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Opiate Receptor Mediation of Ketamine Analgesia

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Previous workers have noted that analgesia produced by ketamine can be antagonized by the narcotic antagonist, naloxone. In order to elaborate further the apparent similarity between ketamine- and narcotic-induced analgesia, the authors examined the effects of ketamine in three standard test systems for the opiate receptor. In a radioligand binding assay using ^{3}H-dihydromorphine, ketamine stereospecifically bound to opiate receptors in rat brain homogenate, (+) ketamine being 2–3 times more potent than the (−) enantiomer of ketamine. In a bioassay for the opiate receptor, using the longitudinal muscle-myenteric plexus of the guinea pig ileum, ketamine inhibited the twitch-like muscular contractions, as do narcotics. However, only the inhibitory effects of (+) ketamine, which in this system also was twice as potent as (−) ketamine, could be partially antagonized by naloxone, suggesting that this enantiomer is responsible for the opiate receptor-related effects of ketamine. *In vivo*, the authors found that ketamine displaces ^{3}H-etorphine, a potent narcotic, from opiate receptors in regional areas of the mouse brain, especially in the thalamic region, but not in the cortex. The results suggest that a significant mechanism of ketamine-induced analgesia is mediated by opiate receptors. (Key words: Analgesics: ketamine; mechanism of action. Anesthetics, intravenous: ketamine. Antagonists, narcotic: naloxone. Receptors: opiate.)

AMONG THE MYRIAD of pharmacologic effects produced by the "dissociative" anesthetic, ketamine, is analgesia.[1,2] Experimentally, subhypnotic doses of ketamine produce a dose-related analgesia both in mice, using a p-phenylquinone writhing test,[3] and in rats, using a hot water tail-flick test to measure analgesia.[4] In both species, the analgesia produced by ketamine was significantly antagonized by administering the narcotic antagonist, naloxone, shortly after they were injected with ketamine. This led both groups of investigators to infer a similarity between ketamine-induced analgesia and the analgesia produced by narcotics. Ryder, Way, and Trevor[3] further speculated that ketamine might produce analgesia by releasing or potentiating endogenous opioid peptides, whose action was then antagonized by naloxone, or, alternatively, that ketamine might interact directly with opiate receptors.

In order to investigate further this last possibility, we have examined the effects of ketamine, both the racemate and its two enantiomers, in two standard *in vitro* opiate receptor systems: a radioligand binding assay using rat brain homogenate, published as a preliminary report,‡ and a bioassay using the longitudinal muscle-myenteric plexus preparation of the guinea pig ileum. In addition, we have tested the ability of ketamine to displace a radioactively labeled narcotic from opiate receptors in regional brain areas of the mouse *in vivo*.

Methods

In Vitro

Radioligand Binding Studies

A modification of the opiate receptor binding assays of Simon *et al.*[5] and Simantov and Snyder[6] was used. Male Sprague-Dawley rats weighing 175–300 g were decapitated, and the brains, minus cerebella, were homogenized in 6 volumes of iced 0.32 M sucrose using a Brinkmann Polytron® (setting No. 7, for 20 s), and centrifuged at 49,000 × g for 15 min. The pellets were resuspended in 60 volumes of iced 0.05 M tris buffer, *p*H 7.4 at 37° C, containing 1×10^{-3} M Mn^{++}. For the assay, each tube contained 2 ml of the diluted brain homogenate, either 5×10^{-10} or 1×10^{-9} M ^{3}H-dihydromorphine (^{3}H-DHM, specific activity of either 43 or 76 Ci/mmol, depending on batch, New England Nuclear Corp), 1×10^{-7} M of either dextrorphan or levorphanol and varying concentrations of morphine, met^5-enkephalin, phencyclidine, (±) ketamine, or the (+) or (−) enantiomers of ketamine in a total volume of 2.14 ml. For the assays of met^5-enkephalin, 50 μg/ml bacitracin was added. All assays were performed in triplicate and incubated at 20° C for 15 min. Incubations were terminated by cooling in an ice-water bath, followed by vacuum filtration on Whatman GF/B® glass fiber circles. These were washed three times with 4 ml iced tris buffer and placed in 10 ml Bray's solution for scintillation counting (Beckman®, Model LS 3133P liquid scintillation counter). Stereospecific binding was determined as the difference in radioactivity between tubes containing dextrorphan and those containing levorphanol. Data were expressed as per cent inhibition of the stereospecific binding in the absence of competing ligand. IC_{50} values (concentrations which inhibit stereospecific ^{3}H-DHM binding by 50 per cent) were calculated from logarithmic regression fitting of the data.

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‡ Finck AD, Ngai SH: A possible mechanism of ketamine-induced analgesia. ANESTHESIOLOGY 51:S34, 1979.

Opiate Receptor Bioassay

The longitudinal muscle-myenteric plexus preparation of the guinea pig ileum was used. This has been described in detail.[7] Briefly, the isolated tissue was suspended in a 5-ml tissue bath at 37° C. The bathing fluid consisted of a modified Krebs solution containing 70 μM hexamethonium bromide and 20 μM choline chloride, gassed with a mixture of 95 per cent O_2 and 5 per cent CO_2. The muscle was placed under 0.3 g tension and stimulated supramaximally with an electrical field stimulus (0.1 Hz, 0.5-ms duration, 80 V) using a Grass Model S8 stimulator connected to platinum electrodes in the bath. The twitch-like contractile response of the muscle was measured isometrically using a strain gauge transducer (Grass Model FT03C) and recorded on a Beckman-Offner Dynograph®. Varying concentrations of morphine, (±) ketamine, (+) ketamine, or (−) ketamine were added to the bath in a volume of 200 μl or less, either in the presence or absence of naloxone, 1×10^{-7} M. All concentrations are expressed as final bath concentrations. Results were expressed as percent inhibition of maximum twitch height.

Three point log dose-response curves were plotted for each drug, and IC_{50} values (concentrations of added drug that inhibit maximal twitch height by 50 per cent) were calculated from log regression fitting of the dose-response data. Results were compared using unpaired Student's *t* test.

In Vivo Pulse-Chase Studies

A modification of the *in vivo* opiate receptor labeling method of Höllt[8-10] was used. Male Swiss-Webster mice weighing 25–30 g were given 5 μCi of ^{3}H-etorphine (1.2 pmols in 0.25 ml normal saline of 15,16-^{3}H-etorphine, 41.4 Ci/mmol, Amersham) iv. In preliminary experiments, the animals were divided into two groups. At 5 min after the pulse injection of ^{3}H-etorphine, one group received subcutaneous injections of dextrorphan, each animal receiving only one dose of either 2, 5, 10, 25, or 50 mg/kg (calculated as base) in 0.25 ml of normal saline. The other group received similar injections of levorphanol at the same times in the same doses.

Twenty minutes after the injection of ^{3}H-etorphine (15 min after the injection of dextrorphan or levorphanol) all animals were killed by cervical dislocation and their brains excised carefully. The cerebellum from each brain was removed, weighed and placed in 0.5 ml of NCS tissue solubilizer (Amersham Corp). Similarly, the remainder of the brain from each animal was weighed, minced, and placed in 1.0 ml of NCS tissue solubilizer. All samples were then digested overnight at 50° C in tightly capped glass scintillation vials. Following digestion, the samples were cooled and 10 ml of Liquifluor scintillation fluid (New England Nuclear) was added, vortexed, and the radioactivity counted in a liquid scintillation spectrometer. DPM for each sample was determined using internal standards. Results were calculated as dpm/mg tissue (wet weight).

Because the cerebellum is devoid of opiate receptors, radioactivity present in the cerebellum of a given animal is nonspecific, *i.e.*, not opiate receptor-related. Thus, the cerebellum of each animal acts as an internal control for the rest of the brain. Accordingly, radioactivity present in the rest of the brain divided by radioactivity present in the cerebellum, expressed as the brain/cerebellum ratio (B/C ratio), was calculated for each animal.

In further experiments, three additional groups of mice were given an intravenous injection of 5 μCi ^{3}H-etorphine at zero time. Five minutes later, one group was injected with 25 mg/kg dextrorphan, a second group was injected with 25 mg/kg levorphanol, and a third group was injected with 50 mg/kg (±) ketamine, all subcutaneously. All drugs were dissolved in 0.25 ml of normal saline. Twenty minutes after the injection of ^{3}H-etorphine, all animals were killed by cervical dislocation and the brains were excised carefully and dissected into the following parts: cerebellum, brainstem, hippocampus, striatum, thalamic region, and cortex, according to the method of Glowinski and Iverson.[11] Each brain part was then weighed and placed in 0.5 ml NCS tissue solubilizer except for the cortex, which was placed in 1.0 ml solubilizer. All samples were then processed as described above. Results were calculated both in terms of dpm/mg tissue (wet weight) for each brain part and also as brain part/cerebellum ratio, and compared using unpaired Student's *t* test.

Results

Radioligand Binding Assay

The abilities of morphine, met^5-enkephalin, phencyclidine, and ketamine to displace ^{3}H-DHM from binding sites in brain homogenate are shown in figure 1, demonstrating differences in potency among these drugs, ranging in concentrations over several thousandfold. A more direct comparison of these potency differences can be made by comparing the IC_{50} values, or concentrations which inhibit ^{3}H-DHM binding by 50 per cent (Table 1). The (+) isomer of ketamine is two to three times more potent than (−) ketamine in this assay. The IC_{50} values obtained for morphine, met^5-enkephalin and phencyclidine are similar to previously published values of approximately 9×10^{-9} M (derived from Ref. 6), 1×10^{-8} M (Ref. 6), and 2.6×10^{-6} M (Ref. 12), respectively, using ^{3}H-DHM as the radioligand, considering minor variations in assay conditions.

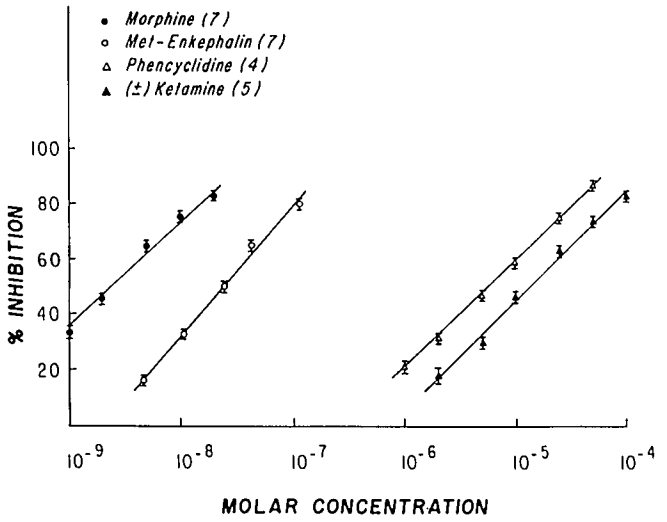

FIG. 1. Effects of morphine, met[5]-enkephalin, phencyclidine, and ketamine in an opiate receptor radioligand binding assay. See text for details. On the ordinate is the per cent inhibition of ^{3}H-dihydromorphine stereospecifically bound to rat brain homogenate caused by each of the drugs, and on the abscissa the final molar concentration of each drug. Number of experiments for each drug is shown in parentheses. Points are mean values, bars are SEM. The concentration of ^{3}H-dihydromorphine was 5×10^{-10} M.

Guinea-pig Ileum Bioassay

The effects of racemic ketamine and its two enantiomers in the guinea-pig ileum bioassay are shown in figure 2. All three drugs produced a dose-related inhibition of twitch height, which was readily reversed by washing. The calculated IC_{50} values are shown in table 2. The IC_{50} value for morphine we obtained is similar to the previously published value of 4.90×10^{-7} M using maximal stimulation.[13] Naloxone (1×10^{-7} M) caused only a partial antagonism of the twitch inhibition produced by (±) ketamine (fig. 2*A*). This same concentration of naloxone was ineffective in antagonizing twitch inhibition produced by (−) ketamine (fig. 2*B*), but was more effective in antagonizing the inhibitory action of (+) ketamine (fig. 2*C*) than the racemic mixture. Higher concentrations of naloxone (up to 1.8×10^{-6} M) tested against racemic ketamine produced only small increases in the degree of antagonism and never completely reversed the inhibitory action of ketamine. A more quantitative analysis of this partial antagonism produced by naloxone is shown in figure 3, depicting three point log dose-response curves for the (+) and (−) isomers of ketamine in the absence and presence of naloxone (1×10^{-7} M). Figure 3*B* shows that naloxone had no effect on the dose-response curve of (−) ketamine. The calculated IC_{50} of (−) ketamine in the presence of naloxone was $7.06 \pm 1.03 \times 10^{-6}$ M, not different from the value of $6.7 \pm 1.4 \times 10^{-6}$ M obtained in the absence of naloxone. However, naloxone did cause a parallel shift to the right of the dose-response curve produced by (+) ketamine, significantly ($P < 0.01$) changing the IC_{50} value from 3.42 ± 0.31 (SEM) $\times 10^{-6}$ M in the absence of naloxone to $5.16 \pm 0.21 \times 10^{-6}$ M in the presence of naloxone, indicating a degree of competitive antagonism toward the (+) enantiomer (fig. 3*A*).

TABLE 1. Molar Concentrations of Drugs in Opiate Receptor Binding Assay, Which Inhibit Binding of ^{3}H-DHM by 50 Per Cent (IC_{50})

Drug Added	IC_{50} [M]
Morphine	$3.1 \pm 0.1 \times 10^{-9}$ (4)
Met[5]-Enkephalin	$2.54 \pm 0.12 \times 10^{-8}$ (7)
Phencyclidine	$5.82 \pm 0.37 \times 10^{-6}$ (4)
(±) Ketamine	$2.30 \pm 0.12 \times 10^{-5}$ (4)
(+) Ketamine	$1.63 \pm 0.74 \times 10^{-5}$ (4)
(−) Ketamine	$4.55 \pm 0.32 \times 10^{-5}$ (4)
Dextrorphan	1.21×10^{-6} (1)

Values are means ± SEM; number of experiments in parentheses. Concentration of ^{3}H-dihydromorphine was 5×10^{-10} M in the experiments using phencyclidine and met[5]-enkephalin, and 1×10^{-9} M for the other drugs tested.

In Vivo "Pulse-Chase" Studies

Results from the preliminary pulse-chase studies are shown in figure 4, demonstrating that levorphanol, but not its inactive stereoisomer, dextrorphan, stereospecifically chased previously administered ^{3}H-etorphine from the brains of mice. This effect was maximal at a dose of 25 mg/kg, decreasing the brain/cerebellum ratio from a control value of 2.12 to 1.19. All doses of levorphanol produced a Straub-tail reaction and an increase in locomotor activity, whereas dextrorphan produced no detectable change in behavior. In subsequent experiments, comparing the relative abilities of levorphanol and racemic ketamine to displace ^{3}H-etorphine from brain areas, 50 mg/kg of ketamine usually produced an initial Straub-tail reaction, followed by a decrease in locomotor activity and marked ataxia. No animals lost their righting reflex, however. The behavioral changes produced when ketamine was used as a "chaser," following a "pulse" of ^{3}H-etorphine were much more pronounced than when the same dose of ketamine was given alone.

Table 3 summarizes the results of actions of dextrorphan, levorphanol, and (±) ketamine in displacing ^{3}H-

TABLE 2. Final Molar Bath Concentrations of Drug Which Inhibit Guinea Pig Ileum Twitch Height by 50 Per Cent (IC_{50})

Drug Added	IC_{50} [M]
Morphine	$4.23 \pm 0.90 \times 10^{-7}$ (6)
(±) Ketamine	$4.89 \pm 0.51 \times 10^{-6}$ (8)
(+) Ketamine	$3.42 \pm 0.31 \times 10^{-6}$ (4)
(−) Ketamine	$6.7 \pm 1.4 \times 10^{-6}$ (4)

Values are means ± SE; number of experiments in parentheses.

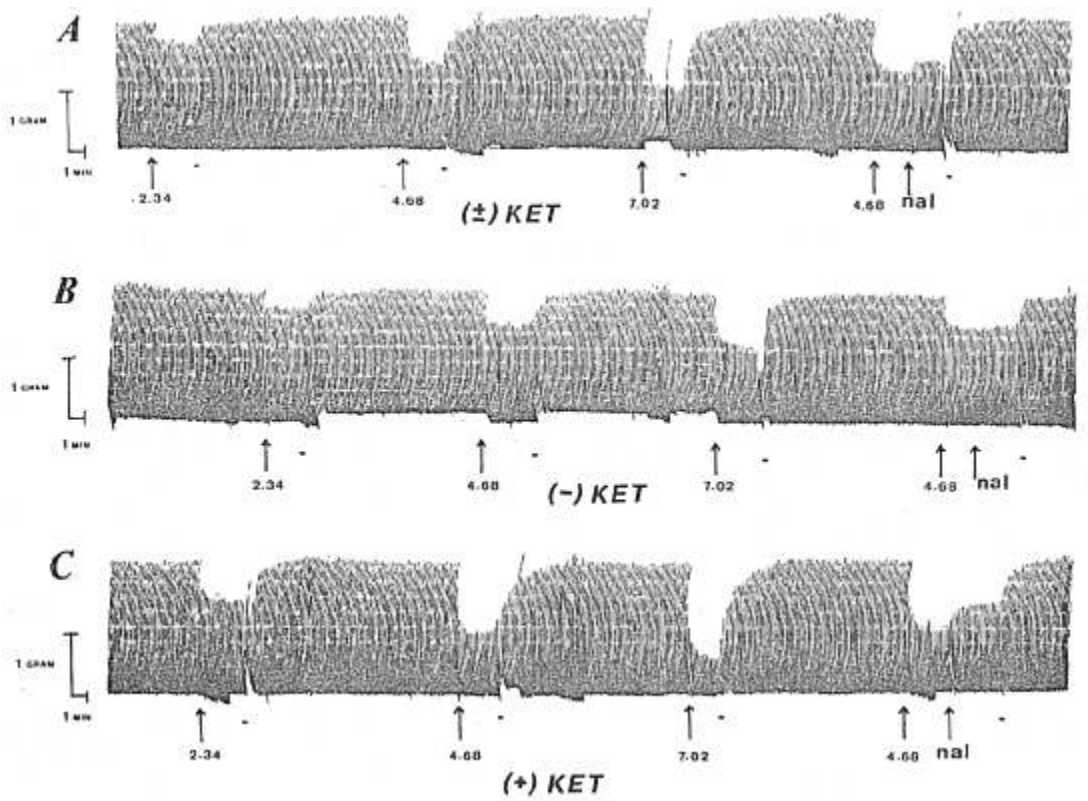

FIG. 2. The abilities of racemic ketamine (panel A), (−) ketamine (panel B), and (+) ketamine (panel C) to inhibit electrically evoked muscle contraction in the guniea pig ileum bioassay. Numbers are final molar bath concentrations $\times 10^{-6}$ of drug added. Nal, naloxone, 1×10^{-7} M. W, washing of the tissue.

etorphine from brain areas examined. Levorphanol significantly displaced ^{3}H-etorphine, using either the raw dpm/mg or the dpm/mg (brain part)/ dpm/mg (cerebellum) ratio data, in which case the cerebellar radioactivity acts as an internal standard for each animal. Both the dpm/mg and the B/C ratios are significantly lower in levorphanol- than in dextrorphan-treated controls. Similarly, ketamine also significantly displaced ^{3}H-etorphine from striatum, hippocampus, brain stem, and thalamic region, but not from cerebellum or cortex.

Discussion

The finding that ketamine binds to opiate receptors in a radioligand binding assay has been reported by other workers. Smith *et al.*[14] using ^{3}H-naloxone as the radioligand found an IC_{50} for racemic ketamine of 1.48×10^{-5} M in a sodium-free system similar to ours, also noting that the (+) enantiomer was approximately three times more potent than the (−) enantiomer in their system. Su *et al.*§ using guinea pig brain homogenate and ^{3}H-naloxone as radioligand found an IC_{50} of 5×10^{-5} M for racemic ketamine, though they did not test the enantiomers. In marked contrast, Fratta et al[15] did not detect ketamine binding to opiate receptors even at concentrations as high as 10^{-4}M. Reasons for this discrepancy are not apparent.

An issue that can be raised is whether the binding of ketamine to opiate receptors in a radioligand binding assay is of any pharmacologic significance, because, as seen in table 1, the concentrations needed are several orders of magnitude higher than the concentrations of morphine or met^5-enkephalin needed to produce the same amount of radioligand displacement. In fact, pharmacologically inactive dextrorphan has a lower IC_{50} than ketamine, though the relevance of binding of this pharmacologically inactive drug[16,17] is obscure in the present context. Perhaps of greater relevance, as shown by Ryder, Way and Trevor,[3] is that the concentration of racemic ketamine measured in mouse brain ranged from 2.5–4.0 μg/g brain tissue at 2–15 min after subcutaneous administration of the median effective *analgesic* (subhypnotic) dose of 7 mg/kg. Recalculation of our IC_{50} values gives a concentration of 5.47 μg/ml in the radioligand binding assay and a concentration of 1.2 μg/ml in the guinea pig ileum bioassay for the racemate. These con-

§ Su TP, Cone EJ, Shannon H, et al: Relative potencies of phencyclidine and analogs in the opiate receptor binding assay. Correlation with relative potencies determined *in vivo* in mouse and rat. Res Comm Subst Abuse 1:85–98, 1980.

centrations are almost an order of magnitude lower than the concentration of 27 μg/g racemic ketamine in rat brain measured by Marietta *et al.*[18] at the time the animals *regained* their righting reflex after a dose of 30 mg/kg, iv. Obviously, no extrapolation can be made between *in vitro* data and the *in vivo* situation other than to lend strong support to the idea that the *in vitro* effects of ketamine reported herein are occurring at pharmacologically relevant concentrations.

The finding that ketamine interacts with opiate receptors in a biologically functional system such as the guniea pig ileum bioassay further strengthens our hypothesis that an interaction of ketamine with opiate receptors is a relevant mechanism of action of this drug. In addition, the data from the guinea pig ileum experiments suggest that the (+) enantiomer of ketamine is the one responsible for any opiate-receptor mediated effects, because only the effects of (+) ketamine were partially antagonized by naloxone.

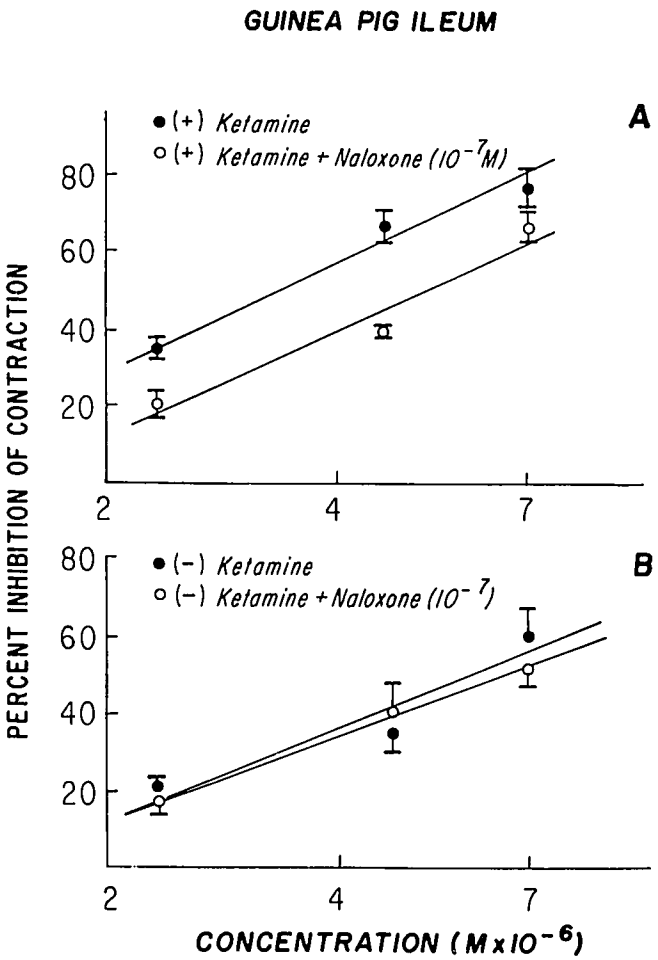

FIG. 3. Log-dose response curves of (+) ketamine (panel A) and (−) ketamine (panel B) in the guinea pig ileum bioassay, both in the absence (closed circles) and presence (open circles) of naloxone, 10^{-7} M, in the bath fluid. Each point is the mean value of four experiments. Bars represent SEM.

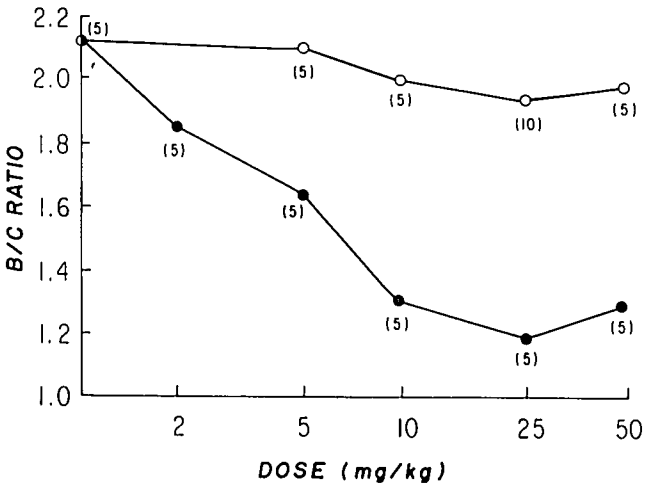

FIG. 4. Displacement of ^{3}H-etorphine in mouse brain by levorphanol or dextrorphan *in vivo*. On the ordinate is whole brain:cerebellum (B/C) ratio of radioactivity (dpm/mg tissue); on the abscissa, the dose of chaser drug administered subcutaneously. Number of mice from which each point is derived is shown in parentheses. ◑ = normal saline; ○ = dextrorphan; ●, levorphanol. SEM for each point are smaller than the plotted points.

Of some concern is the fact that naloxone never fully antagonized the effects of (+) ketamine. A possible explanation of this is that the final neurotransmitter mediating smooth muscle contraction in the guinea pig ileum is acetylcholine. The opiate receptors present in the myenteric plexus appear to modulate cholinergic innervation of the longitudinal smooth muscle.[13,19] One of the properties of the phencyclidine series of compounds, of which ketamine is a member, is a potent anticholinergic effect.[12,20-23] If the inhibition of muscle contraction in the guinea pig ileum caused by ketamine is due to combined opiate-like and anticholinergic actions of the drug, then, when the opiate-receptor mediated component has been antagonized by naloxone, the anticholinergic component is still actively inhibiting contraction. This would explain the apparent inability of naloxone to antagonize fully the effects of ketamine in the guinea pig ileum, and the lack of antagonistic action of naloxone against (−) ketamine (figs. 2*B*, 3*B*).

The *in vivo* pulse-chase studies provide a third separate line of evidence supporting opiate receptor mediation of ketamine analgesia. Ketamine displaces the potent radioactively labeled narcotic agonist, etorphine, from opiate receptors in regional areas of the mouse brain in the intact animal. Ryder, Way, and Trevor[3] studied the analgesic activity of racemic ketamine and its isomers in the mouse using a p-phenylquinone abdominal constriction test to measure analgesia and found the median effective analgesic (subhypnotic) dose of the racemate to be 6.5 mg/kg, subcutaneously. This is a considerably smaller dose than we used (50 mg/kg) in the pulse-chase studies. This discrepancy is probably methodological. No

TABLE 3. The Abilities of Three Drug Treatments to Displace ^{3}H-etorphine from Brain Areas

Brain Part	Dextrorphan, 25 mg/kg, sc				Levorphanol, 25 mg/kg, sc				(±) Ketamine, 50 mg/kg, sc				ΔB/C (Per Cent max)
	N	Weight (mg)	dpm/mg	B/C	N	Weight (mg)	dpm/mg	B/C	N	Weight (mg)	dpm/mg	B/C	
Cerebellum	20	63.4 ± 1.2	81.4 ± 4.3	—	20	60.4 ± 1.3	81.2 ± 3.0	—	15	63.1 ± 1.2	70 ± 5	—	—
Striatum	19	28.1 ± 1.2	206 ± 16	2.54 ± 0.12	20	31.6 ± 1.3	122 ± 5*	1.52 ± 0.05*	14	32.4 ± 1.3	151 ± 14*	2.17 ± 0.15*	36
Hippocampus	15	30.7 ± 1.4	145 ± 12	1.83 ± 0.10	15	27.7 ± 1.7	110 ± 7*	1.37 ± 0.05*	14	28.5 ± 2.0	106 ± 7*	1.50 ± 0.06*	72
Brain stem	19	63.2 ± 1.9	165 ± 10	2.07 ± 0.07	20	68.4 ± 2.0	107 ± 4*	1.31 ± 0.03*	14	66.8 ± 2.4	127 ± 7*	1.80 ± 0.06*	36
Cortex	18	187 ± 4	146 ± 10	1.84 ± 0.07	20	190 ± 5	113 ± 5*	1.40 ± 0.05*	15	188 ± 5	118 ± 6	1.78 ± 0.09	14
Thalamic region	10	81.6 ± 2.3	210 ± 13	2.61 ± 0.11	10	92.0 ± 2.7*	147 ± 14*	1.93 ± 0.15*	10	88.9 ± 2.8	138 ± 12*	1.91 ± 0.14*	100

See text for methodological details. N, number of animals. ΔB/C (per cent max) is the reduction in B/C ratio produced by ketamine divided by the reduction in B/C ratio caused by levorphanol, times 100.

* Significantly different from corresponding dextrorphan-treated group (control group, $P < 0.02$).

published data are available on the analgesic activity of levorphanol in the mouse; however, such data are available in the rat. Fromherz[16] tested the analgesic effects of levorphanol in this species using a Hardy-Wolff thermal stimulus method and found analgesia at doses as low as 1 mg/kg, subcutaneously. Although data obtained in the rat cannot be extrapolated to the mouse, just as a thermal dolorimetry test cannot be compared with an abdominal constriction test, nevertheless it would appear that the dose of levorphanol needed to produce detectable analgesia is also considerably less than the optimal dose we needed (25 mg/kg) to chase labeled etorphine from opiate receptors in the brain. Furthermore, if only the (+) enantiomer of ketamine is active in displacing labeled etorphine from opiate receptors, as suggested by the guinea pig ileum data, the dose of (+) ketamine used as chaser present in the racemate was half the administered dose, *i.e.*, 25 mg/kg. Because the molecular weight of levorphanol base (257 daltons) is similar to that of ketamine (238 daltons), the two drugs, therefore, were administered in approximately equimolar amounts. That levorphanol is intrinsically more potent than ketamine is evident from the last column in Table 3, in which the change in B/C ratio between levorphanol- and dextrorphan-treated animals was considered maximal and the change in B/C ratio produced by ketamine was compared with that produced by levorphanol. Only in the thalamic region did ketamine appear to be equipotent with levorphanol. This is probably not due to a selective accumulation of the drug in this region, because Cohen *et al.*[24] found similar concentrations of ketamine in all brain regions examined at five and ten minutes following an intravenous dose, and this would presumably extend to later time periods. The potency of ketamine in displacing labeled narcotic from opiate receptors in the thalamic region may point toward a selective mechanism of ketamine action if, for example, its action in this region were to "dissociate" incoming stimuli and prevent them from reaching higher brain centers.

Of interest is the fact that Ryder, Way, and Trevor[3] found the (+) isomer of ketamine to be three times more potent (range 2.1–4.3) as an analgesic in the mouse compared with the (−) isomer and yet, the (+) isomer was only one-and-a-half times (range 1.2–1.9) as potent as the (−) isomer as a hypnotic, measured by loss of righting reflex. Similarly, in surgical patients, White *et al.*[25] found that (+) ketamine was approximately 3.4 times more potent as an anesthetic agent than (−) ketamine and more effective as an anesthetic. Side effects commonly associated with narcotic use, such as nausea, and a sense of well-being, were reported more frequently in patients who had received (+) ketamine than (−) ketamine. The pharmacodynamic differences between the two enantiomers of ketamine in the above two studies, as well as

the differences in potency, correlate well with our present results showing that (+) ketamine is 2.7 times more potent than (−) ketamine in radioligand binding assay and twice as potent in the guinea pig ileum bioassay. Unfortunately, the limited amount of isomers available precluded our testing these in the pulse-chase studies.

In summary, previous studies showing that ketamine analgesia is partially antagonized by naloxone, together with the present results showing that ketamine, at pharmacologically relevant concentrations, binds to opiate receptors in two standard *in vitro* test systems and that ketamine displaces radioactively labeled narcotic from opiate receptors *in vivo*, lead us to conclude that a mechanism for the analgesia produced by ketamine is mediated by opiate receptors. Furthermore, it seems that the (+) enantiomer of ketamine is primarily responsible for this action.

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