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Differential effects of ketamine and MK-801 on the induction of long-term potentiation

Stephen Maren,[CA] Michel Baudry and Richard F. Thompson

Neurosciences Program, University of Southern California, Los Angeles, California 90089-2520, USA

KETAMINE and MK-801 are phencyclidine (PCP)-like noncompetitive antagonists of the *N*-methyl-D-aspartate (NMDA) receptor that produce a use-dependent blockade of the NMDA receptor-coupled channel. Recent studies have suggested that the binding properties of these drugs to the NMDA receptor *in-vitro* are different. In the present study, the effects of ketamine and MK-801 on the induction of long-term potentiation (LTP) were compared at perforant path – granule cell synapses in anaesthetized rats. LTP was observed in animals treated with either saline or MK-801, but not in those treated with ketamine. These results reveal that ketamine and MK-801 differentially modulate the induction of LTP, and we propose that this differential modulation may be related to the different binding properties of the drugs.

Key words: Ketamine, MK-801, NMDA receptor, Long-term potentiation, Anaesthetized rat

[CA]Corresponding Author

Introduction

Long-term potentiation (LTP) is a long-lasting enhancement of synaptic efficacy occurring at excitatory synapses in the mammalian brain following brief trains of high-frequency electrical activity applied to afferent pathways.[1] The induction of LTP in the dentate gyrus and hippocampal CA1 region has been shown to be prevented by both competitive and noncompetitive antagonists of the *N*-methyl-D-aspartate (NMDA) receptor/channel complex.[2–7] One class of noncompetitive antagonists consists of phencyclidine (PCP)-like drugs that bind to a site deep within the NMDA receptor-coupled channel.[8,9] Of these, ketamine and MK-801 {(+)-5-methyl-10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5,10-imine maleate} have been reported to effectively prevent LTP induction in area CA1 and the dentate gyrus *in-vivo*, respectively.[2,3] However, differences in physico-chemical properties between ketamine and MK-801 (e.g., ketamine is smaller and more strongly cationic than MK-801 at physiological pH) suggest that the two drugs might interact with the NMDA channel differently, and therefore have differential effects on LTP induction. Indeed, recent studies have suggested that the association rates of the drugs with the NMDA channel *in-vitro* are different. In the present study we have compared the effects of ketamine and MK-801 on the *in-vivo* induction of LTP at rat perforant path-granule cell synapses 15 min after drug administration. Preliminary results have been presented in an abstract.[10]

Methods

Male Long-Evans rats (250–300 g) were anaesthetized with an intraperitoneal (i.p.) injection (60 mg kg^{-1}) of sodium pentobarbital (65 mg ml^{-1}) and mounted in a Kopf stereotaxic frame; the head position was adjusted to place bregma and lambda in the same horizontal plane. After retraction of the scalp, burr holes of approximately 2 mm diameter were drilled unilaterally in the skull for the placement of stimulating and recording electrodes. The recording electrode was implanted in the hilus of the dentate gyrus (3.3 mm posterior, 2.4 mm lateral, and 2.8–3.0 mm ventral to bregma) and the bipolar stimulating electrode in the medial perforant pathway (8.1 mm posterior, 4.4 mm lateral, and 2.5–4.0 mm ventral to bregma). The electrodes consisted of Epoxylite-coated stainless-steel pins with the recording and stimulating surfaces formed by removing the insulation at the tips (tip lengths=50 and 500 μm for the recording and stimulating electrodes, respectively). Body temperature was kept at approximately 37°C with a heating pad; the subjects were not artificially respirated. Surgical anaesthesia was maintained with hourly injections (0.2 ml) of pentobarbital.

Electrophysiological testing (100 μs pulses at 0.05 Hz; voltage adjusted to elicit an approximately 1 mV population spike [PS]) began after stable dentate hilar field potentials had been recorded for at least 30 min. Fifteen minutes prior to high-frequency (HF) perforant path stimulation (ten 30 ms 400 Hz bursts delivered at 5 Hz) animals were injected i.p. with either saline (S; 1 ml; n=7), ketamine HCl (K; 30 mg kg^{-1}; n=7), or MK-801 (M; 1 mg kg^{-1}; n=7). These dosages of ketamine and MK-801 correspond to those that have previously been shown to block the induction of LTP and paroxysmal discharges in the hippocampus.[2,3,11] Moreover, these dosages reflect the different potency ratios of the drugs as NMDA antagonists *in-vivo* and *in-vitro* (MK-801 is much more potent than

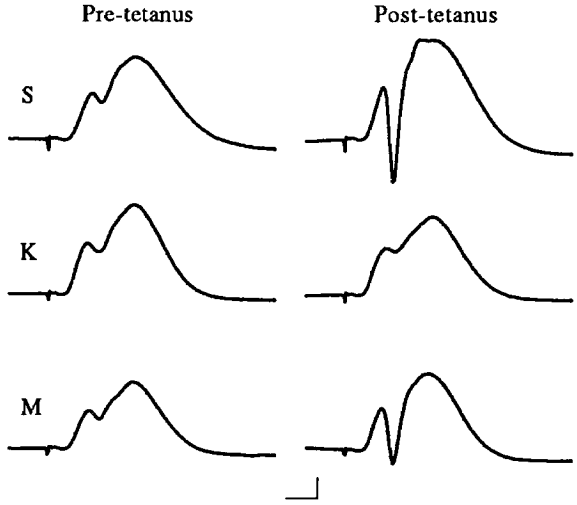

FIG. 1. Pre- and post-tetanus averaged field potentials from representative subjects in each of the treatment groups (S = saline, K = ketamine, M = MK-801). Each trace is an average of five evoked field potentials recorded from the hilar region of the dentate gyrus. Note that the field potentials recorded from subjects treated with saline and MK-801, but not ketamine, exhibited substantial increases (LTP) of both the EPSP slope and PS amplitude following tetanic stimulation. Calibrations: 2.85ms, 2.00mV.

ketamine as an NMDA antagonist.[12,13] In subjects that displayed a diminution in PS amplitude following drug injection, the voltage during HF stimulation was adjusted to assure suprathreshold tetanization (tetanization at a voltage that produced an approximately 1 mV PS).

Input/output (I/O) functions consisting of three averaged field potentials at each of five different stimulation intensities were generated immediately before drug injection (pre-tetanus) and 30 min after tetanization (post-tetanus). For each animal, the pre-tetanus stimulation intensities were adjusted to produce a range of evoked responses. These were as follows: (1) responses subthreshold for PS generation (intensity 1; range = 1.0–5.0 V), (2) responses with an approximately 1 mV PS (intensity 2; range = 1.2–5.5 V), (3) responses with a PS amplitude one-half that of the asymptotic PS (intensity 3; range = 1.4–6.0 V), (4) responses with a PS amplitude three-fourths that of the asymptotic PS (intensity 4; range = 1.6–6.5 V), and (5) responses with the asymptotic PS amplitude (intensity 5; range = 1.8–7.0 V). Two measures, the PS amplitude and excitatory postsynaptic potential (EPSP) slope, were extracted from each averaged I/O response. Representative pre- and post-tetanus averaged evoked responses from subjects in each of the treatment groups are shown in Fig. 1. As a prelude to the statistical analysis, the EPSP and PS measures were normalized for each animal by expressing them as a percentage of the maximum baseline response (the mean maximum baseline responses ± s.e.m. appear in the figure legends). To examine the incidence of LTP, the normalized data for each treatment group were submitted to separate repeated measures, i.e. analyses of variance (ANOVAs) with time (2 levels; pre-tetanus, post-tetanus) and stimulation intensity (5 levels; intensities 1–5).

Results

The averaged EPSP slope and PS amplitude I/O data for each of the three treatment groups are shown in Figs 2 and 3, respectively. ANOVAs performed on the EPSP data revealed significant F ratios for the main effect of time in both the saline ($F_{1,6} = 31.51$, $p < 0.002$) and MK-801 ($F_{1,6} = 9.64$, $p < 0.03$) groups, indicating the occurrence of significant EPSP LTP in these groups 30 min after the delivery of tetanic stimulation. The absence of LTP in animals treated with ketamine was indicated by a non-significant F ratio for the main effect of time ($F_{1,6} = 0.88$, $p > 0.30$). Similarly, potentiation of the PS amplitude was

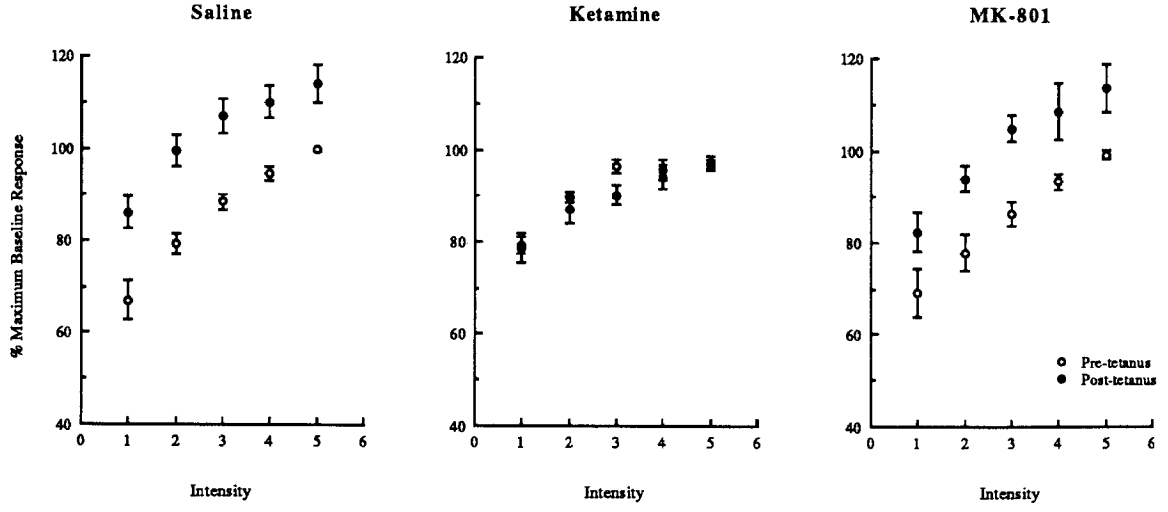

FIG. 2. Averaged EPSP slope I/O functions for the three treatment groups. For each intensity, the I/O data were expressed as a percentage of the maximum baseline (pre-tetanus) response and averaged across the subjects within a group (± s.e.m.). The mean maximum baseline responses ± s.e.m. (mV) for the three groups were as follows: S = 4.68 ± 0.48; K = 4.18 ± 0.60; M = 3.88 ± 0.44.

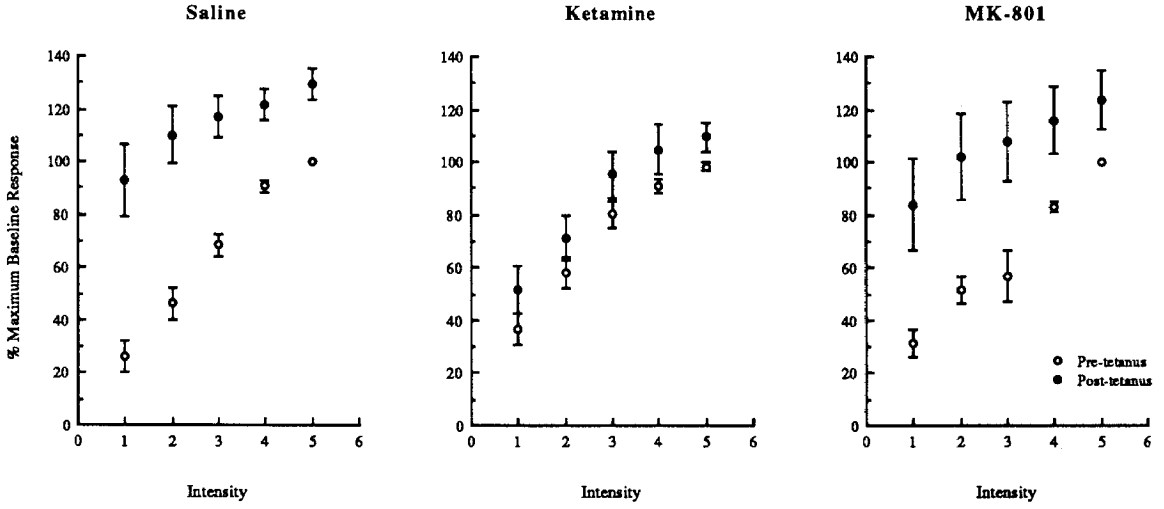

FIG. 3. Averaged PS amplitude I/O functions for the three treatment groups. For each intensity, the I/O data were expressed as a percentage of the maximum baseline (pre-tetanus) response and averaged across the subjects within a group (± s.e.m.). The mean maximum baseline responses ± s.e.m. (mV) for the three groups were as follows: S = 10.96 ± 0.97; K = 9.88 ± 1.11; M = 10.44 ± 1.02.

indicated by a significant main effect of time in the saline ($F_{1,6}$ = 39.29, $p < 0.002$) and MK-801 ($F_{1,6}$ = 9.91, $p < 0.02$), but not the ketamine ($F_{1,6}$ = 4.19, $p > 0.07$) groups.

The analysis of the EPSP data revealed no significant interaction between the factors of time and stimulation intensity in any of the treatment groups, indicating that LTP in the saline and MK-801 group occurred rather uniformly across the range of stimulation intensities. In contrast, for the PS data a significant interaction between the factors of time and stimulation intensity was found in both the saline ($F_{4,24}$ = 16.22, $p < 0.00001$) and MK-801 ($F_{4,24}$ = 5.48, $p < 0.0035$) groups, indicating that PS LTP in these groups did not occur uniformly across the range of stimulation intensities. That is, small PSs evoked by low stimulation intensities were potentiated to a greater degree than larger PSs evoked by high stimulation intensities.

Discussion

The present study indicates that the noncompetitive NMDA receptor antagonist ketamine blocks the induction of LTP in the dentate gyrus of anaesthetized Long-Evans rats. This finding corroborates an earlier report that ketamine blocks LTP induction in area CA1 of anaesthetized Sprague-Dawley rats,[3] and adds further support to the notion that phencyclidine-like noncompetitive NMDA antagonists are powerful inhibitors of LTP induction.[2,6] MK-801, however, failed to block the induction of LTP in the present study. This inability of MK-801 to prevent LTP induction shortly after administration has also been reported by Abraham and Mason.[2]

Given these findings, what factors might contribute to the different actions of ketamine and MK-801 on LTP induction at short intervals following administration? Because both drugs have comparable behavioral effects (e.g., hyperactivity, loss of equilibrium, and ataxia) in the awake animal shortly after administration (Maren, unpublished observations), it is likely that both drugs have reached their central targets by the time the tetanic stimulation is delivered. Furthermore, at the dosages used in the present study, both ketamine and MK-801 act as powerful NMDA antagonists *in-vivo* inhibiting the induction of LTP and paroxysmal discharges in the hippocampus.[2,3,11] Lower doses of the drugs fail to produce these effects, and higher doses are often toxic in anaesthetized animals.[2] Thus, the most likely explanation for the present results is that ketamine and MK-801 interact with the NMDA-gated channel in slightly different manners. That is, although both ketamine and MK-801 bind to the PCP site within the NMDA-gated channel, the compounds may have differential access to this binding site *in-vivo*. In support of this hypothesis, whole-cell voltage clamp experiments conducted in cultured neurons have indicated that the blockade of NMDA-induced currents by ketamine is highly voltage-dependent, whereas that by MK-801 is largely independent of transmembrane potential.[8,14] Furthermore, ketamine appears to bind with the channel much faster than MK-801, reaching equilibrium binding much more rapidly than MK-801.[8,14–16] Indeed, the different binding properties of the two drugs were apparent in the present study as ketamine, but not MK-801, often reduced the amplitude of the baseline stimulus-evoked PS in the 15 min period prior to tetanization (data not shown). Collectively, these results suggest that the association rate of blockade by ketamine is voltage-dependent, whereas that by MK-801 is not. Considering the

structural properties of the two drugs, the difference in the voltage-dependency of association is to be expected as ketamine is smaller and more strongly cationic than MK-801 at physiological pH (pK_as = 8.2 and 7.5, respectively).

Therefore, we propose that the different association rates of ketamine and MK-801 with the NMDA-gated channel account for the results of the present study. More specifically, the binding of MK-801 to the NMDA receptor channel may be slow enough to allow sufficient amounts of calcium to move through the channel during tetanization before the channel gets blocked. Calcium would then trigger LTP by activating some intracellular cascade of events.

Conclusion

In conclusion, ketamine and MK-801 differentially modulate the induction of LTP in the dentate gyrus of anaesthetized rats. This finding illustrates the need for caution when using PCP-like noncompetitive antagonists in electrophysiological and/or behavioral experiments. More importantly, our results should stimulate the search for new NMDA antagonists with a higher affinity than ketamine and a greater association rate than MK-801. Finally, the differential modulation of LTP induction by ketamine and MK-801 might be an important component of the different behavioral effects of these drugs and could be used in experiments designed to evaluate the role of LTP in learning and memory.

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