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Effects of Ketamine on Sensory Perception: Evidence for a Role of N-Methyl-D-Aspartate Receptors[1]

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ABSTRACT

The chiral forms of ketamine were applied as probes for N-methyl-D-aspartate receptor-mediated neurotransmission in humans. Both enantiomers, in clinically relevant concentrations, displaced [^{3}H]dizocilpine (MK 801) from specific binding sites (phencyclidine sites) in membrane fractions of brain homogenates. (*S*)-Ketamine was at least 4 times as potent as (*R*)-ketamine in this respect. In healthy volunteers, the most obvious effect of subanesthetic doses of both enantiomers was altered sensory perception. (*S*)-Ketamine was 4 times as potent as (*R*)-ketamine in reducing pain perception and in causing auditory and visual disturbances. Both enantiomers caused proprioceptive disturbances (feelings of detachment from the body) and slightly reduced the ability to recall objects seen after administration of the drugs. The ability to recall objects seen immediately before drug exposure was unaffected. The results are in accordance with the hypothesis that inhibition of sensory perception by ketamine in subanesthetic concentrations is due to N-methyl-D-asparate receptor blockade. It is suggested that N-methyl-D-asparate receptor-mediated transmission is involved in the processing of sensory information in the human brain.

NMDA receptor gated ion channels (NMDA channels) mediate neurotoxic effects of excitatory amino acids and are probably involved in the pathophysiology of brain ischemia and neurodegenerative disorders (see Olney, 1990 for review). Under experimental conditions NMDA channels serve special neuronal functions like long-term potentiation and developmental plasticity. Fast postsynaptic effects of excitatory amino acid transmitters are believed to be mediated mainly by non-NMDA receptors. However, NMDA receptors also serve fast synaptic transmission (see Davies, 1989). Experimental evidence linking NMDA receptor-mediated neurotransmission to specific brain functions in humans has been lacking.

Ketamine is a PCP-like dissociative anesthetic which binds to a recognition site (the PCP site) in the NMDA channels and inhibits noncompetitively NMDA receptor function (see Lodge and Johnson, 1990). Ketamine also interacts with opiate receptors (Smith *et al.*, 1980) and with the nonopioid *sigma* site which is believed to mediate some of the psychotomimetic effects of PCP-like drugs (see Sonders *et al.*, 1988). In subanesthetic doses, ketamine has been reported to cause analgesia and a peculiar state of altered consciousness (Domino *et al.*, 1965). We have presented evidence that ketamine inhibits pain perception in humans by a nonopioid mechanism (Maurset *et al.*, 1989). By examining the analgesic effects and receptor affinity profiles of the two optical isomers of ketamine, a positive correlation between analgesic potency and PCP site affinity was observed, whereas there was a negative correlation between analgesic potency and *sigma* site affinity (Klepstad *et al.*, 1990). These findings are consistent with the hypothesis that inhibition of pain perception by ketamine is due to NMDA channel blockade, and that the chiral forms of ketamine may serve as probes for NMDA receptor-mediated neurotransmission in humans.

We have now examined the acute effects of four subanesthetic doses of (*R*)- and (*S*)-ketamine on sensory perception, short time memory and mood in human volunteers and compared the relative potencies of the enantiomers for these effects with their relative affinities for the PCP binding site associated with the NMDA receptor operated channels.

Materials and Methods

Ketamine was synthesized in our laboratory and the enantiomers were resolved by a slightly modified version of the previously described method (Klepstad *et al.*, 1990). The final product was identified by gas chromatography/mass spectrometry, optical rotation measurements and high-performance liquid chromatography. The absolute structure of the optical isomers was determined by x-ray chrystallography (Moberg *et al.*, 1991). The stability of each enantiomer in the stock solutions and in blood samples from the test persons was checked by high-performance liquid chromatography using a chiral column (Enantiopac, Pharmacia Fine Chemicals, Piscataway, NJ) under conditions which separated the enantiomers.

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ABBREVIATIONS: NMDA, N-methyl-D-aspartic acid; PCP, phencyclidine; VAS, visual analog scales.

Binding of the ketamine enantiomers to the PCP recognition site in coarse particle fractions from guinea pig and human brain homogenates was examined as described previously (Klepstad *et al.*, 1990), except that 4 nM dizocilpine {(+)-[3-^{3}H]MK-801, New England Nuclear, Boston, MA} was used as radioactive ligand. Autopsy samples from human brain were obtained 24 to 26 hr postmortem and stored at −80°C until used. Relative affinities were calculated from IC_{50} values obtained by plotting the radioligand displacement data according to the method described by Ariëns and Simonis (1964). K_i values, when given, were calculated from the equation

$$K_i = IC_{50}/[1-(L/K_d)]$$

where L is the concentration of the radioactive ligand and K_d the dissociation constant of the radioactive ligand obtained in separate saturation assays.

The *in vivo* effects of the ketamine enantiomers were studied in six male students, age 21 to 28 years. Written informed consent was obtained and the protocol was approved by the official regional ethical committee as an extension of a previous investigation on racemic ketamine (Maurset *et al.*, 1989). The study followed a double blind cross-over design, each test person participating in 10 experiments (two controls and four doses of each ketamine enantiomer).

Graded dose-response relationships for analgesia to experimentally induced ischemic pain were obtained by using VAS extending from 0 (no pain) to 100 mm (intolerable pain) as described previously (Maurset *et al.*, 1989). Briefly, the subjects exercised their forearm muscles to exhaustion by compressing a hand grip strengthener. Blood supply was then shut off by inflating a sphygmomanometer cuff to 100 mm Hg above systolic blood pressure. Pain was assessed by the subjects every 30 sec for 5 min on the VAS. The subjects assessed insobriety (the feeling of having received a drug) by relating the effect to previous experience with alcohol, by using a VAS extending from 0 (sober) to 100 mm (completely drunk or intoxicated). The subjects recorded other effects of ketamine by answering a written questionnaire (in Norwegian) designed to reveal psychopharmacological effects, some of which had been observed in previous studies on ketamine analgesia: altered color perception, reduced visual acuity, changes in hearing, hallucinations, altered body image, feelings of unreality, anxiety, aggression, altered physical strength, dizziness, discomfort, illness and nausea. Dose-response relationships for these effects were expressed as the number of test persons reporting the particular effect at each ketamine dose. The subjects also marked their feelings on 15 100 mm bipolar VAS designed to identify changes in mood, including alertness, contentedness and calmness (adopted from Olajide and Lader, 1985).

Dose-response relationships for amnesia were obtained as follows: 4 min before the start of a ketamine injection a set of eight pictures (figurative and nonfigurative drawings, size about 5 × 10 cm each visible for 5 sec) were presented. Six minutes after the start of a ketamine injection, the subjects were shown a new set of eight pictures. Ten minutes later the subjects were asked to pick the pictures seen during the two presentations from the total set of 23 pictures, presented one at a time.

Results

Displacement of dizocilpine by (*R*)- and (*S*)-ketamine. Both chiral forms of ketamine displaced specifically bound dizocilpine in a coarse membrane fraction from guinea pig forebrains (fig. 1). The K_d for dizocilpine in this preparation was found to be 4 nM, and this ligand concentration was used in the ketamine displacement assays. The K_i values for (*S*)- and (*R*)-ketamine in the displacement experiments shown in figure 1 are 1.2 and 5 μM, respectively. Similar results were obtained by using membranes prepared from autopsy samples of human brain (frontal cortex, occipital cortex and hippocampus) (table 1). The K_d values for dizocilpine in the various autopsy samples were not examined and the results presented in table 1 are therefore expressed as IC_{50} values.

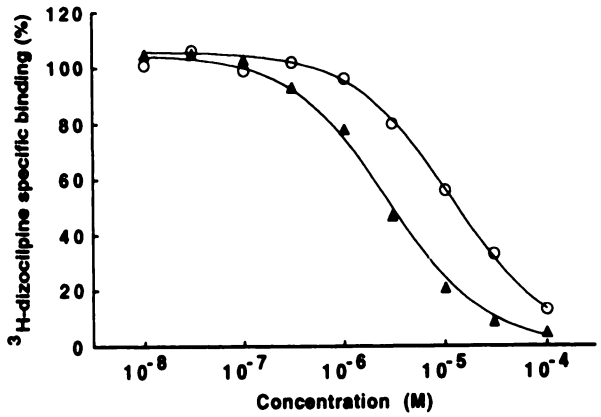

Fig. 1. Displacement of specifically bound [^{3}H]dizocilpine (concentration in assay, 4 nM), by (*R*)-ketamine (○) and (*S*)-ketamine (▲) in a coarse particle fraction from guinea pig brain. Data are from one single experiment (means of triplicate samples). Binding is expressed as percentage of specific binding in the absence of ketamine (total binding, 652 cpm; unspecific binding, 46 cpm).

TABLE 1

(*R*)-Ketamine/(*S*)-ketamine affinity ratios for PCP sites in human brain

Displacement of specifically bound [^{3}H]dizocilpine (MK 801) by (*R*)- and (*S*)-ketamine in coarse particle fractions prepared from autopsy samples from two human brains (cause of death: gastric cancer and cancer of unknown origin, brain metastases not found). The IC_{50} values were calculated from five separate displacement assays, numbers are mean values with S.E.s in parentheses.

	IC_{50}		IC_{50} Ratio
	(*R*)-Ketamine	(*S*)-Ketamine	
	μM		
Brain 1			
Hippocampus	7.2 (1.2)	1.6 (0.2)	4.5
Frontal cortex	8.2 (1.4)	1.5 (0.3)	5.5
Occipital cortex	10.9 (3.3)	2.1 (0.8)	5.2
Brain 2			
Hippocampus	10.0 (3.0)	1.9 (0.6)	5.3
Frontal cortex	13.7 (3.2)	2.8 (1.2)	4.9
Occipital cortex	11.4 (1.2)	1.6 (0.3)	7.1

Effects of (*R*)- and (*S*)-ketamine on sobriety and pain perception. Dose-response relationships of the ketamine enantiomers were examined using 4 times higher doses of (*R*)- than of (*S*)-ketamine. The doses were selected on the basis of previous experience with the analgesic effect of the enantiomers. Doses of 0.05, 0.10, 0.15 and 0.20 mg/kg of (*S*)-ketamine and 0.20, 0.40, 0.60 and 0.80 mg/kg of (*R*)-ketamine were diluted to 2 ml in sterile saline and injected i.v. over a period of 2 min. Maximal effect was observed less than 5 min after starting the injections, and a decline of effect was evident after about 10 min. The test persons experienced the "intoxicating" effect of ketamine as similar to, but not identical with, the effect of alcohol. VAS markings of the subjective sensation of being influenced by a drug (insobriety) confirmed that (*S*)-ketamine was about 4 times more potent than (*R*)-ketamine (fig. 2, top). A similar dose-response relationship was obtained for analgesia to experimentally induced ischemic pain (fig. 2, bottom). The test persons felt the noxious insult, but the appreciation of this insult as painful was reduced by both ketamine enantiomers in a dose-dependent way.

Effects on vision and hearing. Both enantiomers affected

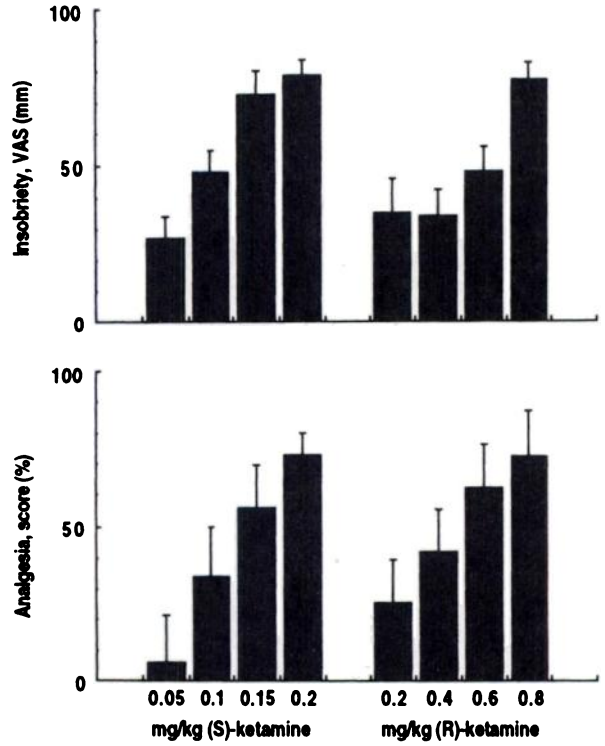

Fig. 2. Effects of ketamine enantiomers in healthy volunteers. Upper panel, effects of four different concentrations of (*S*)- and (*R*)-ketamine on the subjective sensation of being influenced by a drug. Lower panel, ketamine-induced analgesia to experimentally induced ischemic pain. Mean values and S.E.s (n = 6).

vision and hearing. The numbers of test subjects who reported visual and auditory disturbances at various doses are shown in figure 3, top and middle panels. The visual disturbances were primarily described by the test persons as blurred vision. In addition, more subtle changes in visual perception were revealed, including a stereotypic preoccupation with specific objects as well as a sensation of visual field narrowing ("tunnel vision"). The alteration in hearing was characterized by preoccupation with certain presumably "unimportant" sounds like the ticking of a clock or the sound from a centrifuge. The test persons often reported that these sounds were louder than before and that shifting the attention to another sound required unusual "effort."

Effects on somatosensory perception. The test persons were able to recognize touch and pressure and could differentiate between cold and warm objects applied to the skin. These sensory qualities were not systematically tested. Impaired recognition of limbs or body parts was often experienced by the test persons. A sensation like "floating in the air" or a feeling of detachment from the body was often reported. For the higher doses, (*R*)-ketamine appeared to be less efficient than (*S*)-ketamine in causing these proprioceptive disturbances when equianalgesic doses were compared (fig. 3, bottom).

Effects on short time memory, alertness and mood. There was no impairment of the ability to recall a set of pictures shown before ketamine was given. The recall of a similar set shown after ketamine injection was slightly reduced by both enantiomers (fig. 4). The results of the mood rating scales showed dose-dependent effects for the alert/drowsy group of ratings only. At equianalgesic doses, (*S*)-ketamine appeared to be more potent than (*R*)-ketamine in reducing the scores for

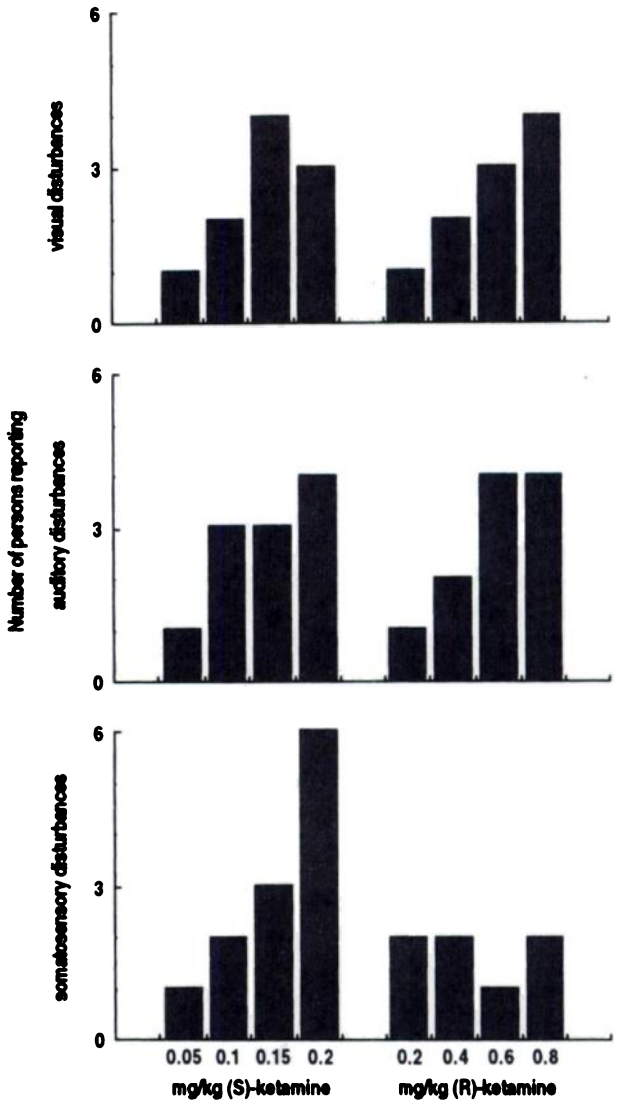

Fig. 3. Number of test persons (n = 6) reporting disturbances of vision (upper panel), hearing (middle panel) and proprioception (lower panel) after four different doses of (*S*)- and (*R*)-ketamine.

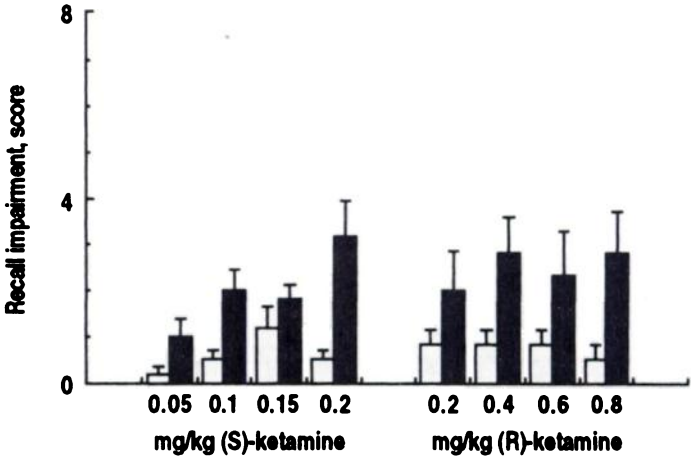

Fig. 4. Effect of four different doses of (*S*)- and (*R*)-ketamine on recall: number of missed recognitions of figures presented before (open columns) and after (black columns) ketamine injection. Mean values and S.E.s (n = 6). Number of possible misses = 8.

alertness (data not shown). All test persons remained awake, cooperative and were able to perform the various tasks according to the protocol. The test persons were unable to discriminate between the two optical isomers of ketamine at this low concentration range.

Discussion

Both chiral forms of ketamine displaced [3-^{3}H]dizocilpine which is a relatively selective ligand for the PCP recognition site associated with the NMDA channel. The present investigation confirms that (*S*)-ketamine has higher affinity for PCP sites than (*R*)-ketamine, the affinity of (*S*)-ketamine being about 4 times higher than that of (*R*)-ketamine in guinea pig forebrain. The ratios found in autopsy samples from various regions of human brain were in the same range as those found in freshly prepared homogenates from guinea pig brain.

In previous investigations both ketamine enantiomers have been found to have higher affinity for the NMDA channel (PCP recognition site) than for the other ketamine binding sites presently known (Øye *et al.*, 1991). A K_i value of 0.6 μM for racemic ketamine in human brain was reported by Tam and Zhang (1988) who used dizocilpine as radioactive ligand. By using TCP (a thienyl analog of PCP) as radioligand, we found slightly higher values, about 0.9 μM for (*S*)-ketamine and 2.5 μM for (*R*)-ketamine (Øye *et al.*, 1991). At higher concentrations (*S*)- and (*R*)-ketamine interact with opioid *mu* receptors (K_i values 11 and 28 μM, respectively) and *sigma* sites (K_i values 131 and 19 μM, respectively) (Øye *et al.*, 1991).

Ketamine injected i.v. is distributed rapidly to the brain, and concentrations in brain tissue have been reported to be equal to, or higher than, concentrations in blood (Gole *et al.*, 1990). Surgical anesthesia is obtained at serum concentrations above 4 to 5 μM racemic ketamine. Analgesia is obtained by 1/10 to 1/5 of an anesthetic dose. Accordingly, the effects of subanesthetic doses of ketamine in humans are likely to occur at concentrations which are sufficient to block a substantial fraction of the NMDA channels. At higher (anesthetic) doses other receptors, in particular opioid *mu* receptors and *sigma* sites, may contribute to the pharmacological effects of ketamine.

The present study confirms that both (*S*)- and (*R*)-ketamine inhibit pain perception, and that (*S*)-ketamine is about 4 times more potent in this respect. The graded dose-response relationships of analgesia and insobriety were closely similar. Furthermore, the quantal dose-response relationships for disturbance of vision and hearing nearly overlapped the graded dose-response curves for analgesia and insobriety. Thus, the relative order of potency of the enantiomers in inhibiting sensory perception corresponds to their relative affinities for PCP sites and not to their relative affinities for *sigma* sites. As shown previously, the effect of ketamine on pain perception in this concentration range is not inhibited by naloxone, indicating that opioid receptors are not involved (Maurset *et al.*, 1989). The potency ratio for inhibition of sensory perception and the affinity ratio for the PCP site were of similar magnitude. It is therefore suggested that these disturbances of sensory perception reflect NMDA channel blockade.

The dose-response relationships for proprioceptive disturbances revealed an apparent discrepancy between the effects of the enantiomers, (*R*)-ketamine in the higher doses causing less disturbance of proprioception than (*S*)-ketamine. This apparent difference may be related to the fact that (*R*)-ketamine caused less drowsiness than (*S*)-ketamine. The "floating" and "out of the body" feelings recorded here as proprioceptive disturbances appeared to be associated with drowsiness. This is in line with previous studies on the *anesthetic* properties of the ketamine enantiomers (White *et al.*, 1980). These authors found (*R*)-ketamine inferior to (*S*)-ketamine as a general anesthetic because of its excitatory properties. The excitatory properties of (*R*)-ketamine may be related to its affinity for the *sigma* site (Klepstad *et al.*, 1990).

The slight effect of the enantiomers on short time memory occurred in parallel with the general perceptive disturbances. The apparent amnesia may therefore not reflect a selective impairment of memory, but may rather reflect the general perceptive disturbances described above. At the higher doses used in clinical anesthesia, ketamine is generally believed to cause "reliable amnesia."

Ketamine inhibits NMDA receptor-mediated neuronal excitation, and the (*S*)-enantiomer is the more potent inhibitor (Lodge *et al.*, 1982; Anis *et al.*, 1983). The molecular requirements for inhibition of synaptic transmission at the NMDA receptor by PCP site ligands correlate with *in vivo* behavioral actions of these drugs in animals (see Quirion *et al.*, 1988). Studies in animal models thus indicate that at least some of the effects of ketamine are due to PCP site-mediated inhibition of the NMDA subtype of glutamate receptors.

The present investigation confirms that (*S*)-ketamine has higher affinity than (*R*)-ketamine for the PCP binding site, the affinity ratios found being about 4 in guinea pig brain and about 5 in most autopsy samples from human brain. Both (*R*)- and (*S*)-ketamine interfered with pain perception as well as the perception of several other sensory qualities. These effects were caused by doses which are expected to give serum (and brain) concentrations within the PCP site occupancy range of the enantiomers. The relative potencies of the enantiomers for inhibition of sensory perception corresponded to their relative affinities for the PCP site. The present findings indicate that inhibition of sensory perception by subanesthetic doses of ketamine is due to PCP site-mediated blockade of NMDA receptor operated channels. This is consistent with the hypothesis that the NMDA type of glutamate receptors serve synaptic transmission in neuronal pathways involved in the processing of sensory information in the central nervous system. In these pathways Ca^{++} influx through NMDA operated channels may serve to link synaptic transmission to neuronal plasticity and thus constitute a mechanism for the continuous modulation of the nervous system by sensory afferent signals.

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