Title : A POSSIBLE MECHANISM OF KETAMINE-INDUCED ANALGESIA

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Introduction. One of the actions of ketamine is analgesia, induced at subhypnotic doses in the absence of significant behavioral effects. Ryder, Way and Trevor reported that in mice the analgesia produced by subhypnotic doses of ketamine was antagonized by pretreatment of the animals with the narcotic antagonist, naloxone.[1] One proposed explanation of this observation is that ketamine might produce analgesia by causing a release of endorphins which are then antagonized by naloxone. An alternative explanation is that ketamine might interact directly with opiate receptors to produce analgesia. To test this latter hypothesis, we examined the action of ketamine in a standard stereospecific opiate receptor binding assay.[2, 3]

Methods. Brains minus cerebella of decapitated male Sprague-Dawley rats (175-250 g) were homogenized in 6 volumes of iced 0.32 M sucrose with a Brinkmann Polytron, centrifuged at 49,000 x g for 15 min, and the pellets resuspended in 60 volumes of iced 0.05 M tris buffer, pH 7.4 at 37° C, containing 1 mM Mn^{++}. Assays were performed in triplicate at 20° C for 15 min using 2 ml aliquots of the diluted brain homogenate, $5x10^{-10}$ M ^{3}H-dihydromorphine (3H-DHM, specific activity 43 Ci/mmol, New England Nuclear), 10^{-7} M levorphanol or dextrorphan, and varying concentrations of morphine, met^5-enkephalin, phencyclidine or ketamine. Bacitracin 50 µg/ml was added to the incubation mixture for the assay of met^5-enkephalin. Incubations were terminated by cooling in an ice-water bath for at least 15 min, followed by vacuum filtration on Whatman GF-B glass fiber circles, which were washed thrice with 4 ml portions of iced tris buffer and placed in 10 ml Bray's solution for scintillation counting. The difference in radioactivity between samples containing dextrorphan and those containing levorphanol was determined as stereospecific binding between ^{3}H-DHM and opiate receptors. In the absence of competing ligand, this difference averaged 1300 CPM, accounting for > 70% of total binding. Addition of one of the four drugs being studied inhibited stereospecific binding, expressed as percent inhibition.

Results. Figure 1 depicts log concentration-inhibition curves for the four drugs tested. The IC_{50} values (concentrations which inhibit stereospecific ^{3}H-DHM binding by 50%) based on logarithmic regression fitting of the data, are: morphine, 2.5×10^{-9} M; met^5-enkephalin, 2.5×10^{-8} M; dl-phencyclidine, 5.9×10^{-6} M; and dl-ketamine, 1.3×10^{-5} M. The calculated slopes of the regression lines for morphine, phencyclidine and ketamine are not significantly different from each other by one-way analysis of variance.

Discussion. The IC_{50} values obtained for morphine, met^5-enkephalin and phencyclidine are very similar to previously published values of approximately 9×10^{-9} (derived from Ref 3, Fig 4), 1×10^{-8} (Ref 3) and 2.6×10^{-6} M (Ref 4), respectively, using ^{3}H-DHM as the radioligand, considering minor variations in assay conditions. The parallelism of the dose-response curves for morphine, phencyclidine and ketamine suggests that they are all competing for the same stereospecific binding site, in spite of the fact that binding affinity varies 5000-fold. No extrapolation of these in vitro results can be made to the in vivo situation. However, in the report by Ryder, Way and Trevor,[1] the measured brain concentrations of racemic ketamine ranged from 2.5 - 4 µg/g 2-15 min after subcutaneous administration of the median effective analgesic (subhypnotic) dose of 7 mg/kg. Recalculation of IC_{50} of ketamine from our data gives a concentration of 3.6 µg/ml. The fact that the in vitro IC_{50} in our study is of a similar order of magnitude as concentrations of ketamine found in brain after a subhypnotic analgesic dose suggests that ketamine-induced analgesia is mediated by a direct action of ketamine on opiate receptors.

References

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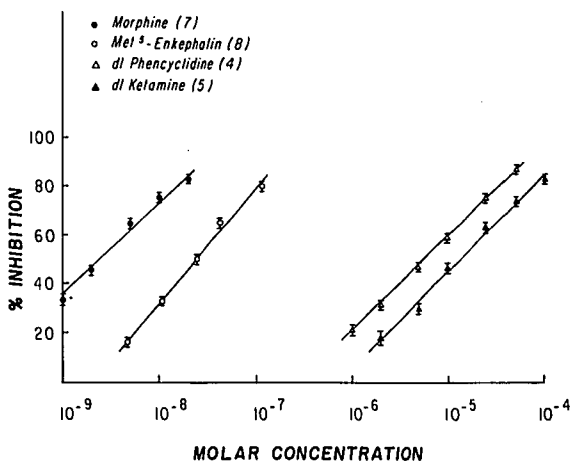

Figure 1. Log concentration versus inhibition of stereospecific ^{3}H-DHM binding for morphine, met^5-enkephalin, dl-phencyclidine and dl-ketamine. Values are mean ± SE of number of assays shown in parentheses. Calculated slopes of regression lines are: morphine, 16.9; met^5-enkephalin, 20.2, dl-phencyclidine, 16.6; ketamine, 17.3