readers, for personal use only, 6 months from the cover date of the issue.

References

1. Fragen RJ, Caldwell N: Comparison of dezocine (WY 16, 225) and meperidine as postoperative analgesics. Anesth Analg 1978; 57:563–6

2. Sun Q, Zhou W, Wu B, Ji MH, Peng YG: Dezocine: A novel drug to prevent fentanyl-induced cough during general anesthesia induction? J Anesth 2012; 26:470

3. Gharagozlou P, Hashemi E, DeLorey TM, Clark JD, Lameh J: Pharmacological profiles of opioid ligands at kappa opioid receptors. BMC Pharmacol 2006; 6:3

4. Gal TJ, DiFazio CA: Ventilatory and analgesic effects of dezocine in humans. ANESTHESIOLOGY 1984; 61:716–22

5. Mumby SM, Linder ME: Myristoylation of G-protein alpha subunits. Methods Enzymol 1994; 237:254–68

6. Wildman DE, Tamir H, Leberer E, Northup JK, Dennis M: Prenyl modification of guanine nucleotide regulatory protein gamma 2 subunits is not required for interaction with the transducin alpha subunit or rhodopsin. Proc Natl Acad Sci U S A 1993; 90:794–8

7. Roth BL, Baner K, Westkaemper R, Siebert D, Rice KC, Steinberg S, Ernsberger P, Rothman RB: Salvinorin A: A potent naturally occurring nonnitrogenous kappa opioid selective agonist. Proc Natl Acad Sci U S A 2002; 99:11934–9

8. Iversen L, Gibbons S, Treble R, Setola V, Huang XP, Roth BL: Neurochemical profiles of some novel psychoactive substances. Eur J Pharmacol 2013; 700:147–51

9. Bikadi Z, Hazai E: Application of the PM6 semi-empirical method to modeling proteins enhances docking accuracy of AutoDock. J Cheminform 2009; 1:15

10. Liu R, Perez-Aguilar JM, Liang D, Saven JG: Binding site and affinity prediction of general anesthetics to protein targets using docking. Anesth Analg 2012; 114:947–55

11. Manglik A, Kruse AC, Kobilka TS, Thian FS, Mathiesen JM, Sunahara RK, Pardo L, Weis WI, Kobilka BK, Granier S: Crystal structure of the μ-opioid receptor bound to a morphinan antagonist. Nature 2012; 485:321–6

12. Wu H, Wacker D, Mileni M, Katritch V, Han GW, Vardy E, Liu W, Thompson AA, Huang XP, Carroll FI, Mascarella SW, Westkaemper RB, Mosier PD, Roth BL, Cherezov V, Stevens RC: Structure of the human κ-opioid receptor in complex with JDTic. Nature 2012; 485:327–32

13. Gabrielsen M, Kurczab R, Ravna AW, Kufareva I, Abagyan R, Chilmonczyk Z, Bojarski AJ, Sylte I: Molecular mechanism of serotonin transporter inhibition elucidated by a new flexible docking protocol. Eur J Med Chem 2012; 47:24–37

14. Singh SK, Piscitelli CL, Yamashita A, Gouaux E: A competitive inhibitor traps LeuT in an open-to-out conformation. Science 2008; 322:1655–61

15. Schlessinger A, Geier E, Fan H, Irwin JJ, Shoichet BK, Giacomini KM, Sali A: Structure-based discovery of prescription drugs that interact with the norepinephrine transporter, NET. Proc Natl Acad Sci U S A 2011; 108:15810–5

16. Yamashita A, Singh SK, Kawate T, Jin Y, Gouaux E: Crystal structure of a bacterial homologue of Na$^+$/Cl$^-$–dependent neurotransmitter transporters. Nature 2005; 437:215–23

17. Zhou Z, Zhen J, Karpowich NK, Goetz RM, Law CJ, Reith ME, Wang DN: LeuT-desipramine structure reveals how antidepressants block neurotransmitter reuptake. Science 2007; 317:1390–3

18. Stewart JJ: MOPAC: A semiempirical molecular orbital program. J Comput Aided Mol Des 1990; 4:1–105

19. Morris GM, Goodsell DS, Huey R, Olson AJ: Distributed automated docking of flexible ligands to proteins: Parallel applications of AutoDock 2.4. J Comput Aided Mol Des 1996; 10:293–4

20. Morris GM, Huey R, Lindstrom W, Sanner MF, Belew RK, Goodsell DS, Olson AJ: AutoDock4 and AutoDockTools4: Automated docking with selective receptor flexibility. J Comput Chem 2009; 30:2785–91

21. Solis FJ, Wets RJB. Minimization by random search techniques. Math Oper Res 1981; 6:19–30

22. Chen JC, Smith ER, Cahill M, Cohen R, Fishman JB: The opioid receptor binding of dezocine, morphine, fentanyl, butorphanol and nalbuphine. Life Sci 1993; 52:389–96

23. Malis JL, Rosenthale ME, Gluckman MI: Animal pharmacology of Wy-16,225, a new analgesic agent. J Pharmacol Exp Ther 1975; 194:488–98

24. Downing JW, Brock-Utne JG, Barclay A, Schwegmann IL: WY 16225 (dezocine), a new synthetic opiate agonist-antagonist and potent analgesic: Comparison with morphine for relief of pain after lower abdominal surgery. Br J Anaesth 1981; 53:59–64

25. Gharagozlou P, Demirci H, David Clark J, Lameh J: Activity of opioid ligands in cells expressing cloned mu opioid receptors. BMC Pharmacol 2003; 3:1

26. Rowlingson JC, Moscicki JC, DiFazio CA: Anesthetic potency of dezocine and its interaction with morphine in rats. Anesth Analg 1983; 62:899–2

27. Jacobs AM, Youngblood F: Opioid receptor affinity for agonist-antagonist analgesics. J Am Podiatr Med Assoc 1992; 82:520–4

28. Chavkin C: The therapeutic potential of κ-opioids for treatment of pain and addiction. Neuropsychopharmacology 2011; 36:369–70

29. Kraus ML, Alford DP, Kotz MM, Levounis P, Mandell TW, Meyer M, Salsitz EA, Wetterau N, Wyatt SA; American Society Of Addiction Medicine: Statement of the American Society Of Addiction Medicine Consensus Panel on the use of buprenorphine in office-based treatment of opioid addiction. J Addict Med 2011; 5:254–63

30. Chowdhury S, Chowdhury AN: Emergence of buprenorphine addiction. J Indian Med Assoc 1990; 88:294

31. Roberts DM, Meyer-Witting M: High-dose buprenorphine: Perioperative precautions and management strategies. Anaesth Intensive Care 2005; 33:17–25

32. Bryson EO, Lipson S, Gevirtz C: Anesthesia for patients on buprenorphine. Anesthesiol Clin 2010; 28:611–7

33. Chern SYS, Isserman R, Chen L, Ashburn M, Liu R: Perioperative Pain Management for Patients on Chronic Buprenorphine: A Case Report. J Anesth Clin Res 2012; 3:10

34. Zhang H, Shi YG, Woods JH, Watson SJ, Ko MC: Central kappa-opioid receptor-mediated antidepressant-like effects of nor-Binaltorphimine: Behavioral and BDNF mRNA expression studies. Eur J Pharmacol 2007; 570:89–96

35. Morgan D, Cook CD, Smith MA, Picker MJ: An examination of the interactions between the antinociceptive effects of morphine and various mu-opioids: The role of intrinsic efficacy and stimulus intensity. Anesth Analg 1999; 88:407–13

36. Obara G, Toyohira Y, Inagaki H, Takahashi K, Horishita T, Kawasaki T, Ueno S, Tsutsui M, Sata T, Yanagihara N: Pentazocine inhibits norepinephrine transporter function by reducing its surface expression in bovine adrenal medullary cells. J Pharmacol Sci 2013; 121:138–47

37. Hartrick CT: Noradrenergic reuptake inhibition in the treatment of pain. Expert Opin Investig Drugs 2012; 21:1827–34

38. Rojo ML, Rodríguez-Gaztelumendi A, Pazos Á, Díaz Á: Differential adaptive changes on serotonin and noradrenaline transporters in a rat model of peripheral neuropathic pain. Neurosci Lett 2012; 515:181–6

39. Carr GV, Bangasser DA, Bethea T, Young M, Valentino RJ, Lucki I: Antidepressant-like effects of kappa-opioid receptor antagonists in Wistar Kyoto rats. Neuropsychopharmacology 2010; 35:752–63

40. Chartoff E, Sawyer A, Rachlin A, Potter D, Pliakas A, Carlezon WA: Blockade of kappa opioid receptors attenuates the development of depressive-like behaviors induced by cocaine withdrawal in rats. Neuropharmacology 2012; 62:167–76

41. Gillman KW, Parker MF, Silva M, Degnan AP, Tora GO, Lodge NJ, Li YW, Lelas S, Taber M, Krause RG, Bertekap RL, Newton AE, Pieschl RL, Lengyel KD, Johnson KA, Taylor SJ, Bronson JJ, Macor JE: Design, optimization, and *in vivo* evaluation of a series of pyridine derivatives with dual NK1 antagonism and

SERT inhibition for the treatment of depression. Bioorg Med Chem Lett 2013; 23:407–11

42. Joensuu M, Tolmunen T, Saarinen PI, Tiihonen J, Kuikka J, Ahola P, Vanninen R, Lehtonen J: Reduced midbrain serotonin transporter availability in drug-naïve patients with depression measured by SERT-specific [(123)I] nor-beta-CIT SPECT imaging. Psychiatry Res 2007; 154:125–31

43. Merrill JO, Von Korff M, Banta-Green CJ, Sullivan MD, Saunders KW, Campbell CI, Weisner C: Prescribed opioid difficulties, depression and opioid dose among chronic opioid therapy patients. Gen Hosp Psychiatry 2012; 34:581–7

44. Jamison RN, Edwards RR, Liu X, Ross EL, Michna E, Warnick M, Wasan AD: Relationship of negative affect and outcome of an opioid therapy trial among low back pain patients. Pain Pract 2013; 13:173–81

45. Valkanoff TA, Kline-Simon AH, Sterling S, Campbell C, Von Korff M: Functional disability among chronic pain patients receiving long-term opioid treatment. J Soc Work Disabil Rehabil 2012; 11:128–42

46. Husain MM, Rush AJ, Trivedi MH, McClintock SM, Wisniewski SR, Davis L, Luther JF, Zisook S, Fava M: Pain in depression: STAR*D study findings. J Psychosom Res 2007; 63:113–22