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Ketamine Reverses the Fentanyl-induced Increase in NMDA-mediated Synaptic Transmission in CA1 Area of the Rat Hippocampus

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INTRODUCTION

Tissue damage associated with surgical lesions may produce hyperalgesia (exaggerated nociceptive response to noxious stimulation), allodynia (nociceptive response to innocuous stimulation), and persistent pain. The establishment of these phenomena reflects a sensitization of both peripheral and central neuronal systems.

Opiates, such as fentanyl, are known to exert an analgesic effect as a response to strong and potentially tissue damaging stimuli. However, subsequent to their analgesic effect opiates activate a pronociceptive NMDA-dependent mechanism leading to long-lasting hyperalgesia. Several studies have shown that ketamine, the selective non-competitive NMDA receptor antagonist that binds to the phencyclidine (PCP) recognition site, is effective in reducing fentanyl-induced hyperalgesia.

According to the Gray-McNaughton theory the hippocampal formation responds to aversive events (e.g., pain) by amplifying them in order to lead the organism toward a behavior that is adaptive to the worst possible outcome. This process is accompanied or can be induced by anxiety. It has been also shown that electrical stimulation of the dorsal hippocampus affects nociception without being aversive. Furthermore, intracellular in vivo recordings have demonstrated that a subpopulation of CA1 pyramidal neurons of the rat hippocampus responds to peripheral noxious shocks. Interestingly, the amplitude of the recorded EPSPs increased with higher intensity electrical shocks.

The aim of the present study was to investigate the hippocampal neuronal sensitization to fentanyl treatment in nonsuffering rats. The consequence of ketamine administration prior to fentanyl treatment, on this phenomenon, was also investigated.

METHODS

Male Wistar rats were randomly selected and separated into three groups: group I: animals treated with saline, group II: animals treated with fentanyl (4 injections, 80 μg/kg per injection, subcutaneously every 15 min), group III: animals treated with ketamine (1 injection, 10 mg/kg subcutaneously) 30 min prior to fentanyl treatment (4 injections, 80 μg/kg per injection, subcutaneously every 15 min). The animals were deeply anaesthetized with ether before decapitation. The brain was excised and placed into ice-cold, oxygenated (95% O_2 and 5% CO_2) artificial cerebrospinal fluid (ACSF) containing (in mM): 124 NaCl; 4 KCl; 2 $MgSO_4$; 2 CaCl; 1.25 NaH_2PO_4; 26 $NaHCO_3$; 10 glucose at pH 7.4. Both hippocampi were dissected however, only the extreme dorsal parts of each were used. Slices, 500 μm, were cut using a McIlwain tissue chopper and were transferred in an interface-type recording chamber. Intracellular current clamp recordings were made from hippocampal neurons using microelectrodes that were filled with 2M potassium acetate. On- and off-line data acquisition and analysis was accomplished using an Axolab 1100 interface (Axon Instruments) between an Axoclamp HS-2A preamplifier and a personal com-

puter utilizing pCLAMP 5.03 software programs (Axon Instruments). Data were stored as analog signals for later analysis. The bridge balance was carefully monitored throughout the experiments and adjusted when necessary. Intracellular recordings were made mostly from neurons in CA1b area of the pyramidal cell layer and were considered acceptable only if they exhibited a steady membrane potential, in the absence a negative DC current, and overshooting action potentials. Synaptic responses were evoked by orthodromic stimulation of the Schaffer collateral pathway using a bipolar tungsteen electrode placed in CA1 stratum radiatum at a distance of approximately 0.5 mm from the recording electrode. Electrical stimuli were delivered at a frequency of 0.05 Hz. The NMDA receptor mediated response was pharmacologically isolated by the presence of 25 μM 6-cyano-7-nitro-quinoxaline-2,3-dione disodium (CNQX), to block AMPA receptors, 20 μM Biccuculine, to block $GABA_A$ receptors and 500 μM (3-aminopropil)(diethoxymethyl)phosphinic acid (CGP 35348), to block $GABA_B$ receptors. All drugs were obtained from Tocris Cookson, UK. Data were expressed as mean ± S.E.M. In all cases n=number of neurons. The unpaired student t-test was used for statistical comparisons between groups of animals. Statistical significance was determined at the level of $p < 0.05$.

RESULTS

Recordings were obtained from three different groups of animals: group I) rats treated with saline, group II) fentanyl-treated rats, group III) fentanyl-and ketamine-treated rats. The passive membrane properties: resting membrane potential (group I: -63.2±0.6 mV, group II: -62.4±0.4 mV, group III: -62.6±0.7 mV), input resistance (group I: 51.9±5.0 MΩ, group II: 49.8±4.2 MΩ, group III: 50.2±4.7 MΩ), and time constant (group I: 19.5±2.0 ms, group II: 21.4±0.9 ms, group III: 20.1±1.8 ms), were similar in all groups of neurons. The amplitude of the EPSPs in the fentanyl-treated group was significantly larger (5.67±0.63 mV, n=7, MP= -65 mV) as compared to the saline-treated group (3.6±0.69 mV, n=7, MP=-65 mV) ($p< 0.05$). In ketamine treated animals the amplitude of EPSPs (3.86±1.5 mV, n=5, MP=-70) was significantly smaller as compared to the fentanyl-treated group (8.77±1.47 mV, n=7, MP=-70) ($p< 0.05$).

CONCLUSION

The present data show for the first time that in vivo ketamine treatment reverses the augmentation of NMDA-mediated synaptic responses induced by fentanyl in CA1 pyramidal neurons of the dorsal hippocampus. These results may explain the ability of ketamine to reverse the fentanyl-induced hyperalgesia reported in behavioral studies. Ketamine is a commonly administered anaesthetic in clinical procedures. It exhibits, however, several side effects. Developing NMDA receptor antagonists with fewer side effects than ketamine may be beneficial for the prevention or reduction of post-surgical pain sensitization.