Abstract The sensitivity of 5-HT$_{1A}$ serotonin receptors and $\alpha_2$-adrenoceptors (autoreceptors and heteroreceptors) modulating brain monoamine synthesis was investigated in rats during morphine treatment and after naloxone-precipitated withdrawal. The accumulation of 5-hydroxytryptophan (5-HTP) and 3,4-dihydroxyphenylalanine (DOPA) after decarboxylase inhibition was used as a measure of the rate of tryptophan and tyrosine hydroxylation in vivo. Acute morphine (3–100 mg/kg, 1 h) increased the synthesis of 5-HTP/5-HT in various brain regions (15%–35%) and that of DOPA/dopamine (DA) in striatum (28%–63%), but decreased the synthesis of DOPA/noradrenaline (NA) in hippocampus and cortex (20%–33%). Naloxone (2–60 mg/kg, 1 h) did not alter the synthesis of 5-HTP or DOPA in brain. Tolerance to the inhibitory effect of morphine on DOPA/NA synthesis and a sensitization to its stimulatory effects on DOPA/DA and 5-HTP/5-HT synthesis were observed after chronic morphine and/or morphine-withdrawn rats. In morphine-dependent rats (tolerant and withdrawn states) the inhibitory effects of the 5-HT$_{1A}$ agonists 8-OH-DPAT and buspirone (0.1 mg/kg, 1 h), and that of the $\alpha_2$-adrenoceptor agonist clonidine (0.1 mg/kg, 1 h) on the synthesis of 5-HTP/5-HT were potentiated (25%–50%). Moreover, the effect of 8-OH-DPAT was antagonized by WAY 100135, a selective 5-HT$_{1A}$ antagonist. In morphine-dependent rats (tolerant state), the inhibitory effects of clonidine on the synthesis of DOPA/NA (hippocampus, hypothalamus) and DOPA/DA (striatum) also were potentiated (35%–55%). In summary, we conclude that morphine addiction is associated with supersensitivity of 5-HT$_{1A}$ serotonin receptors and $\alpha_2$-adrenoceptors (autoreceptors and heteroreceptors) that modulate the synthesis of monoamines in brain.

Keywords 5-HT$_{1A}$-Autoreceptors · $\alpha_2$-Autoreceptors · $\alpha_2$-Heteroreceptors · Receptor supersensitivity · 5-HTP/5-HT synthesis · DOPA/dopamine synthesis · DOPA/noradrenaline synthesis · Morphine addiction · Rat brain

Introduction

A growing body of literature suggests that biogenic amine systems are involved in the behavioral and physiological effects of opiates. Neurochemical evidence is consistent with the inference that some effects of opioids like nociception are mediated, at least in part, by activation of serotonergic neurons (Roberts 1984), because the synthesis, release and metabolism of serotonin (5-HT) are affected by morphine administration (Way et al. 1968; García-Sevilla et al. 1978; Tao and Auerbach 1994). Also 5-HT could be involved in the behavioral and physiological rewarding aspects of opiates (Smith et al. 1987), and development of opiate withdrawal (Berthold et al. 1989; Akaoka and Aston-Jones 1993). Several lines of biochemical and pharmacological evidence also provide support for the involvement of the noradrenergic system in the physical dependence and in the expression of the somatic symptoms of opiate withdrawal. Various studies have reported changes in brain noradrenaline (NA) and metabolites during opiate dependence and abstinence (Done et al. 1992; Rossetti et al. 1993; Silverstone et al. 1993). The main noradrenergic structure mediating the expression of opioid abstinence seems to be the locus coeruleus (LC; Maldonado et al. 1992). The activation of the LC during morphine withdrawal (Aghajanian 1978) is due to an up-regulation of the cyclic AMP transduction system, although afferent projections containing excitatory amino acids (glutamate), derived from the nucleus paragigantocellularis, seem also to be involved in...
this activation (Rasmussen and Aghajanian 1989; Akaoka and Aston-Jones 1991; Nestler 1992). In this context brain 5-HT selectively attenuates excitation of LC neurons mediated by excitatory amino acids, and agents which increase serotonergic neurotransmission attenuate the hyperactivity of LC induced by naloxone-precipitated withdrawal in chronic morphine-treated rats (Akaoka and Aston-Jones 1993). Moreover, 5-HT1A receptor agonists like 8-OH-DPAT, buspirone and ipsapirone were found to suppress jumping induced by naloxone in morphine-dependent mice (Berthold et al. 1989). On the other hand, it is well known that the α2-adrenoceptor agonist clonidine improves the symptomatology and biochemical changes associated with opiate withdrawal (Aghajanian 1978; Maldonado 1997). Although chronic opiate administration and morphine withdrawal have been reported to induce adaptive changes in α2-adrenoceptors (increased density), the results obtained are controversial (Vicentini et al. 1983; García-Sevilla et al. 1986; Ulibarri et al. 1987 and references therein). With regard to 5-HT1A serotonin receptors, very little is known on possible adaptive changes after chronic opiate administration.

Monoaminergic neurons possess presynaptic inhibitory 5-HT1A serotonin receptors and α2-adrenoceptors that regulate the synthesis of 5-HT and NA in brain. These receptors are called synthesis-modulating autoreceptors or heteroreceptors and they operate through the negative control of the rate-limiting enzymes tryptophan hydroxylase and tyrosine hydroxylase (Esteban et al. 1999; Sastre-Coll et al. 1999). The modulation of these receptors regulating the synthesis of 5-HT and NA following chronic morphine has not been reported yet.

Therefore, the aim of this study was to assess in vivo the sensitivity of presynaptic 5-HT1A-autoreceptors and α2-adrenoceptors/heteroreceptors modulating monoamine synthesis in the rat brain after chronic morphine treatment and during morphine withdrawal precipitated by naloxone. A preliminary report of a portion of this work was given at the eighth annual meeting of the Spanish Society of Neuroscience (Sastre-Coll et al. 2000).

**Materials and methods**

**Drug treatment of animals.** Male Sprague-Dawley rats (weighing 220–260 g) were used. The animals were housed individually under controlled environmental conditions (22°C; 70% humidity and a 12-h light/dark cycle) with free access to standard food and tap water. For the acute treatments the animals received intraperitoneally (i.p.) a single injection of morphine (3, 10, 30, 100 mg/kg, 1 h) or naloxone (2, 20, 60 mg/kg, 1 h). After this injection (30 min) the animals received the aromatic L-amino acid decarboxylase inhibitor NSD 1015 (3-hydroxybenzylhydrazine HCl, 100 mg/kg, i.p.) and then were sacrificed after another 30 min. For the chronic treatments, the rats were injection i.p. three times daily (at 08:00 h, and then were sacrificed after another 30 min. For the chronic treatment, naloxone (2 mg/kg, i.p., 2 h)-precipitated withdrawal was induced in one group of rats, which resulted in various withdrawal reactions like wet dog shakes, diarrhea, weight loss, teeth chattering and others (data not shown). A challenge dose of morphine (100 mg/kg) was tested in these animals. Another series of experiments was designed to test the in vivo sensitivity of 5-HT1A autoreceptors modulating 5-HT synthesis, α2-autoreceptors modulating NA synthesis and α2-heteroreceptors modulating 5-HT/dopamine (DA) synthesis in brain. Groups of rats received i.p. a single dose of 0.9% NaCl vehicle, the 5-HT1A receptor agonist 8-OH-DPAT (0.1 mg/kg) and buspirone (1 mg/kg) or the α2-adrenoceptor agonist clonidine (0.1 mg/kg) 1 h after the last injection of vehicle, morphine or naloxone. Elapsed 30 min after the administration of 8-OH-DPAT, buspirone or clonidine, the animals received NSD 1015 (100 mg/kg) and then were sacrificed after another 30 min. To assess receptor sensitivity in brains of morphine-dependent rats (expected supersensitivity), low doses of the agonists DPAT and clonidine (0.1 mg/kg) were chosen which induce adaptive changes in catecholaminergic neurons mediated by excitatory amino acids, and agents that attenuate the synthesis of 5-HT and catecholamines, were determined simultaneously by measuring the accumulation of 5-hydroxytryptophan (5-HTP) and 5-hydroxyindoleacetic acid (EC 1.1.1.28) by a maximally effective dose of NSD 1015 (3-hydroxybenzylhydrazine HCl, 100 mg/kg, i.p.; Carls-son et al. 1972; Nissbrandt et al. 1988). The 5-HTP and DOPA accumulation method is the most commonly used assay system to monitor the in-vivo rate of tryptophan and tyrosine hydroxylation in the brain. The synthesis of 5-HTP and DOPA was measured in four brain regions enriched in 5-HT, NA or DA nerve terminals (hippocampus, cerebral cortex, hypothalamus and striatum). The 5-HTP and DOPA formed from endogenous tryptophan and tyrosine, respectively, were then determined by HPLC with electrochemical detection (ED).

**Brain samples and HPLC analyses.** The rats were killed by decapitation and the brains were quickly removed and dissected on an ice-cold plate into the hippocampus, parieto-occipital cortex, hypothalamus and striatum. Fresh brain regions were weighed and placed individually into cold tubes which contained 1 ml of 0.4 M HClO4, 0.01% K2EDTA and 0.1% Na2SO4 and were homogenized with an Ultra-Turrax homogenizer (Type Tp 18/10). The homogenate was then centrifuged at 40,000 g for 15 min at 4°C. The resulting supernatant was filtered through 0.45-µm syringe filters (Sartorius; Aldrich Chemical, Milwaukee, Wis., USA) and various aliquots were injected into the HPLC system for the 5-HTP, DOPA, 5-HT, NA, DA, 5-hydroxyindolacetic acid (5-HIIA), 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) determination. The levels of precursor amino acids, monoamines and metabolites were determined by HPLC-ED as described previously (Sastre-Coll et al. 1999 for representative chromatograms). Aliquots (10 µl) of the purified supernatants from brains of rats were subjected to HPLC on a Spherisorb S3 ODS1 C18 reversed-phase column (3-µm particle size range, 4.6 mm × 10 cm) coupled to a Tracer ODS2 C18 (2–5 µm particle size range) pre-column (Teknokroma). The mobile phase consisted of 0.1 M KH2PO4, 211