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Anxiolytic-Like and Antinociceptive Effects of MK-801 Accompanied by Sedation and Ataxia in Primates

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RUPNIAK, N. M. J., S. BOYCE, S. TYE, G. COOK AND S. D. IVERSEN. *Anxiolytic-like and antinociceptive effects of MK-801 accompanied by sedation and ataxia in primates.* PHARMACOL BIOCHEM BEHAV 44(1) 153–156, 1993. – Anxiolytic-like and antinociceptive activities of the noncompetitive NMDA receptor antagonist MK-801 (dizocilpine) were compared with sedative and ataxic side effects in primates. Administration of MK-801 (0.1 mg/kg) caused taming in cynomolgus monkeys and increased tail withdrawal latencies in squirrel monkeys; both effects were accompanied by sedation and ataxia. These findings are discussed in relation to the possible therapeutic uses of NMDA antagonists and differences in the behavioural effects of such compounds in primate and rodent species.

NMDA receptor Analgesia Anxiety Sedation

NMDA receptor antagonists have been proposed as potential therapeutic agents for epilepsy, stroke, anxiety, and pain. However, it is not clear which of these represent viable clinical targets for such drugs. Anxiolytic-like properties of competitive and noncompetitive NMDA receptor antagonists have been demonstrated in a range of rodent models, including social interaction, elevated plus-maze (6), separation-induced ultrasonic vocalisation (10), and conflict paradigms (1,3). Unlike benzodiazepines, these agents do not induce sedation in rodents and induce locomotor *stimulation* rather than ataxia (17). However, in contrast to these effects in rodents the noncompetitive NMDA receptor antagonists MK-801, phencyclidine (PCP), and ketamine and the competitive antagonist CGS 197555 all induced sedation and ataxia in primates (2, 9,13). Further, in primates NMDA antagonists did *not* exhibit anticonflict activity and decreased nonpunished responding, suggesting sedation (3,14).

Further evidence for marked species differences in the behavioural effects of NMDA antagonists emerges from their apparent antinociceptive activity in primates but not rodents. Ketamine induces analgesia in man (11,15) and primates (8). Similarly, MK-801 and CGS 17555 increased tail withdrawal latencies from a thermal stimulus in rhesus monkeys (8,9). In contrast, antinociceptive effects were *not* demonstrated using similar antinociception screens in rodents (5,18,20). One explanation for such species differences may be that sedation and ataxia may contribute to the apparent analgesic activity of these compounds in primates. In the present study, we directly compared the anxiolytic-like, antinociceptive, and sedative/ataxic effects of MK-801 in primates to identify more clearly the likely therapeutic uses and limitations of NMDA antagonists in man.

METHOD

Taming

Subjects were five individually housed, adult, male cynomolgus monkeys (*Macaca fascicularis*; 5.5–8.0 kg). Benzodiazepines induce taming in this species (16). Aggressive (or submissive, depending upon the individual animal) behaviours were rated on a scale of 0–5 using a modification of the procedure described by Cook and Mineka (4). The monkey entered a transport cage and sat on one side of a Wisconsin General Testing Apparatus facing an opaque screen, which could be raised or lowered by the experimenter. On the other side of the apparatus, an experimenter sat behind a black curtain and a camera enabled continuous recording of the monkey's behaviour when the opaque screen was raised. A session consisted of eight trials separated by an intertrial interval of 60 s. The following aversive stimuli were presented to the monkey in random order, one at a time, for 10 s with the opaque screen raised: a live ribbon snake, the experimenter wearing a

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face visor and leather gauntlets, a monkey-like glove puppet, a sound track of monkey vocalisations, a broom handle inserted into the transport cage, the experimenter wearing a wolf-like face mask, a blow-out party whistle, and a streamer party popper. At the end of each stimulus presentation, the screen was again lowered until the next trial. The monkey's reaction to the stimuli was scored from the videotape by an observer blind to treatment. Scores for each stimulus were summed to give a maximum possible aggression score of 40.

Sedation and ataxia were rated in the same animals, each on a scale of 0–5 (maximum possible total score = 10) according to the ease with which the animal could be aroused, time taken to retrieve a food reward, and time taken to enter or leave the home cage.

Tail Withdrawal Latency

Three adult, male squirrel monkeys (*Saimiri sciureus*; 0.7–1.1 kg) were habituated to primate chairs for at least 30 min prior to behavioural testing. The method was based upon the warm-water tail withdrawal procedure described for rhesus monkeys (7). The tail was shaved using a hair clipper and the lower 12 cm immersed in a vacuum flask filled with water at either 40 or 55°C. The latency for monkeys to withdraw their tails from water at 55°C was taken as a measure of nociception provided the following criteria were satisfied. At the start of each session (pretreatment baseline), each monkey was required to leave his tail in tepid water (40°C) for at least 18 s, and withdraw his tail from warm water (55°C) within 5 s of immersion. Animals not meeting these criteria were excluded from testing on that day. If these criteria were met, monkeys received an appropriate drug treatment and tail withdrawal latencies from water at 55°C were recorded every 30 min for up to 3 h thereafter. The maximum period for which a monkey's tail was immersed in water at 55°C was 15 s. Monkeys were randomly tested using tepid water to ensure that the 18-s criterion was maintained during each session.

Throughout the session, ataxia was rated on a scale of 0–3 (absent, mild, moderate, or severe). The maximum score included a slumped posture and inability to retrieve and consume a food tidbit when offered.

Drug Administration

MK-801 (dizocilpine; Merck Sharp & Dohme, West Point, PA) was dissolved freshly each day in 0.9% saline using an injection volume of 0.1 ml/kg. Four doses in the range 0.0125–0.1 mg/kg were examined in random order such that every animal received each treatment. For taming experiments using cynomolgus monkeys, MK-801 was administered subcutaneously 30 min prior to behavioural testing once weekly. For antinociceptive screening, the time of peak effect was determined following intramuscular administration during twice-weekly testing sessions. At least 2 days elapsed between drug treatments.

Statistical Analysis

Taming and sedation/ataxia scores in cynomolgus monkeys were summed and subjected to square root or logarithmic transformation as necessary to achieve normality and homogeneity of variance prior to one-way analysis of variance (ANOVA) with repeated measures followed by two-tailed paired contrast analysis. Tail withdrawal latencies in squirrel monkeys were meaned over the period of peak effect and subjected to one-way ANOVA with repeated measures followed by paired *t*-tests with posthoc adjustment.

RESULTS

Taming Effect of MK-801

The response of individual cynomolgus monkeys to the aversive stimuli differed markedly. The object that elicited the most consistent and vigorous aggressive reaction from all subjects was a glove puppet resembling a rhesus monkey. Animals would typically lunge toward the puppet, thrusting the head forward with teeth bared, mouth open, often accompanied by gutteral vocalisation and cage shaking. Responses to other objects ranged from absent (depending upon the object/subject combination) to facial grimacing, retreat or approach, threats, or avoidance.

Treatment with MK-801 (0.0125–0.1 mg/kg, SC, 30 min previously) caused a dose-related reduction in aggression scores that reached significance at the highest two doses examined ($F = 9.11, p < 0.001$; Fig. 1). This effect was accompanied by motor incoordination (ataxia) and apparent drowsiness indicated by hypoactivity and intermittent eye closure with the head down ($F = 5.44, p = 0.006$; Fig. 1). There was a clear correlation between the reduction in aggression scores and the induction of sedation/ataxia ($r = -0.99$).

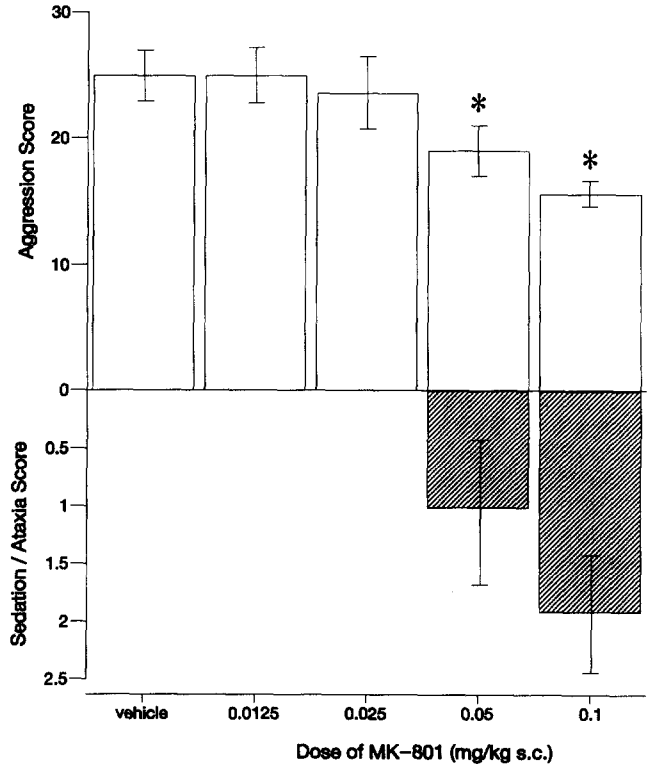

FIG. 1. Taming effect of MK-801 in cynomolgus monkeys. Values are total aggression or sedation/ataxia scores 30 min following treatment with MK-801 (0.0125–0.1 mg/kg, SC). Data were subjected to one-way analysis of variance with repeated measures followed by paired contrast analysis. *$p \leq 0.05$ compared with vehicle treatment.

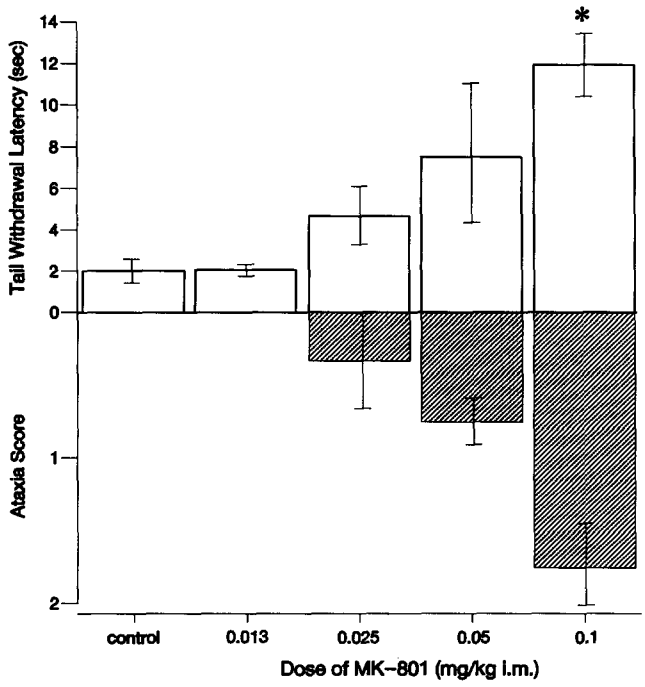

FIG. 2. Antinociceptive effect of MK-801 in squirrel monkeys. Values are the mean peak latencies to withdraw the tail from immersion in water at 55°C and peak ataxia scores (rated on a scale of 0–3) 1–2 h after treatment with MK-801 (0.0125–0.1 mg/kg, IM). Data were subjected to one-way analysis of variance with repeated measures followed by paired *t*-tests. $*p \leq 0.05$ compared with untreated control values.

Effect of MK-801 on Tail Withdrawal Latency

Throughout the study, vehicle-treated squirrel monkeys consistently withdrew their tails from water at 55°C with a mean latency of 2 s. Administration of MK-801 increased tail withdrawal latency, which was maximal between 60–120 min after treatment. At the time of peak effect, there was a dose-dependent increase in latency ($F = 51.21$, $p = 0.006$) but only the effect of the highest dose of MK-801 (0.1 mg/kg, IM) reached significance (latency = 12 ± 2 s; $F = 5.40$, $p = 0.01$; Fig. 2). Like the cynomolgus monkeys, squirrel monkeys became moderately to severely ataxic in this dose range ($F = 10.41$, $p = 0.001$; Fig. 2) and there was again a clear correlation between antinociception and ataxia scores ($r = 0.99$).

DISCUSSION

We directly compared the anxiolytic-like and antinociceptive effects of MK-801 with the induction of sedation and ataxia in primates. In cynomolgus monkeys, MK-801 reduced aggressive reactions to a range of aversive stimuli. This is the first demonstration of anxiolytic-like activity of MK-801 in primates. However, this effect coincided with sedation and ataxia, which might have made animals less responsive in the absence of any specific change in emotionality. Similarly, in squirrel monkeys MK-801 induced ataxia at "tranquillising" doses (3). Although evidence from rodent experiments suggests a true anxiolytic effect of MK-801 (1,3,6,10), anticonflict effects of NMDA antagonists were *not* obtained in primates (14). Noncompetitive NMDA antagonists do not appear to offer any therapeutic advantages over existing anxiolytics because of their PCP-like and sedative effects in primates (2,13). Alternative approaches to reduce NMDA-mediated neurotransmission, such as interaction with the modulatory glycine site, may eliminate these undesirable effects.

In rodents, there is evidence for a clear dissociation between the behavioural effects of glycine antagonists compared with direct NMDA antagonists. For example, the glycine antagonists 7-chlorokynurenic acid and HA-966 failed to induce motor stimulation even at doses up to fivefold greater than the anticonvulsant range (12). Moreover, anxiolytic-like activity was reported for the glycine receptor partial agonist 1-aminocyclopropanecarboxylic acid and the glycine antagonists 7-chlorokynurenic acid (19) and 5,7-dichlorokynurenic acid (10) in the absence of motor stimulation typical of NMDA antagonists. Further studies in primates are essential to evaluate more fully the properties of these compounds.

We also examined the antinociceptive effects of MK-801 in squirrel monkeys. In agreement with a similar study using rhesus monkeys (8), MK-801 increased tail withdrawal latency using a thermal antinociception screen. Again, this effect was highly correlated with the induction of motor impairment by MK-801. In man, ketamine, which like MK-801 is a noncompetitive NMDA antagonist, was effective in relieving experimental ischemic pain (11,15) and postoperative pain after oral surgery (15) at doses well below the sedative dose range. For certain therapeutic applications where analgesia is required in conjunction with sedation, or in cases of severe pain where use of opioids is undesirable because of tolerance, or potential circulatory or respiratory complications, antagonists at the NMDA site may be valuable analgesic agents. However, the side effect profile of such compounds will clearly be critical. In a single primate study published to date using the competitive NMDA antagonist CGS 19755, antinociception was apparent at doses 10 times lower than those inducing ataxia (9). Whether glycine antagonists will demonstrate an even greater therapeutic index in primates remains to be determined.

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