

CHANGES OCCUR IN CENTRAL ADRENORECEPTOR FUNCTION FOLLOWING LONG-TERM MORPHINE TREATMENT AND DURING MORPHINE WITHDRAWAL

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ABSTRACT

Radioligand binding techniques were used in combination with *in vivo* electrophysiological recording to characterize changes in beta adrenoreceptor activity in various brain areas in rats treated chronically with morphine. Following chronic morphine treatment, the maximum number of specific binding sites for <sup>3</sup>H-dihydroalprenolol (<sup>3</sup>H-DHA) in parietal cortex and hippocampus showed a biphasic change, indicating an initial increase and decrease (relative to controls) in beta adrenoreceptors in these regions with time during withdrawal. No appreciable changes were observed in the dissociation constants for <sup>3</sup>H-DHA binding. The changes in cortical beta adrenoreceptor density found in early (8 hr) and later phases (32 hr) of withdrawal were paralleled by a selective increase and decrease, respectively, in cortical neuron sensitivity to noradrenergic stimulation. These results suggest a possible linkage between changes in central adrenoreceptor function and the formation and/or expression of opiate dependence.

INTRODUCTION

Considerable evidence indicates that significant components of the opiate withdrawal syndrome may be due to increased central noradrenergic activity. For example, activation of the locus coeruleus (LC) in subhuman primates by means of either electrical or pharmacological stimulation elicits a profile of behavioral and physiological effects strikingly similar to those observed during opiate withdrawal (1). Both morphine and clonidine, an alpha<sub>2</sub> adrenergic agonist, have been shown to block these effects of LC activation (1) and to suppress the acceleration in LC neuron firing and norepinephrine (NE) turnover in brain that occurs during acute opiate withdrawal (1,2). Clonidine has also been shown to alleviate opiate withdrawal symptoms in animals and man (3); an effect which has been attributed to its inhibitory influence on LC-NE neuronal activity. These findings have served to focus attention on central noradrenergic neurons, particularly those of the LC, as a "final common pathway" for the manifestation of the opiate abstinence syndrome. Nevertheless, the finding in recent studies of increases and decreases in beta and alpha<sub>2</sub> adrenergic receptors, respectively, in various brain regions in rats treated chronically with morphine (4,5,6) raises the additional possibility that

changes in central adrenoceptor function may contribute to or reinforce the effects occurring in opiate-sensitive noradrenergic neurons. In the present study, radioligand binding techniques were used in conjunction with in vivo electrophysiological recording to determine whether changes in brain beta adrenoceptors that result from chronic morphine treatment are reflected in corresponding alterations in postsynaptic neuronal sensitivity to noradrenergic stimulation. Experiments were carried out during early and late stages of morphine withdrawal to assess the possible relationship of changes in central adrenoceptor function to the abstinence syndrome.

#### METHODS

Groups of eight Sprague-Dawley male rats (175-225g) were injected with morphine sulfate or saline, i.p., every 8 hr for 14 days, with the dosage of the narcotic ranging from 10 mg/kg, t.i.d., on the first 3 days to 100 mg/kg, t.i.d., on the last 2 days. Animals used for the binding assays were killed by decapitation either 8 hr (chronic treatment group) or 32 hr (withdrawal group) after the last injection and homogenates prepared from the parietal cortex, hippocampus, and cerebellum. The brain areas isolated from six rats were homogenized in 5 ml of ice-cold 5mM Tris-sucrose buffer (pH 7.4), centrifuged at 1000 x g for 1 min, and the supernates were saved and re-centrifuged at 40,000 x g. The crude membrane pellet was then re-homogenized in the same buffer and centrifuged again at 40,000 x g. The final pellet was resuspended in 50 mM Tris-incubation buffer adjusted to pH 8.0. For the determination of total beta adrenergic receptor binding, 1 ml aliquots of the neural membranes were incubated in duplicate for 30 min at 25°C with various concentrations of <sup>3</sup>H-DHA (.1 - 25.6nM range, sp. act. 34.1 Ci/mmol). Nonspecific binding was determined by addition of propranolol 10<sup>-5</sup>M to the incubation. The incubations were terminated by rapid filtration under vacuum through Whatman GF/C glass fiber filters and by washing with two 10 ml aliquots of Tris-incubation buffer at 25°C.

The remaining animals from each morphine- and saline-treatment group were prepared for in vivo electrophysiological recording at the same time that the binding assays were initiated. Following induction of anesthesia with halothane (0.75% in air), the rats were fixed in a stereotaxic frame and a craniotomy performed to expose a 2mm x 3mm circumscribed area of the parietal cortex. Five-barreled glass micropipets (3-5 megohm) were used to record extracellularly the discharge of single identified cerebrocortical neurons and to apply chemical substances in their immediate vicinity by microiontophoresis. The neural signals were amplified and displayed using conventional techniques and ratemeter records of spike discharge constructed to determine neuronal chemosensitivity to the following test substances: 0.5 M 1-NE, pH 4.0, 0.25 M 1-isoproterenol (ISO), pH 4.5 and .1M GABA, pH 3.5. Changes in postsynaptic adrenoceptor sensitivity following prolonged opiate treatment were assessed by comparing the mean threshold current of iontophoretically applied NE required to inhibit the spontaneous firing of cerebrocortical neurons recorded in chronic morphine-treated and morphine-withdrawn rats versus saline-treated controls.

#### RESULTS

Chronic administration of morphine resulted in significant increases in the maximum number of specific binding sites ( $B_{max}$ ) for <sup>3</sup>H-DHA in the

parietal cortex (33.9%,  $p < .0005$ ) and hippocampus (12.8%,  $p < .06$ ), but not in cerebellum. This enhancement in  $^3\text{H}$ -DHA binding was observed 8 hr following cessation of morphine treatment. In comparison, there was a marked reduction in the maximum number of  $^3\text{H}$ -DHA binding sites in parietal cortex (-14.7%,  $p < .005$ ) and hippocampus (-26.4%,  $p < .005$ ) during late withdrawal. These changes in beta receptor number occurred without appreciable change in the  $K_p$ 's for  $^3\text{H}$ -DHA binding and were not observed following acute treatment of rats with morphine 30 mg/kg, t.i.d., for 1 day.

Microiontophoretic testing revealed that the changes in  $^3\text{H}$ -DHA binding found in parietal cortex during early (8 hr) and later phases (32 hr) of withdrawal were paralleled by a concomitant increase and decrease, respectively, in postsynaptic sensitivity of cerebrocortical neurons to noradrenergic stimulation (Fig. 1). In tests on 46 cell-pairs from 13 experiments, the mean threshold currents of NE (27.3  $\pm$  1.4 nA, S.E.M.) and ISO (25.1  $\pm$  2.0 nA, S.E.M.) required for depression of neurons in chronic morphine-treated rats were significantly less ( $p < .001$ ) than those of control cells (41.5  $\pm$  1.9 nA, NE; 41.1  $\pm$  2.3 nA, ISO). In subsequent experiments (N=12) on 51 cell-pairs, significantly higher ( $p < .001$ ) levels of iontophoretic NE (45.9  $\pm$  2.1 nA, mean  $\pm$  S.E.M.) and ISO (49.2  $\pm$  2.4 nA, mean  $\pm$  S.E.M.) were required to inhibit the firing of neurons recorded in 32 hr withdrawn rats compared to cells in saline-treated controls (23.0  $\pm$  1.4 nA, NE; 30.7  $\pm$  1.6 nA, ISO). No significant differences in chemosensitivity to the direct inhibitory effects of GABA were observed between cells recorded in control, chronic morphine-treated and withdrawn animals.

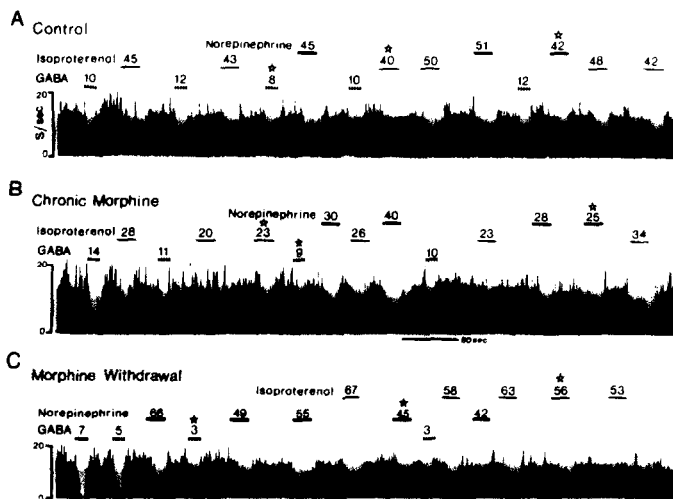


Fig. 1. Continuous ratemeter records from an experiment comparing responsiveness to iontophoretically applied NE (thick bars), ISO (thin bars) and GABA (dashed lines) between cerebrocortical neurons from a saline control (A), chronic morphine-treated (B) and morphine-withdrawn rat (C). The cells recorded from the morphine-treated (B) and morphine-withdrawn animal (C) show a higher and lower sensitivity, respectively, to the inhibitory actions of both NE and ISO than does the control cell (A).

#### DISCUSSION

The results of this study confirm and extend earlier findings (4,5) that chronic, but not acute, administration of morphine results in an increased

number of beta adrenoreceptors in rat cerebral cortex. Using electrophysiological recording methods in combination with radioligand binding techniques, we were able to demonstrate that the increase in cortical beta adrenoreceptors produced by chronic morphine treatment was paralleled by an enhanced responsiveness of cerebrocortical neurons to NE. Neurons in chronic morphine-treated animals appeared hypersensitive as well to the direct inhibitory action of the beta selective agonist ISO, but not to GABA. It seems likely, therefore, that the increase in cerebrocortical neuron sensitivity to noradrenergic stimulation following long-term morphine administration reflects the emergence of supersensitivity in postsynaptic beta adrenoreceptors, rather than alterations in catecholamine re-uptake or a nonspecific change in cellular excitability. The time course of this observed change in beta receptor activity would support a linkage between the occurrence of beta-adrenergic supersensitivity and the opiate dependent state. Whether such changes in central adrenoreceptor sensitivity contribute in a significant way in the manifestation of the withdrawal syndrome, however, remains unclear. The fact that beta adrenoreceptors in brain are not increased, but rather markedly reduced, during late withdrawal (32 hr) at a time when the primary abstinence syndrome is fully expressed suggests that beta adrenergic mechanisms are not crucial to the maintenance of opiate withdrawal.

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