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The cocaine-like behavioral effects of meperidine are mediated by activity at the dopamine transporter

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Abstract

Meperidine has atypical opioid receptor agonist effects and shares some structural features with the phenyltropane (WIN) analogs of cocaine. In combination with 0.1 mg/kg naltrexone, meperidine produced cocaine-like discriminative stimulus effects in monkeys, whereas morphine was inactive. Both cocaine and meperidine inhibited [^{3}H]dopamine uptake in chopped rat caudate putamen with comparable potencies; meperidine differed from cocaine in that its effects could be characterized as having predominantly a single high-affinity component. Morphine was not active in inhibiting [^{3}H]dopamine uptake, indicating that the effect of meperidine was not via a classic μ-opioid receptor agonist action. Further, meperidine but not morphine displaced [^{3}H]WIN 35,428 (2β-carbomethoxy-3β-(4-fluorophenyl)tropane) binding. These data suggest that the actions of meperidine that are atypical of opioids are due to activity at the dopamine transporter. In addition, meperidine appears to interact predominantly with the high-affinity component of the dopamine transporter, and this high-affinity component may be the site of importance for the production of cocaine's behavioral effects.

Keywords: Dopamine transporter; Cocaine; Meperidine

1. Introduction

Differences between the pharmacology of meperidine (*N*-methyl-4-phenyl-4-carboethoxypiperidine) and other μ-opioid receptor agonists have been documented for some time. For example, chronic exposure to meperidine can produce central nervous system stimulant effects such as tremor and convulsions (Andrews, 1942; Himmelsbach, 1942) as well as a hyperflexia and increased susceptibility to startle (Himmelsbach, 1942) that do not generally occur with μ-opioid receptor agonists such as morphine (Martin and Sloan, 1977). Further, certain similarities have been noted between the symptoms of meperidine intoxication and that of cocaine or atropine (Polonio and Lisbon, 1947), whereas differences between meperidine and morphine withdrawal syndromes have also been reported (Isbell, 1955). A detailed comparison of morphine and meperidine in non-dependent human subjects with a history of opioid abuse revealed further differences between meperidine and morphine (Jasinski and Nutt, 1973; Jasinski and Preston, 1986; see also Zacny et al., 1993). Across a range of doses that were equivalent in producing miosis and subject scores of liking, meperidine produced greater subjective symptoms related to stimulant effects ('drive' and 'nervous'), as well as some other effects (e.g. 'sleepy', 'stomach turning' and 'drunken') that were either not produced by morphine, or were evident to much less of an extent.

In animals, results of several studies have indicated that the effects of high doses of meperidine are relatively resistant to antagonism by nalorphine (Huggins et al., 1950; Miller and Anderson, 1954; Winter and Flataker, 1956). For example, high doses of meperidine as well as μ-opioid receptor agonists, such as heroin and *d*-propoxyphene, can produce convulsions that are

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antagonized by naloxone. However, the antagonism of the meperidine effect is incomplete, even at high doses of naloxone, and is less than the antagonism of the effects of heroin or *d*-propoxyphene (Gilbert and Martin, 1975).

Other studies have indicated that the effects of meperidine at doses that are below toxic levels can also be resistant to opioid antagonism, and may be mediated by non-opioid mechanisms. For example, Cox and Weinstock (1964) showed that the antagonism of hotplate analgesic effects of meperidine by nalorphine was not competitive, and distinctly different from that for other receptor agonists, such as morphine and methadone. In contrast, the production of lenticular opacities produced by meperidine conformed to a competitive model. The authors concluded that at least some of the actions of meperidine were mediated by non-opioid mechanisms. Similar conclusions were reached by Leander and colleagues in studies of the stimulant-like effects of meperidine on operant behavior (e.g. Leander and McMillan, 1977; Witkin et al., 1983). Several previous studies had suggested that the non-opioid effects of meperidine might be mediated through an inhibition of the uptake of serotonin (Carlsson and Lindquist, 1969; Fahim et al., 1972). However, meperidine analogs that do not inhibit serotonin uptake have non-opioid behavioral effects similar to those of meperidine (Leander, 1978). Further, stimulation of serotonergic activity generally is not considered to produce behavioral stimulation (e.g. Glennon and Lucki, 1988).

Meperidine shares several structural features with cocaine and, even more closely, the phenyltropane analogs of cocaine, a prototype of which is WIN 35,065-2 (2β-carbomethoxy-3β-phenyltropane). The piperidine ring of meperidine is also a component of the tropane rings of cocaine and WIN 35,065-2. Further, all three of the compounds possess an *N*-methyl group. WIN 35,065-2 has a phenyl ring attached to the 3-position on the tropane ring, which is analogous to the phenyl substituent on the 4-position of the piperidine ring of meperidine. The commonality in the structures of meperidine and the congeners of cocaine, coupled with the distinctive pharmacological profile of meperidine, suggests that the non-opioid actions of meperidine might be due to a cocaine-like pharmacological action. Because many of the behavioral effects of cocaine are thought to be mediated via the inhibition of dopamine uptake (Ritz et al., 1987), the present study examined the cocaine-like behavioral effects of meperidine and potential actions of meperidine at the dopamine transporter. These studies were done in an attempt to better understand the neurochemical mechanisms that underly the behavioral effects of meperidine as they relate to cocaine and the dopamine transporter.

2. Materials and methods

2.1. Chemicals

Chemicals and reagents were obtained from the following sources: [^{3}H]dopamine (specific activity ranged from 47–50 Ci/mmol) from Amersham Corp. (Arlington Heights, IL, USA); [^{3}H]WIN 35,428 (2β-carbomethoxy-3β-(4-fluorophenyl)tropane 1,5-naphthalene disulfonate; specific activity 82.1 Ci/mmol) from New England Nuclear (Boston, MA, USA); morphine sulfate from Merck and Co. (Rahway, NJ, USA); cocaine hydrochloride and meperidine hydrochloride from Sigma Chemical Co. (St. Louis, MO, USA); naltrexone from The National Institute on Drug Abuse (Rockville, MD, USA).

2.2. Cocaine discrimination studies

2.2.1. Subjects

Five adult squirrel monkeys (*Saimiri sciureus*) served as subjects. They were fed a daily food ration (Purina Monkey Chow supplemented with Teklad Monkey Diet) that was sufficient to maintain them at approximately 90% of their unrestricted-feeding weight throughout the course of the study. Water was always available in the individual home cages. All monkeys had been studied previously under experimental procedures similar to those described below.

2.2.2. Apparatus

Subjects were handled according to the general procedures outlined by Kelleher et al. (1963). During experimental sessions, subjects were seated and restrained loosely about the waist in Plexiglas chairs (Hake and Azrin, 1963), which were placed within ventilated, sound-attenuating chambers. Continuous white noise was present in the chambers at all times to mask extraneous sounds. On the front panel of each chair were two response keys (Model 121-05, BRS/LVE, Laurel, MD, USA); a downward force of 20 g or more on either key produced an audible click and was recorded as a response. Behind the clear front panel of the chair were three pairs of stimulus lamps (28 V d.c.); each pair had colored lenses different from the other pairs, and could be independently illuminated. A food-pellet dispenser (Model D-1, Ralph Gerbrands Co., Arlington, MA, USA) delivered 190-mg food pellets (banana flavored, BioServ, Frenchtown, NJ, USA) to the subject through an opening in the front panel of the chair.

2.2.3. Procedures

Experimental sessions were conducted at approximately the same time daily, 5 days per week. Subjects were seated in the restraint chair, which was then

placed within the sound-attenuating isolation chamber. Before each session, subjects received an intramuscular injection of either saline or cocaine (0.3 mg/kg). Cocaine and saline injections were delivered in a double alternation sequence across sessions. When subjects received cocaine, 30 consecutive responses on only one of the keys produced food which was followed by a 20-s timeout during which all stimulus lights were out and responses had no scheduled consequences. When subjects received saline, 30 consecutive responses on the alternate key produced food. Sessions started with a 5-min timeout and ended after 20 food presentations or 15 min, whichever occurred first.

Testing began once subjects reliably responded appropriately to the pre-session injection. The criterion for testing was four consecutive sessions with greater than 85% correct responses over the entire session and before the first food presentation of the session. Before test sessions, subjects were injected with one of several doses of morphine or meperidine. In studies of naltrexone antagonism, an intramuscular injection of naltrexone (0.1 mg/kg) was administered immediately before an injection of either morphine or meperidine. The dose of naltrexone was selected based on previous reports showing that it is sufficient to antagonize μ-opioid actions produced by morphine in this species (e.g. Goldberg et al., 1981). Each dose was typically examined once in each of five subjects and doses were tested in a mixed sequence. Test sessions were identical to training sessions with the exception that, during test sessions, 30 consecutive responses on one of either of the response keys produced food presentation. Test sessions were only conducted if subjects met the training criterion as outlined above on the two immediately preceding training sessions. When subjects failed to meet the criterion, training continued until they did so for two consecutive training sessions (one saline and one cocaine session).

2.2.4. Statistical analysis

The behavioral effects of drugs were evaluated in terms of the percent of cocaine-appropriate responses emitted (responses on the cocaine-appropriate lever divided by the total number of responses emitted $\times$ 100), and in terms of the rate of responding (number of responses divided by elapsed time, excluding the 5 min timeout at the beginning of the session and the 20 s timeout periods that followed each presentation of food). Lever selection for any subject failing to respond at a rate of at least 0.02 responses per second (corresponding to the completion of one fixed-ratio requirement) was considered to be an unreliable indicator and not included in the calculation of mean drug-appropriate responding at that dose. If more than half of the subjects tested at a given dose failed the response rate requirement, no mean value was calculated for percentage of drug-appropriate responding at that dose. For statistical assessments of effects, analysis of variance and Dunnett's post-hoc tests were used to determine if values following various treatments were different from saline values.

2.3. [^{3}H]Dopamine uptake

Male Sprague-Dawley rats (200–250 g, Taconic, Germantown, NY, USA) were killed by decapitation and their brains removed to an ice-cooled dish for dissection of the caudate putamen. [^{3}H]Dopamine uptake was measured essentially as described previously (Izenwasser et al., 1990). The tissue was weighed and chopped into 225 μm slices on a Sorvall TC2 tissue slicer with two successive cuts at an angle of 90°. The strips of tissue were suspended in oxygenated modified Krebs-Hepes buffer (NaCl, 127 mM; KCl, 5 mM; NaH_2PO_4, 1.3 mM; $MgSO_4$, 1.2 mM; $CaCl_2$, 2.5 mM; Hepes acid, 15 mM; glucose, 10 mM; adjusted to pH 7.4) which was pregassed with 95% O_2/5% CO_2 and warmed to 37°C. The tissue was rinsed three times, each in approximately 15 volumes of drug-free buffer. Aliquots of tissue slice suspensions were then incubated in buffer in glass test tubes at 37°C to which morphine, meperidine, cocaine or no drug was added, as appropriate. After a 5 min incubation period in the presence of drug, [^{3}H]dopamine (final concentration 15 nM) was added to each tube. The uptake incubation period was initiated with the addition of [^{3}H]dopamine and terminated after 5 min by the addition of 2 ml of ice-cold buffer to each tube and filtration under reduced pressure over glass fiber filters (presoaked in 0.1% polyethyleneimine in water). Each filter was rinsed three times with 2 ml cold buffer. The filters were placed in scintillation vials to which 1 ml methanol and 2 ml 0.2 M HCl were added to extract the [^{3}H]dopamine. The vials were heated for 2 h in a 70°C oven and then left overnight to cool. 10 ml of Beckman Ready Value Scintillation Cocktail was added to each vial and radioactivity was determined by liquid scintillation spectrometry. The reported values represent specific uptake (total minus non-specific binding to filters).

2.4. [^{3}H]WIN 35,428 binding

Male Sprague-Dawley rats (200–250 g, Taconic, Germantown, NY, USA) were decapitated and their brains removed to an ice-cooled dish for dissection of the caudate putamen. The tissue was homogenized in 30 volumes ice-cold modified Krebs-Hepes buffer (as above) using a Teflon/glass homogenizer and centrifuged at 20 000 $\times g$ for 10 min at 4°C. The resulting pellet was then washed two more times by resuspension in ice-cold buffer and centrifugation at 20 000 $\times g$

for 10 min at 4°C. Fresh homogenates were used in all experiments.

Binding assays were conducted in modified Krebs-Hepes buffer on ice, as previously described (Izenwasser et al., 1993b). The total volume in each tube was 0.5 ml and the final concentration of membrane after all additions was approximately 0.3% (w/v) corresponding to 200–300 μg of protein/sample. Increasing concentrations of the drug being tested were added to triplicate samples of membrane suspension. Five minutes later, [^{3}H]WIN 35,428 (final concentration 1.5 nM) was added and the incubation was continued for 1 h on ice. The incubation was terminated by the addition of 3 ml of ice-cold buffer and rapid filtration through Whatman GF/B glass fiber filter paper (presoaked in 0.1% bovine serum albumin in water to reduce non-specific binding) using a Brandel Cell Harvester (Gaithersburg, MD, USA). After filtration, the filters were washed with three additional 3 ml washes and transferred to scintillation vials. Absolute ethanol (0.5 ml) and Beckman Ready Value Scintillation Cocktail (2.75 ml) were added to the vials which were counted the next day at an efficiency of about 36%. Under these assay conditions, an average experiment yielded approximately 6000 dpm total binding per sample and approximately 250 dpm nonspecific binding. Nonspecific binding was defined as binding in the presence of 100 μM cocaine. B_{max}, K_D and K_i values were derived from 14-point competition assays using increasing concentrations of unlabeled compounds (0.05 nM to 100 μM) against 1.5 nM [^{3}H]WIN 35,428. Protein values were determined using a modification of the Lowry procedure (Peterson, 1977).

Displacement data were analyzed by the use of the nonlinear least squares curve-fitting computer program LIGAND (Munson and Rodbard, 1980). Nonspecific binding was less than 0.5% of total binding. Data from replicate experiments were modeled together to produce a set of parameter estimates and the associated standard errors of these estimates. Single-site models ($K_{0.5}$) are reported unless a two-site model was considered significantly better as determined by LIGAND according to the F test at $P \leq 0.05$. When a two-site model was preferred, data are expressed as K_{hi} and K_{lo}, representing the high- and low-affinity components, respectively.

2.5. Molecular modeling

Molecular modeling studies were performed using the SYBYL software package (Tripos, version 6.1a) installed on an IRIS Indigo workstation running IRIX 5.2. Meperidine and WIN 35,065-2 were sketched and then energy minimized by MAXMIN2 using the Powell method. Charges were calculated using the Gasteiger-Huckel method. RMS molecular fitting was done using four points: the nitrogen of the tropane ring of WIN 35,065-2 with the nitrogen of meperidine; the 3-position carbon of WIN 35,065-2 with the analogous 4-position carbon of meperidine; the 1′-carbon of the phenyl rings attached to the 3-position of WIN 35,065-2 and the 4-position of meperidine; and the 4′-position carbon of the phenyl rings of both molecules. Fitting other substituents such as the 1-position carbon of WIN with the analogous 2-position carbon of meperidine or attempting to superimpose the ester functions did not result in a good fit between these two molecules.

3. Results

3.1. Cocaine discrimination studies

Neither meperidine (Fig. 1, top panel, open circles) nor morphine (Fig. 1, top panel, open triangles) alone produced a substitution for cocaine in animals trained to discriminate cocaine from saline. The maximum substitution with either drug was approximately 30% at a dose of 1.0 mg/kg. There were no significant effects of treatment for meperidine ($F(4,17) = 0.904$, $P = 0.4835$) or morphine ($F(3,15) = 2.465$, $P = 0.1022$). Higher doses could not be tested because response rates were dramatically reduced or eliminated in the majority of subjects by the two drugs, rendering the assessment of lever selection unreliable (Fig. 1, bottom panel, open symbols). Effects of meperidine on response rates were significant ($F(4,16) = 3.735$, $P = 0.0249$), with the dose of 3.0 mg/kg producing a significant decrease (Dunnett $q = 3.196$, $P < 0.05$). Effects of morphine on response rates were significant ($F(4,16) = 17.387$, $P < 0.0001$), with doses of 1.0 (Dunnett $q = 5.064$, $P < 0.01$) and 3.0 (Dunnett $q = 6.128$, $P < 0.01$) mg/kg producing significant decreases.

Pretreatment with 0.1 mg/kg naltrexone produced an approximate 10-fold shift to the right in the morphine dose-effect curve. The maximum substitution with morphine in combination with naltrexone was approximately 40% at a morphine dose of 30 mg/kg, comparable to the 30% obtained with 1.0 mg/kg morphine alone (Fig. 1, top panel, compare open and filled triangles). There were no significant effects of treatment for morphine with naltrexone ($F(5,22) = 2.051$, $P = 0.1107$). Higher doses of morphine in combination with naltrexone could not be tested because response rates were dramatically reduced or eliminated in the majority of subjects. The effects of morphine on response rates were also shifted to the right by naltrexone which allowed the testing of higher doses than could be tested alone (Fig. 1, bottom panel, compare open and filled triangles).

Pretreatment with 0.1 mg/kg naltrexone increased the effectiveness of meperidine in substituting for co-

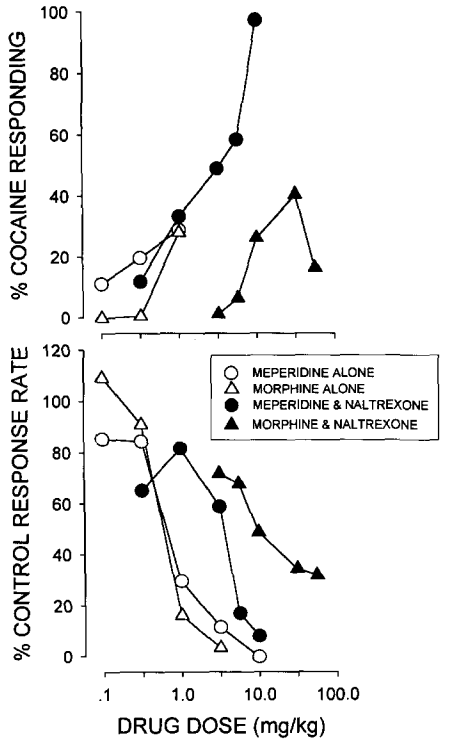

Fig. 1. Substitution of meperidine and morphine, alone and in combination with 0.1 mg/kg naltrexone for cocaine in squirrel monkeys trained to discriminate cocaine (0.3 mg/kg) from saline. Abscissae: drug dose in mg/kg. Ordinates for upper panel: percentage of responses on the cocaine-appropriate lever. Ordinates for lower panel: response rate as a percentage of the rate obtained after saline injections. Open points: effects of meperidine (circles) or morphine (triangles) when administered alone; filled points: effects of each drug when administered with naltrexone (0.1 mg/kg). The average percentage (±S.E.M.) of cocaine-appropriate responses during training sessions in which saline or cocaine was administered was 1.89 (±3.21) or 98.78 (±2.12), respectively. The average response rate (in responses per second) during training sessions in which saline or cocaine was administered was 3.33 (±0.81) or 1.89 (±0.60), respectively. Each point represents the effect in 5–6 squirrel monkeys. Note that neither drug substituted for cocaine when administered alone, whereas meperidine substituted for cocaine in subjects also treated with naltrexone.

caine. The maximum substitution of meperidine in combination with naltrexone approximated 100% at a meperidine dose of 10 mg/kg, compared to 30% at 1.0 mg/kg after meperidine alone (Fig. 1, top panel, compare open and filled circles). Effects of meperidine in combination with naltrexone on the percentage of cocaine-appropriate responses were significant ($F(5,22) = 5.553$, $P = 0.0019$), with doses of 5.6 (Dunnett $q = 3.014$, $P < 0.05$) and 10.0 (Dunnett $q = 4.434$, $P < 0.01$) mg/kg significantly greater than saline. Higher doses of meperidine in combination with 0.1 mg/kg naltrexone could not be tested due to the decreases in response rates. The effects of meperidine on response rates were also shifted to the right by naltrexone which allowed the testing of higher doses than those of meperidine alone that could be tested (Fig. 1, bottom panel, compare open and filled circles). However, the shift in the meperidine dose-effect curve was less than that obtained for morphine. Naltrexone alone did not substitute for cocaine in any of the subjects (data not shown).

3.2. [^{3}H]Dopamine uptake

Cocaine inhibited [^{3}H]dopamine uptake in chopped striatum with a broad, shallow inhibition curve suggesting two components of [^{3}H]dopamine uptake (Fig. 2), as shown previously (Izenwasser et al., 1990, 1992). Meperidine also inhibited [^{3}H]dopamine uptake but unlike cocaine only inhibited approximately 20% of the total uptake (Fig. 2). Naltrexone had no effect on the inhibition of dopamine uptake by meperidine (data not shown). Morphine, at concentrations up to 100 μM, had no effect on [^{3}H]dopamine uptake, suggesting that the effects of meperidine were not due to actions at opioid receptors (Fig. 2).

3.3. [^{3}H]WIN 35,428 binding

Under the binding conditions used, two components of [^{3}H]WIN 35,428 binding are apparent in fresh homogenates of rat caudate putamen with the high-affinity component comprising approximately 18% of the total specific binding (Izenwasser et al., 1994). Cocaine inhibited [^{3}H]WIN 35,428 binding with a curve that was best fit by a two-site model. Analysis of the data revealed a high-affinity component ($K_{hi} = 32$ nM) and a low-affinity component ($K_{lo} = 388$ nM) (Izenwasser et al., 1994). Meperidine significantly competed against binding of [^{3}H]WIN 35,428 with an affinity > 1 μM, in a monophasic manner.

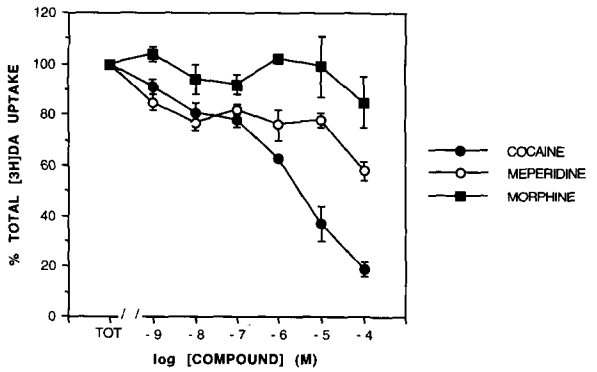

Fig. 2. Effects of cocaine (filled circles, $n = 5$), meperidine (open circles, $n = 5$), and morphine (filled squares, $n = 3$) on [^{3}H]dopamine uptake in chopped rat caudate putamen. Values represent percentage of total uptake ± S.E.M. from triplicate samples in each of n independent experiments. Total uptake did not differ significantly between experiments. Mean total uptake across experiments was 1.16 ± 0.24 pmol/mg protein.

4. Discussion

In the present study, meperidine in combination with a μ-opioid receptor antagonist dose of naltrexone completely substituted for cocaine in squirrel monkeys trained to discriminate cocaine from saline. A similar substitution was not obtained with the prototype μ-opioid receptor agonist morphine. In addition, meperidine produced an inhibition of dopamine uptake in chopped tissue from rat caudate with a maximal effect less than that obtained with cocaine, which inhibits dopamine uptake in this preparation through a two-component mechanism. The effects of meperidine suggest that it inhibits dopamine uptake through a single high-affinity component.

The substitution of meperidine for cocaine appears to be mediated by a mechanism unrelated to opioid receptors. Morphine, a prototypic μ-opioid receptor agonist, did not significantly substitute for cocaine, and the effects of meperidine were not antagonized by naltrexone. Indeed, significant substitution for cocaine by meperidine was only observed in the presence of a μ-receptor antagonist dose of naltrexone. These results indicate that the opioid agonist effects of meperidine interfere with the expression of its cocaine-like effects in the discrimination procedure. Thus, it is likely that an action by meperidine at μ-opioid receptors resulted in predominant disruptions in responding that precluded the testing of higher doses. The antagonism by naltrexone of the effects on response rates allowed for the testing of the higher doses that substituted for cocaine. The present assessment of the effects of meperidine is similar to that suggested for levorphanol in which its dextrorphan- or phencyclidine-like effects were obscured by its more potent opioid actions (Bertalmio and Woods, 1992).

As noted above, a number of previous studies have documented stimulant-like effects of meperidine. Himmelsbach (1943) in his classic studies of meperidine dependence noted that "At the outset patients receiving 75 mgm. q.i.d. liked the effects of Demerol. Such effects were described as being 'like Nembutal or Cocaine'..." (p. 6). More recently, Witkin et al. (1983) examined the effects on learned operant behavior of meperidine, when administered alone or with an opioid receptor antagonist dose of naloxone. When given alone, meperidine generally decreased rates of responding. When administered with naloxone, however, meperidine increased rates of responding in a manner similar to that produced by stimulant drugs such as cocaine (e.g. Barrett, 1974). Further, a similar stimulant effect was obtained in morphine-tolerant subjects (Witkin et al., 1983). The substitution of meperidine for cocaine in the present behavioral study, its inhibition of dopamine uptake, and its displacement of [^{3}H]WIN 35,428 suggest that the differences in the pharmacology of meperidine and more typical μ-opioid receptor agonists are due at least in part to meperidine's stimulant effects mediated by actions at the dopamine transporter. Previous studies have shown that dopamine transporters in rats and monkeys, as measured both by binding and dopamine uptake, are virtually identical (Boja and Kuhar, 1989; Madras et al., 1989a, b; Boja et al., 1990). Binding affinities and IC_{50} values for dopamine uptake inhibition are the same in both species, thus allowing us to draw conclusions based on the findings in the present study, even though different species were used in the behavioral and biochemical studies.

It is not possible at this time (due to the lack of compounds that are selective for either the high- or low-affinity component of dopamine uptake) to definitively determine to which component meperidine is binding. It is important to remember though, that the 'high' and 'low' affinity components of dopamine uptake are so named based on the affinities of cocaine, and that it might be possible for a different compound to have a high affinity for the low-affinity component. Even though meperidine appears to have a low-affinity for binding, it appears that it is acting selectively through the component of dopamine uptake inhibition for which cocaine has a high affinity. First, the inhibition of dopamine uptake by meperidine is distinguished from that for other dopamine uptake inhibitors (Izenwasser et al., 1994) by its large plateau (over a 1000-fold range of concentrations) that resembles the high-affinity component of the cocaine inhibition curve. Second, the maximal inhibition by meperidine was approximately 20%, a number consistent with the percentage (18%) of total binding attributed to the high-affinity component of [^{3}H]WIN 35,428 binding (Madras et al., 1989b; Izenwasser et al., 1994).

The present in vitro data suggest that the high-affinity component of the interaction of cocaine with the dopamine transporter may play an important role in producing the characteristic behavioral effects of cocaine. Meperidine, which appears to inhibit only this component, produced behavioral effects similar to those of cocaine. Further, the locomotor stimulant effects of a number of dopamine uptake inhibitors have in vivo potencies that are better correlated with their affinities for the high-affinity component of WIN 35,428 binding than with the low-affinity component (Izenwasser et al., 1994). Moreover, the potencies of dopamine uptake inhibitors in substitution for cocaine in a discrimination procedure were better correlated with affinities for the high-affinity component of WIN 35,428 binding than they were the low-affinity component (unpublished data).

Structure-activity relationships that have been described for a large series of cocaine and WIN analogs have demonstrated the importance of the 3-position

aryl substituent and its discrete distance from the *N*-methyl group of the tropane ring for optimal dopamine transporter binding (Carroll et al., 1992). The importance of the 2-methyl-ester of the cocaine and WIN analogs continues to be a matter of debate (Carroll et al., 1992; Kozikowski et al., 1992; Kelkar et al., 1994) and this function may primarily serve to position the aryl portion of the molecule optimally. As seen in Fig. 3, when meperidine and WIN 35,065-2 are energy minimized and the molecules are superimposed as described in the Materials and methods section, significant overlap results. Since the energy minima structure for meperidine results in a twist conformation of the piperidine ring rather than the chair conformation of the tropane ring of WIN 35,065-2, the fit is not exact but several key structural features of both molecules are closely aligned. Although the atoms that were chosen to be fitted are arbitrary, these are considered important for binding of WIN 35,065-2 to the cocaine recognition site on the dopamine transporter (Carroll et al., 1992). The superimposition of these molecules in the manner described may not represent the way these molecules interact at the dopamine transporter; however, based on what is known about the structural requirements of the tropane analogs, it is a reasonable hypothesis. Notably, the ester functions are not closely aligned, which may be the reason that meperidine binds with significantly lower potency to the dopamine transporter than WIN 35,065-2, or why meperidine does not seem to recognize the low-affinity component of the dopamine transporter. Interestingly, cocaine and the WIN series of compounds that demonstrate binding and inhibition of dopamine uptake via high- and low-affinity components of the dopamine transporter possess these common structural features with meperidine and thus may provide clues to chemical moieties that are required for recognizing the high-affinity component of the dopamine transporter.

A previous study from this laboratory has shown that a number of σ receptor ligands bind to the dopamine transporter and inhibit dopamine uptake in a monophasic manner that appears to correspond to

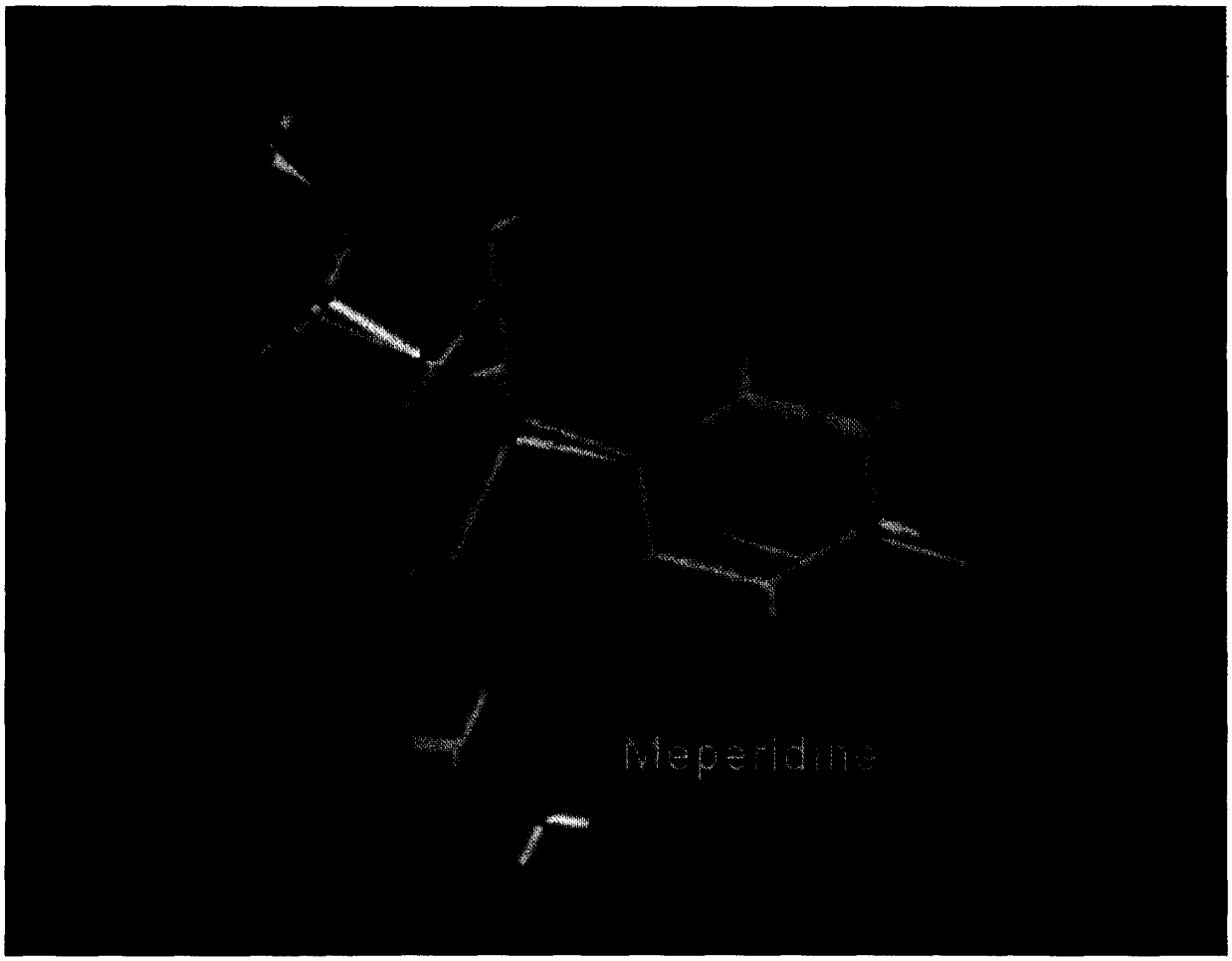

Fig. 3. Superimposition of the energy-minimized structures of meperidine and WIN 35,065-2, using SYBYL.

the low-affinity component of cocaine (Izenwasser et al., 1993a). In addition, these compounds have behavioral effects that are distinctly different from those of cocaine. For example, they are not effective stimulants of locomotor activity (Menkel et al., 1991; Witkin et al., 1993), do not substitute for cocaine discriminative stimulus effects (Witkin and Acri, 1995; also unpublished data from our laboratory), and have been reported to antagonize these behavioral effects (Menkel et al., 1991; Cook et al., 1992; Witkin et al., 1993). That these compounds are structurally distinct from meperidine and cocaine supports the idea that discrete common chemical features of meperidine and cocaine or its analogs are required for binding to the high-affinity component.

The present findings, in conjunction with previous results, suggest that there are functional differences associated with the high- and low-affinity components of cocaine's inhibition of dopamine uptake that result in distinctive behavioral effects. Previous results suggest that actions mediated by the low-affinity component do not result in cocaine-like behavioral effects, but rather may interfere with cocaine-like activity. Thus, the low-affinity component may play a regulatory role in the mediation of dopamine transport. In contrast, behavioral effects of cocaine such as its psychomotor stimulant and discriminative stimulus, or subjective, effects appear to be mediated by the high-affinity component. To the extent that these latter effects are related to drug abuse, the high-affinity component may be more intimately involved in actions that confer the abuse liability of cocaine than is the low-affinity component.

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