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Psychotomimesis Mediated by κ Opiate Receptors

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The κ opioid agonists are analgesics that seem to be free of undesired morphine-like effects. Their dysphoric actions observed with the κ agonist cyclazocine are thought to be mediated by an action at σ-phencyclidine receptors. The benzomorphan κ agonist MR 2033 is inactive at σ-phencyclidine receptors. In male subjects, the opiate-active (−)-isomer, but not the (+)-isomer, elicited dose-dependent dysphoric and psychotomimetic effects that were antagonized by naloxone. Thus, κ opiate receptors seem to mediate psychotomimetic effects. In view of the euphorigenic properties of μ agonists, our results imply the existence of opposed opioid systems affecting emotional and perceptual experiences.

THE OBSERVATION THAT BENZOMORPHAN κ opioid agonists have analgesic properties but do not produce the drug-seeking behavior or cardiovascular and respiratory depression seen with morphine and other agonists at μ opiate receptors has raised the possibility that such compounds could be developed for use by humans (*1, 2*).

Detailed clinical studies of the actions of κ agonists have been performed with the benzomorphan derivative cyclazocine (*3*). This opioid, in addition to its κ agonistic activity, has antagonistic properties at μ opiate receptors (*1–3*). Moreover, in humans, cyclazocine and another benzomorphan, *N*-allylnormetazocine (Smith Kline & French 10047), elicit psychotomimetic and dysphoric effects similar to those of the dissociative anesthetics phencyclidine (PCP) and ketamine (*4*). Pharmacological studies suggested that these effects of the κ agonists are mediated by σ-PCP receptors (*1, 5, 6*). These latter receptors were distinguished from μ, δ, and κ opiate receptors by their resistance to the opiate antagonist naloxone, which classifies them as nonopiate receptors (*7*). In addition, PCP receptors displayed preference for (+)-isomers, whereas opiate receptors show stereospecificity for (−)-isomers (*6, 8*). During experiments designed to evaluate endocrine actions (*9*) of the potent benzomorphan κ agonist MR 2033 (*10*), which is inactive at σ-PCP receptors (*6*), we observed aversive and psychotomimetic effects. Because this observation contradicted the view that σ-PCP receptors mediate psychotomimetic effects of κ agonists, we undertook the present studies to characterize the type of receptor involved. Our results suggest that κ receptors elicit aversive and psychotomimetic actions in humans.

Thirty healthy male volunteers (24 to 62 years old) participated in the experiments. An intravenous cannula was inserted 60 minutes before the drug was administered and the subjects remained in a recumbent position throughout the experiment. Fifteen minutes before intravenous drug injection, the subjects completed five evaluation scales related to mood and physical experiences (*11*). Observer rating scales were used to assess psychotic symptoms and abnormal behavior (*12*). The same evaluation scales were again completed 30 and 90 minutes after drug treatment. Each subject first received either saline or 10 mg of naloxone followed immediately by MR 2033 (the racemic mixture), MR 2034 [the opiate-active (−)-isomer], or MR 2035 [the opiate-inactive (+)-isomer]. Subjects were unaware of which treatment they received.

In a first set of experiments, five subjects received MR 2033 (3.5 μg/kg) after being treated with either saline or naloxone. This relatively low dose was followed by an increase in nonspecific bodily complaints such as weakness, sweating, vertigo, and dizziness as well as an increase in anxiety (as assessed by the psychopathological evaluation scales). Three of five subjects experienced, respectively, racing thoughts, feelings of body distortion, and severe discomfort. These effects were blocked by prior treatment with naloxone (10 mg).

These experiments indicated that MR 2033–induced changes in subjective experience which were sensitive to naloxone and thus probably related to opiate receptors. To further characterize these subjective effects, six men in each of three groups were treated with the opiate-active (−)-isomer (MR 2034) (1.9 or 3.8 μg/kg) or with 10 mg of naloxone prior to injection of the dose of MR 2034 (3.8 μg/kg).

The lower dose of MR 2034 slightly increased and the higher dose considerably increased abnormal behavior; these symptoms were prevented by prior treatment with naloxone (Fig. 1). All subjects showed psychotomimetic symptoms after treatment with MR 2034. In particular, the high dose resulted in somesthetic changes and disturbances in the perception of space and time. Abnormal visual experiences reported by most subjects consisted of moving lines or walls or color phenomena. There were symptoms of depersonalization, derealization, and loss of self-control. For example, one subject had frequent episodes of unmotivated and uncontrolled laughter during a 90-minute period. Two subjects became unaware of the experimental situation for periods of 20 to 30 minutes and later described their experiences as dreamlike. Although pseudohallucinations were reported, true hallucinations did not seem to occur. Most subjects became intermittently disoriented, but remembered the experimental situation when asked by the observers. This disorientation was probably due to preoccupation with perceptual and cognitive alterations

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rather than to a general deterioration of cognitive function. These psychotomimetic effects were most pronounced in the first 30 minutes after drug injection. They were frequently followed by a second phase of marked sedation lasting for about 90 minutes (Fig. 1C). No psychotomimetic effects and little sedation were noted in subjects treated with naloxone (Fig. 1, B and C). Another subject who became disoriented and experienced brightly colored visual phenomena was given 10 mg of naloxone 15 minutes after being injected with the higher dose of MR 2034. The symptoms were reversed within 2 minutes of the naloxone treatment.

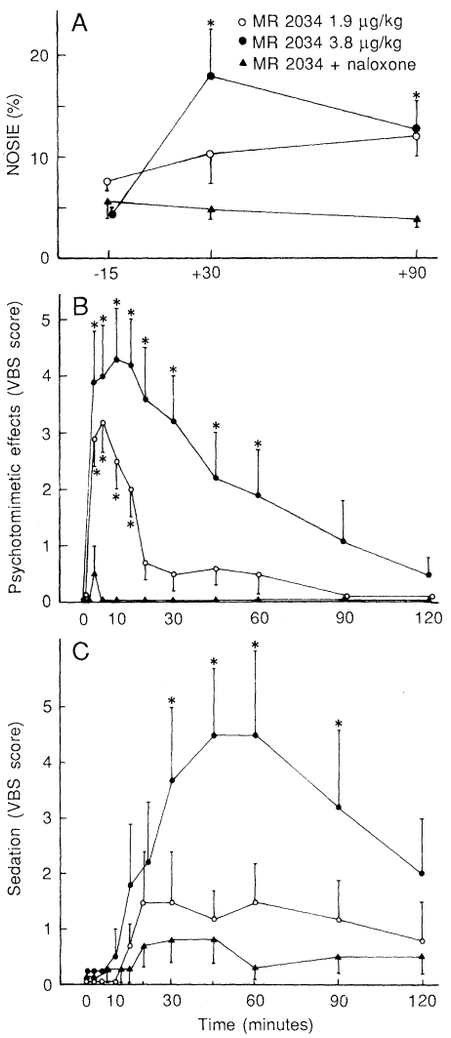

Fig. 1. (A) Psychopathological effects of MR 2034 with or without naloxone (10 mg) in normal volunteers ($n = 6$ per group) (*18*). Data are from a scale of abnormal behavior (NOSIE, nurses' assessment scale for inpatient observation) (*12*). (B) Psychotomimetic effects as assessed by the VBS-scale (Verlaufs-Beurteilungs-Skala, course-assessment scale) (*12*), an eight-point rating scale that can be adapted to specific individual symptoms of the probands. Items evaluated were (i) disturbance of the perception of space, (ii) disturbance of the perception of time, (iii) abnormal visual experience, (iv) disturbance of body image perception, (v) depersonalization, (vi) derealization, (vii) loss of self-control, and (Fig. 1C) sedation. Data are indicated as means ± SEM. Statistical analysis was by Dunn's test (Bonferroni test). Asterisks indicate significant differences ($P < 0.05$) from subjects receiving MR 2034 + naloxone.

Self-rated bodily complaints such as weakness, sweating, vertigo, difficulty in focusing, and dizziness occurred after the higher dose of MR 2034; these complaints did not occur in subjects treated with naloxone. Similarly, the increase in anxiety assessed by a self-rating scale (Fig. 2A) was prevented by naloxone treatment.

These experiments demonstrate that the subjective actions of MR 2034 are mediated by opiate receptors. They did not exclude, however, the possibility of an independent psychotomimetic effect of the (+)-isomer MR 2035, particularly at higher doses. To test this possibility, two subjects were each treated with MR 2035 at doses of 3.8, 7.6, and 14.2 μg/kg. Neither of these subjects reported the subjective effects or displayed any abnormal behavior, thus demonstrating that the subjective experiences are confined to the opiate-active (−)-isomer.

To compare the effects of the κ agonist to those elicited by a typical μ agonist, three subjects (two of whom had previously received the κ agonist) were treated with a dose of fentanyl (100 μg) sufficient to produce analgesia. The subjects completed the psychopathological evaluation scales as before. All subjects reported an increase in well-being and also some sedation and dizziness. There were no significant changes in anxiety or bodily complaints, and neither abnormal behavior nor psychotomimetic effects were seen. Thus, it seems unlikely that the observed effects of the κ agonist were mediated by μ receptors. This conclusion is in agreement with several studies demonstrating that MR 2034 is functionally inactive at μ receptors in vivo (*2, 9, 13*). However, the control is necessary because MR 2034, in addition to its high affinity for κ sites, also interacts with μ opiate binding sites in vitro (*14*).

Our data suggest that activation of κ opiate receptors elicits psychotomimetic effects in humans. This probably limits the clinical usefulness of these benzomorphans as analgesics despite their other favorable properties.

Psychotomimetic effects of PCP are likely to be mediated by nonopiate receptors as suggested by the low affinity of PCP to κ opiate receptors in the human brain (*14*) and by the very low binding affinity of naloxone for σ PCP receptors (*5*). Dysphoric and psychotomimetic actions of cyclazocine and *N*-allylnormetazocine in humans may involve κ opiate receptors for which

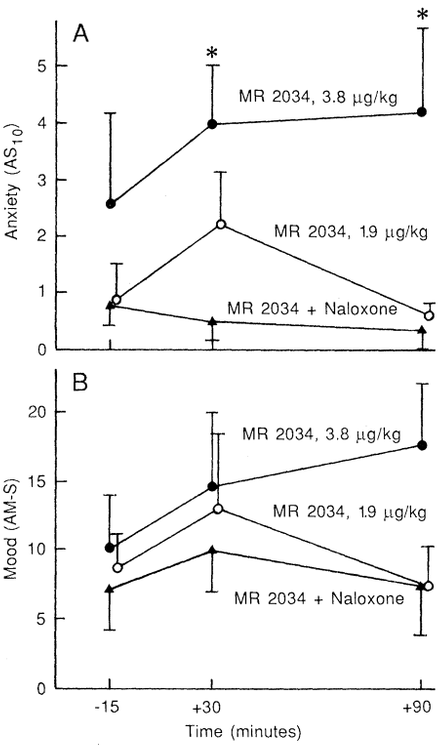

Fig. 2. Effects of MR 2034 with or without naloxone on anxiety and mood as assessed by (A) an anxiety scale (AS_{10}) and (B) an adjective mood scale (AM-S); high values represent deterioration of psychic states (*11*). Asterisks indicate significant differences from treatment with MR 2034 + naloxone. The self-rating scales were completed in approximately 10 minutes at $t = -15$ and $t = 90$ minutes; approximately 30 minutes was required at $t = 30$ minutes, and two of seven subjects in the group receiving the dose at 3.8/μg/kg were unable to complete the questionnaire at this time.

these compounds have high affinity in the human brain (*14*), although further experiments with naloxone will be required to confirm this possibility.

A recent examination of motivational properties of μ and κ agonists in rats showed that μ agonists mediate preference conditioning whereas κ agonists had aversive properties; these results suggest opposite functions of the two opioid systems (*15*). In humans, endogenous opioid systems are activated by arousing emotional situations (*16*). Also in humans, μ agonists produce euphorigenic actions (*17*) that appear to be opposed to the dysphoric effects of the κ agonist we studied. The endogenous opioid systems associated with μ and κ receptors may serve opposite functions in processes affecting emotional and perceptual experiences.

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18. The volunteers were healthy male medical doctors or students (mean age 27 ± 1.5 years, $n = 30$) who gave written informed consent to participate in the study. They had no histories of previous major illness and were evaluated as healthy by routine clinical examination.
19. We thank R. Welchering and A. Wendl for help during the experiments and A. Yassourides for statistical advice.

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Transplantation of Fetal Hematopoietic Stem Cells in Utero: The Creation of Hematopoietic Chimeras

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Transplantation of normal, immature, fetal hematopoietic cells into a preimmune fetal recipient with a congenital hemoglobinopathy may allow partial reconstitution of normal hemoglobin production without the complications associated with postnatal bone marrow transplantation (immunosuppression and the occurrence of graft versus host disease). In order to test this hypothesis the naturally occurring polymorphism at the β-hemoglobin locus of the sheep was used as a marker for engraftment and hematopoietic chimerism. Intraperitoneal injection of allogeneic fetal stem cells into normal fetal lambs resulted in hematopoietic chimerism in three of four surviving recipients. This chimerism has been sustained for 6 months after birth and 9 months after engraftment, without evidence of graft versus host disease, and without the use of immunosuppressive therapy.

THE RECONSTITUTION IN UTERO OF cell lines that are either deficient or defective with normal cells has considerable theoretical appeal and potential clinical application. Treatment of congenital hematopoietic diseases by the creation of hematopoietic chimerism through stem cell reconstitution with normal cells represents a potential method of alleviating the clinical manifestations of some of these diseases. However, current methods of postnatal transplantation of stem cells are hampered by the complications of immunosuppression and the occurrence of graft versus host disease (GVHD) (*1*). These problems result from the immunocompetence of the recipient or graft or both and thus may be avoidable if grafting is performed before immunocompetence is well established. Transplantation of hematopoietic stem cells derived from an early gestational fetus into an immunologically immature fetal recipient offers such a possibility.

Our goal in this study was to produce an animal model of hematopoietic chimerism by the in utero transplantation of fetal hematopoietic stem cells. We chose sheep for the following reasons: (i) the sheep has two naturally occurring alleles of the β-hemoglobin locus (A and B), which can be used as definitive markers of successful engraftment and chimerism (*2*); (ii) gestation in the pregnant ewe is sufficiently long (term, 145 days) to allow experimental manipulation in the first trimester; (iii) the immune status of the fetal lamb is relatively well documented (*3–5*), permitting accurate selection of preimmune donors and recipients; and (iv) we have extensive experience with fetal surgery in the sheep (*6*). We report here that transplantation of fetal hematopoietic cells into preimmune fetuses resulted in the creation of hematopoietic chimeras. This chimerism has persisted for 6 months after birth and 9 months after engraftment without the occurrence of GVHD.

Pretyped homozygous AA and BB Dorset-Merino sheep (*Ovis aries*), from separate herds in Minnesota and California, respectively, were used for breeding stock. We transplanted hematopoietic stem cells obtained from the combined livers of 35- to 50-day-old fetal AA sheep (two males and one female) into eight 45- to 65-day-old homozygous BB fetuses by injecting the cells intraperitoneally. Access to the recipient was achieved by exposure of the uterus through a midline laparotomy incision. The layers of the myometrium and chorion were transversely incised, taking care to leave the amnion intact. The fetus was visualized and gently manipulated into an amniotic bubble. Donor cells (1 to 2 ml per fetus) were injected intraperitoneally through a 22-gauge needle. The fetus was returned to the primary amniotic space, and the myometrium was closed in a double layer. The pregnancy was allowed to proceed to term and vaginal delivery.

In order to prepare fetal liver hematopoietic cells, slices of liver were washed [three times in heparinized α-minimum essential medium (MEM) + 10% fetal sheep serum] and passed through a wet screen mesh under a constant stream of α-MEM. The cell suspensions were passed through stainless steel mesh screens of progressively smaller pore sizes until a single cell suspension was obtained (final pore size, 0.2 mm). The mixture was allowed to stand at room temperature for 5 minutes and the top two-thirds of the cell suspension was removed, pelleted by

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