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THE INTERACTION OF KETAMINE WITH THE OPIATE RECEPTOR

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SUMMARY

The analgesic effect of the anesthetic agent ketamine HCl is inhibited in rats by the narcotic receptor antagonist naloxone. Racemic (±) ketamine HCl also displaced ^{3}H-naloxone in an opiate receptor binding-assay. The potency of ketamine in the assay was reduced nearly six-fold by sodium suggesting that the drug interacts as an agonist. However, some activity as an antagonist was not ruled out. The interaction of ketamine HCl with the opiate receptor was stereospecific with the (+) salt being more effective than the (-) salt. The stereoselective nature of the interaction is consistent with other studies (1) demonstrating that (+) ketamine HCl has a greater analgesic effect than the (-) salt.

Ketamine [2-(0-chlorophenyl)-2-(methylamino) cyclohexanone HCl] is an intravenous anesthetic agent that produces profound analgesia in man and animals (1-4). Ryder and coworkers (1) recently demonstrated that the analgesic effect of ketamine as determined in the phenylquinone - writhing test was attenuated by the narcotic receptor antagonist naloxone. This observation suggests that the endogenous opiate neuronal pathways in the central nervous system may be involved in the analgesic action of ketamine.

One explanation for an interaction of ketamine with endogenous opiate mechanism might be that the drug is an agonist of the opiate receptor. In fact, several of the phencyclidine like structural homologues of ketamine have been shown to bind to opiate receptors (5). Consequently, we have evaluated the ability of ketamine to interact with opiate receptors using a standard opiate receptor binding assay with ^{3}H-naloxone as the radioligand. In addition, we reexamined the analgesic action of ketamine in rats and its sensitivity to the narcotic receptor antagonist naloxone.

METHODS

Male Sprague-Dawley rats (200-250 g) received from Hilltop Laboratory Animals, Inc., Scottsdale, PA were used. Opiate receptor binding was determined as described by Pert and Snyder (6) with modifications by Childers and coworkers (7). Briefly, a rat brain minus the cerebellum was placed in 40 volumes of ice cold

0.05 M Tris-HCl buffer, pH 7.7 (adjusted at 25°C) and homogenized using a Brinkman Polytron (setting 5, 20 seconds). The homogenate was centrifuged at 40,000 X g for 12.5 minutes and the pellet was resuspended in fresh buffer (10 mg original tissue/ml). The brain membrane tissue was then incubated at 37°C for 40 minutes and recentrifuged as above. After resuspending the final pellet (as above), aliquots (1.8 ml) were added to incubation tubes containing 0.05 ml of ^{3}H-naloxone (final concentration 1.4 nM, S.A. 8.23 Ci/m mole, New England Nuclear), one of at least 5 concentrations of test drugs and additional buffer as required to bring the final incubation volume to 2 ml. Some tubes contained 1 µM levallorphan in order to determine the degree of non-specific binding (see below). All tubes were incubated at 25°C for 20 minutes after which the tissue was collected by vacuum filtration on Millipore AP 25 glass-fiber filters (binding of ^{3}H-naloxone to the filters was negligible). The filters were rapidly rinsed 3 times with 5 ml aliquots of ice cold Tris-HCl buffer and were then vigorously shaken with 10 ml of Hydromix scintillation fluor (Yorktown Research) and counted in a Packard Tri-Carb scintillation spectrometer.

Specific opiate receptor binding was calculated by subtracting the value for radioactivity in samples containing 1 µM levallorphan (see above) from the values obtained in its absence. Data were expressed as percent inhibition of control binding (^{3}H-naloxone, specific receptor binding in the absence of competing drugs). IC 50 values (concentration of a drug displacing 50 percent of the specific ^{3}H-naloxone binding) were determined using logarithmic - probability paper (8).

The analgesic effect of ketamine was evaluated using a modification of the rat tail - flick method developed by D'Amour and Smith (9). The test was conducted by placing the tail of a rat on a board over a 1 cm long slit through which passed the light from a high intensity projection bulb. The time required for the rat to remove his tail from the heat source was recorded in seconds and expressed as the tail - flick latency. A 15 seconds maximum exposure was used to avoid damaging the tail tissue. Animals were tested before and several times after the administration of drugs. Averaging the last 3 of 4 latencies prior to drug administration gave the control tail - flick latency for each rat. Statistical comparisons were made using Dunnett's t-test (10).

RESULTS

The analgesic effect of racemic (±) ketamine HCl. The effect of racemic (±) ketamine HCl on the tail - flick latency occurring 5-7 minutes after the intraperitoneal administration of the drug is shown in figure 1.

A dose-dependent increase in latency was observed. The analgesic effect was maximal 5-15 minutes after drug administration and had a duration of approximately 1 hour with a sub-anesthetic dose (80 mg/kg, ip) and 1.5 hour with an anesthetic (160 mg/kg, ip) dose of the drug. No analgesia was detected with 40 mg/kg of ketamine.

The narcotic antagonist naloxone (10 mg/kg, ip) administered 10 minutes prior to the injection of ketamine prevented the anal-

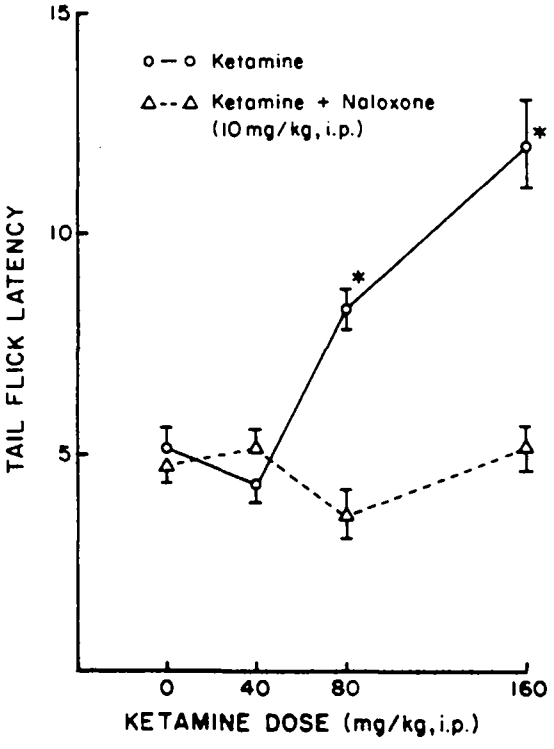

FIG. 1

The effect of ketamine on tail flick latency in seconds is shown 5-7 minutes after drug administration. Each point on the graph represents the mean and standard error of the mean from at least 6 rats. The control point (0 ketamine) is a mean derived from all of the animals tested. The asterisk represents significant ($p<0.05$) increases in tail-flick latency.

gesia (Fig. 1). Lower doses of naloxone were also effective. The lowest dose tested was 0.625 mg/kg and provided about 62 percent inhibition of the response to ketamine (6 rats).

The interaction of (±) ketamine HCl with the opiate receptor. (+) Ketamine HCl caused a concentration - dependent inhibition of the binding of ^{3}H-naloxone to opiate receptors on rat brain membranes (Fig. 2). Compared to morphine, levorphanol or naloxone, however, ketamine was less effective in inhibiting ^{3}H-naloxone binding. IC 50 values (concentration inhibiting ^{3}H-naloxone binding by 50 percent) are shown in table 1. The concentration - inhibition curves for each drug were essentially parallel (see slopes in figure 2, determined by least squares regression analysis) suggesting that ketamine and the opiates were competing for the same binding sites

The stereospecificity of ketamine for opiate receptor binding was also evaluated using enriched isomers of the drug. It has been estimated that the isomers available to us might be 10-20 percent cross - contaminated (i.e., the (-) salt contains some

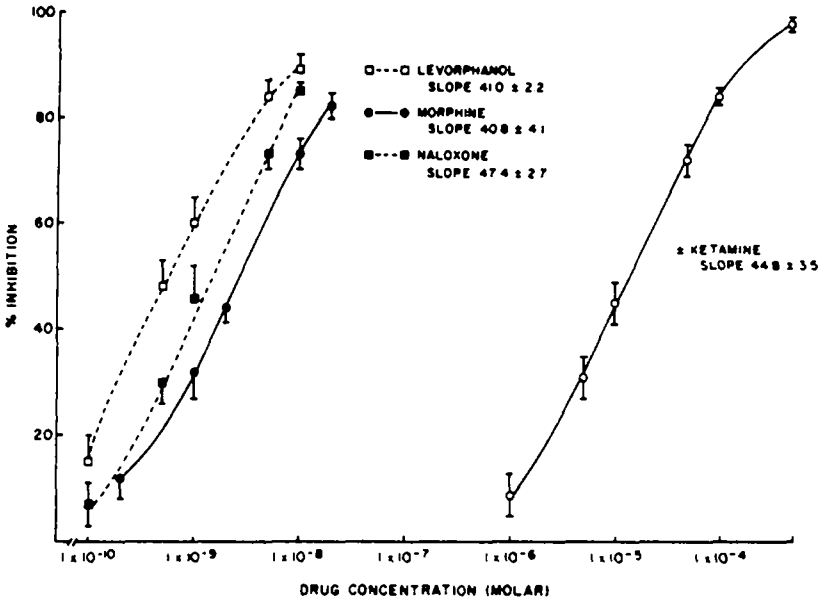

FIG. 2

The effect of opiates and ketamine on ^{3}H-naloxone binding. Each point represents the mean ± the standard error of the mean from at least 5 experiments run in duplicate. The assay was conducted by adding 1.8 ml of the brain membrane fraction in 0.05 M Tris-HCl buffer (pH 7.7) to tubes containing 0.05 ml of ^{3}H-naloxone (1.4 nM final concentration), 0.05 ml of the appropriate drug dilution and additional buffer to bring the final volume to 2 ml. No sodium was included. Some tubes contained 1 µM levallorphan in order to determine the degree of non-specific ^{3}H-naloxone binding. All tubes were incubated at 25°C for 20 minutes.

(+) salt and vice versa) (personal communication E.F. Domino and Warner-Lambert/Parke Davis, Inc.). However, the enriched isomers did show a difference in their ability to inhibit ^{3}H-naloxone binding. The predominately (+) salt had an IC 50 value of 7.0 ± 0.98 µM and the IC 50 for the (-) salt was 23.8 ± 1.7 µM.

It has been shown that the ability of opiate receptor agonists to inhibit ^{3}H-naloxone binding is significantly reduced in the presence of sodium. whereas opiate antagonists are only slightly affected. Mixedagonist - antagonists have intermediate responses to sodium. This relationship was studied with racemic ketamine and compared to values obtained with morphine, levorphanol and naloxone (Table 1). IC 50 values obtained when the binding assay was run with 100 mM NaCl in the incubation medium were compared to data derived using the standard condition (see methods).

TABLE 1

Comparison of the relative abilities of opiates and (±) ketamine HCl to displace ^{3}H-naloxone in the presence and absence of sodium.

Drug	IC 50 [Na=0]	IC 50 [Na=100mM]	Na RATIO $\frac{\text{IC 50 [Na=100mM]}}{\text{IC 50 [Na=0]}}$
	nM	nM	
MORPHINE	3.60±0.89	103.0±28.0	33.0±7.3
LEVORPHANOL	0.98±0.20	21.0±4.8	19.3±2.9
NALOXONE	1.50±0.12	1.90±0.31	1.31±0.33
	µM	µM	
±KETAMINE HCl	14.8±2.01	75.7±0.85	5.8±0.97

IC 50 values were determined by log probit plot analysis. Values shown are the mean ± standard error of the mean.

It was observed that ketamine was about 6 times less effective in displacing ^{3}H-naloxone when Na+ was present in the incubation medium. The relative shifts observed in the potency of the opiates studied were similar to those reported by other investigators.

DISCUSSION

Our data suggest that the analgesic effect of ketamine is at least partially related to an ability of the drug to act as an agonist at the opiate receptor in the central nervous system. Ketamine - induced analgesia was prevented by the narcotic receptor antagonist naloxone and ketamine displaces ^{3}H-naloxone in the opiate receptor binding assay. The affinity of ketamine for opiate receptor binding was much lower than that of classical opiate agonists. However, unlike the inactive opiate dextrorphan which also has a low affinity (8 µM;IC50) in the same assay (6), ketamine appears to stimulate opiate mediated responses. Preliminary studies from our laboratory demonstrate that ketamine produces a naloxone - sensitive reduction in the electrically induced twitch of the guinea pig ileum as do other narcotic agonists (12).

The inhibition of ketamine - induced analgesia by naloxone does not preclude the involvement of other neuronal systems in the antinociceptive action of the drug. In fact, our studies in progress suggest that the neurotransmitters serotonin and norepinephrine may also be involved, since specific receptor blockers for these putative neurotransmitters attenuate the analgesic action of ketamine (13). Therefore it is possible that several neuronal systems may be involved in the action of the drug on pain perception and reaction. Some of these systems may converge on opiate pathways making their physiological effects sensitive to naloxone as well.

In our experiments, the effect of sodium to reduce the binding affinity of ketamine for the opiate receptor by only six-fold may indicate that the drug has antagonist as well as agonist properties (7, 14). On the other hand, the significance of sodium shift ratios of potency have not been analyzed for compounds with binding affinities as low as ketamine, and other studies will be required to assess the drug's antagonistic activity. Furthermore, some compounds with low sodium shift ratios may only exhibit agonist activity as has been demonstrated for ethylketocyclazocine (14). However classical opiates with higher affinities and mixed agonist - antagonist activities usually do have degrees of shift in potency comparable to that of ketamine. Included among these compounds are levorphanol, phenazocine, methionine enkephalin and ketocyclazocine with "sodium ratios" of 15, 13, 6, and 3.3, respectively.

The observation that ketamine was less effective than the classical opiates in inhibiting ^{3}H-naloxone binding does not compromise the importance of its interaction at the opiate receptor. The concentration of ketamine in the brain of rats after an anesthetic dose of the drug ranges from 5-100 µg/g tissue (15). The concentration range required in vitro to inhibit binding was 0.27 to 27.4 µg/ml. However, a comparison of in vitro results to those obtained in vivo should be made with caution, and an evaluation of the binding of ketamine to the opiate receptor in vivo should be done.

Although ketamine is used clinically as the racemic salt of HCl, it has been demonstrated in mice that the (+) salt of the drug is about 3 times more potent as an analgesic (1). Our observation that the (+) salt is at least 3 times more active in displacing ^{3}H-naloxone is therefore consistent with the observed pharmacological difference. However, it should be noted that upon conversion to their respective hydrochlorides the free bases of the isomers of ketamine change their rotational signs (personal communication, Bristol Laboratories). Therefore, the (+) ketamine HCl is in fact the levorotatory free base. Consequently, ketamine appears to display the same type of stereospecific interaction with the receptor as do the opiate narcotics with the levorotatory form of the drugs being preferred. The fact that the (-) salt (dextrorotatory free base) had the ability to interfere with ^{3}H-naloxone binding may be an artifact related to its potential cross - contamination with the (+) salt. In fact, if a 20 percent contamination is assumed, then it would appear that nearly all of the activity resides in the levorotatory form of the free base of ketamine.

Recently, it has been suggested that some of the behavioral effects of ketamine and other phencyclidine derivatives may be related to an interaction of these drugs with high affinity at specific phencyclidine receptors (16, 17). In view of the slightly higher apparent affinity of ketamine (IC 50, 7 µM) for phencyclidine binding sites relative to its binding to the opiate receptor, an evaluation of the role of phencyclidine receptors in ketamine - induced analgesia may be warranted. Phencyclidine receptors could be associated with neuronal

patnways involved in the mediation of pain, but as yet there is no data to support this suggestion.

In conclusion, we have shown that ketamine HCl interacts with the opiate receptor in a stereospecific manner. This interaction is at least partially responsible for the analgesic effect of the drug, since ketamine analgesia is reversed by naloxone.

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