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A Single Exposure to μ-Opioid Receptor agonist Fentanyl *in vivo* is Sufficient to Induce long-term Reduction in GABA-mediated Synaptic Transmission Recorded from Hippocampal CA1 Pyramidal Neurons in an *in vitro* Slice Preparation

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INTRODUCTION

The physiological and pharmacological actions of opioids have widely concerned the scientific community during the last decades. Opioid treatment is regarded as the most effective therapy for alleviating moderate to severe pain. However, the administration of opioids has been related to chronic (1-3) as well as acute (4-7) physical dependence and addiction.

In the hippocampus opioids have been found to increase neuronal activity. Opioid receptors in the hippocampal area are found almost exclusively on interneurons (8-10) and their activation hyperpolarize hippocampal interneurons and/or inhibit GABA release leading to increased excitation of principal hippocampal neurons via a disinhibitory mechanism.

Until now, research has focused on the acute effects observed following opioid superfusion on hippocampal synaptic transmission in the *in vitro* slice preparation. It was the purpose of the present study to examine the effect of *in vivo* administration of fentanyl on the inhibitory transmission of pyramidal neurons of CA1 a major output area of the rat hippocampus.

METHODS

Male Wistar rats were randomly selected and divided into two groups: animals treated with saline and animals treated with fentanyl (4 injections, 80 μg/kg per injection, subcutaneously, every 15 min). One day after treatment the animals were decapitated, 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 at 7.4. Both hippocampi were dissected, however, only the extreme dorsal parts of each were used. Transverse slices, 500 μm thick were cut and then transferred in an interface-type recording chamber where they were maintained at a constant temperature of 32±0.2ºC for at least 1.5 h before recording and were continuously superfused with humidified (with 95% O_2 and 5% CO_2) ACSF.

Synaptic responses were evoked by orthodromic stimulation of the Schaffer collateral pathway using a bipolar tungsten electrode placed in CA1 stratum radiatum at a distance of approximately 0.5 mm from the recording electrode.

Drugs.- All drugs were applied by superfusion in the ACSF and introduced into the recording chamber by means of a three-way stopcock. Isolated $GABA_A$ IPSPs were recorded in the presence of the ionotropic excitatory amino acid (6-cyano-7-nitro-quinoxaline-2,3-dione-disodium, CNQX, 10 μM), DL-AP5 50 μM and $GABA_B$ (CGP55845A 1 μM) receptor antagonists. All drugs were purchased from Tocris, Neuroamin, UK.

Statistical analysis.- All data were expressed as mean±S.E.M; in all cases *n*=number of neurons. Data were analyzed statistically using the unpaired Student's *t* test. Statistical significance was determined at the level of $p \leq 0.05$.

RESULTS

Intracellular recordings were made from saline- and fentanyl-treated animals under control conditions and in the presence of cocktail containing excitatory amino acid receptor antagonists and $GABA_B$ receptor antagonists.

Under standard conditions the resting membrane potential and the input resistance were similar between saline- and fentanyl-treated animals (-63.4 ±0.46 mV, n=20 and 47.2±3.06 MΩ, n=17; -63.6±0.6 mV n=12 and 46.8±4.6 MΩ, n=10 respectively).

Multiphasic postsynaptic potentials elicited after orthodromic stimulation of the Schaffer collaterals pathway were recorded at resting membrane potential. No differences were found regarding the elicited subthreshold EPSPs and $GABA_B$ receptor me-

diated IPSPs between the two populations (Saline-treated EPSP: 8.3±1.2 mV, n=18, s-IPSP: 4.97±0.7 mV, n=18; Fentanyl-treated EPSP: 6.8± 0.6 mV, n=12, s-IPSP: 3.06±0.5 mV, n=12). However the peak amplitude of fast IPSPs ($GABA_A$ receptor mediated) was significantly smaller in fentanyl-treated animals (4.2±0.69 mV, n=12), than in saline-treated animals (6.49±0.58 mV, n=18, $p<0.05$). Furthermore, significantly lower stimulus intensities were required to evoke just subthreshold synaptic responses in slices from fentalyl, than from saline-treated animals (saline: 46.8±10.2 Volts, n=10; fentanyl: 22.2±4.2 Volts, n=9; $p<0.05$).

In the presence of cocktail f-IPSPs were recorded at a membrane potential of -58 ± 0.49 mV, n=14 in saline-treated and -57.5±0.56 mV, n=8 in fentanyl-treated animals. No difference was found regarding the input resistance (56.7±3.5 MΩ, n=13 saline treated; 51.5±2.9 MΩ, n=8 fentanyl-treated). Under conditions eliciting maximal response, f-IPSPs were significantly different between the two groups of animals. In saline treated animals the value of f-IPSP peak amplitude was 10.9±0.5 mV, n=15, whereas in fentanyl-treated animals 8.4±0.7, n=9, ($p<0.05$).

CONCLUSIONS

The present data show for the first time that acute *in vivo* fentanyl treatment produces reduction of the $GABA_A$-receptor mediated inhibition that is persistent 24 hours after the administration of the opioid.

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