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PERIPHERAL AND SPINAL 5-HT RECEPTORS PARTICIPATE IN THE PRONOCICEPTIVE AND ANTINOCICEPTIVE EFFECTS OF FLUOXETINE IN RATS

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Abstract—The role of 5-HT receptors in fluoxetine-induced nociception and antinociception in rats was assessed. Formalin produced a typical pattern of flinching and licking/lifting behaviors. Local peripheral ipsilateral, but not contralateral, pre-treatment with fluoxetine (0.3–3 nmol/paw) increased in a dose-dependent fashion 0.5% formalin-induced nociception. In contrast, intrathecal pretreatment with fluoxetine (0.3–3 nmol/rat) prevented nociception induced by formalin. The peripheral pronociceptive effect of fluoxetine was prevented by the 5-HT_{2A} (ketanserin, 3–10 pmol/paw), 5-HT_{2B} (3-(2-[4-(4-fluorobenzoyl)-1-piperidinyl]ethyl)-2,4(1H,3H)-quinazolinedione (+) tartrate, RS-127445, 3–10 pmol/paw), 5-HT_{2C} (8-[5-(2,4-dimethoxy-5-(4-trifluoromethylphenylsulphonamido) phenyl-5-oxopentyl]1,3,8-triazaspiro[4.5] decane-2,4-dione hydrochloride, RS-102221, 3–10 pmol/paw), 5-HT_3 (ondansetron, 3–10 nmol/paw), 5-HT_4 ([1-[2-methylsulphonylamino ethyl]-4-piperidinyl]methyl 1-methyl-1H-indole-3-carboxylate, GR-113808, 3–100 fmol/paw), 5-HT_6 (4-iodo-N-[4-methoxy-3-(4-methyl-1-piperazinyl)phenyl]benzene-sulfonamide hydrochloride, SB-258585, 3–10 pmol/paw) and 5-HT_7 ((R)-3-(2-(2-(4-methylpiperidin-1-yl) ethyl) pyrrolidine-1-sulfonyl) phenol hydrochloride, SB-269970, 0.3–1 nmol/paw), but not by the 5-HT_{1A} (N-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-N-2-pyridinylcyclohexanecarboxamide maleate, WAY-100635, 0.3–1 nmol/paw), 5-$HT_{1B/1D}$ (N-[4-methoxy-3-(4-methyl-1-piperazinyl)phenyl]-2'-methyl-4'-(5-methyl-1,2,4-oxadiazol-3-yl)-1,1'-biphenyl-4-carboxamide hydrochloride hydrate, GR-127935, 0.3–1 nmol/paw), 5-HT_1B (1'-methyl-5-[[2'-methyl-4'-(5-methyl-1,2,4-oxadiazol-3-yl)biphenyl-4-yl]carbonyl]-2,3,6,7-tetrahydrospiro[furo[2,3-*f*]indole-3,4'-piperidine hydrochloride, SB-224289, 0.3–1 nmol/paw), 5-HT1D (4-(3-chlorophenyl)-α-(diphenylmethyl)-1-piperazineethanol hydrochloride, BRL-15572, 0.3–1 nmol/paw) nor 5-HT_{5A} ((*N*-[2-(dimethylamino)ethyl]-*N*-[[4'-[[(2-phenylethyl)amino]methyl] [1,1'-biphenyl]-4-yl]methyl]cyclopentanepropanamide dihydrochloride, SB-699551, 1–3 nmol/paw), receptor antagonists. In marked contrast, the spinal antinociceptive effect of fluoxetine was prevented by the 5-HT_{1A} (WAY-100635, 0.3–1 nmol/rat), 5-$HT_{1B/1D}$ (GR-127935, 0.3–1 nmol/rat), 5-HT_{1B} (SB-224289, 0.3–1 nmol/rat), 5-HT_{1D} (BRL-15572, 0.3–1 nmol/rat) and 5-HT_{5A} (SB-699551, 1–3 nmol/rat), but not by the 5-HT_{2A} (ketanserin, 3–10 pmol/rat), 5-HT_{2B} (RS-127445, 3–10 pmol/rat), 5-HT_{2C} (RS-102221, 3–10 pmol/rat), 5-HT_3 (ondansetron, 3–10 nmol/rat), 5-HT_4 (GR-113808, 3–100 fmol/rat), 5-HT_6 (SB-258585, 3–10 pmol/rat) nor 5-HT_7 (SB-269970, 0.3–1 nmol/rat), receptor antagonists. These results suggest that fluoxetine produces nociception at the periphery by activating peripheral 5-$HT_{2A/2B/2C/3/4/6/7}$ receptors. In addition, intrathecal fluoxetine produces antinociception by activation of spinal 5-$HT_{1A/1B/1D/5A}$ receptors.

Key words: fluoxetine, formalin test, mechanism of action, nociception, 5-HT receptors.

INTRODUCTION

Serotonergic descending pathway from rostral ventromedial medulla (RVM) to the dorsal horn is crucial to spinal nociceptive processing (Millan, 2002; Suzuki et al., 2004; Heinricher et al., 2009). Once released 5-hydroxytryptamine (5-HT) can exert facilitatory (Bee and Dickenson, 2007; Wei et al., 2010) or inhibitory (Zhao et al., 2007; Braz and Basbaum, 2008) influences onto dorsal horn neurons depending on the spinal 5-HT receptor subtype activated and on the cellular distribution of such receptors (Sommer, 2006). For instance, intrathecal injection of 5-HT produces antinociception (Yaksh and Wilson, 1979) while

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Abbreviations: ANOVA, one-way analysis of variance; AUC, area under the number of flinches against time curves; 5-HT, 5-hydroxytryptamine; BRL-15572, 4-(3-chlorophenyl)-α-(diphenylmethyl)-1-piperazineethanol hydrochloride; DRG, dorsal root ganglion; GR-113808, [1-[2-methylsulphonylamino ethyl]-4-piperidinyl] methyl 1-methyl-1H-indole-3-carboxylate; GR-127935, N-[4-methoxy-3-(4-methyl-1-piperazinyl)phenyl]-2′-methyl-4′-(5-methyl-1,2,4-oxadiazol-3-yl)-1,1′-biphenyl-4-carboxamide hydrochloride hydrate; RS-102221, 8-[5-(2,4-dimethoxy-5-(4-trifluoromethylphenyl sulphonamido)phenyl-5-oxopentyl]1,3,8-triazaspiro[4.5] decane-2,4-dione hydrochloride; RS-127445, 3-(2-[4-(4-fluorobenzoyl)-1-piperidinyl]ethyl)-2,4(1H,3H)-quinazolinedione(+)tartrate; SB-224289, 1′-methyl-5-[[2′-methyl-4′-(5-methyl-1,2,4-oxadiazol-3-yl) biphenyl-4-yl]carbonyl]-2,3,6,7-tetrahydrospiro[furo[2,3-*f*]indole-3,4′-piperidine hydrochloride; SB-699551, (*N*-[2-(dimethylamino)ethyl]-*N*-[[4′-[[(2-phenylethyl)amino]methyl][1,1′-biphenyl]-4-yl]methyl]cyclopentanepropanamide dihydrochloride; SB-258585, 4-iodo-N-[4-methoxy-3-(4-methyl-1-piperazinyl)phenyl]benzene-sulfonamide hydrochloride; SB-269970, (R)-3-(2-(2-(4-methylpiperidin-1-yl)ethyl)pyrrolidine-1-sulfonyl)phenol hydrochloride; SSRIs, selective serotonin reuptake inhibitors; WAY-100635, N-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-N-2-pyridinylcyclohexane-carboxamide maleate.

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depletion of 5-HT reduces the antinociceptive effect of morphine (Sawynok and Reid, 1989) suggesting an inhibitory role for the descending serotonergic pathway. In contrast, depletion of spinal 5-HT reduces formalin-induced nociception (Svensson et al., 2006; Wei et al., 2010) indicating a facilitatory role. All 5-HT receptor subtypes (5-$HT_{1–7}$) are expressed in the dorsal root ganglion (DRG) and spinal dorsal horn (Pierce et al., 1996; Wu et al., 2001; Doly et al., 2004; Liu et al., 2005). Previous studies have shown that activation of the spinal 5-HT_{1A} (Mjellem et al., 1992; Oyama et al., 1996; Jeong et al., 2012), 5-HT_{1B} (Jeong et al., 2004; Liu et al., 2007) and 5-$HT_{1B/1D/1F}$ (Nikai et al., 2008) receptors attenuates nociception in models of inflammatory pain. Contrariwise, there is evidence that activation of spinal 5-$HT_{2/3/6/7}$ receptors (Oyama et al., 1996; Kjørsvik et al., 2001; Sasaki et al., 2001; Rocha-González et al., 2005; Castañeda-Corral et al., 2009) increases formalin-induced nociception.

Selective serotonin reuptake inhibitors (SSRIs), as fluoxetine, have been used as a first-line therapy for treating chronic pain in humans (Sindrup and Jensen, 1999; Crowell et al., 2004). Systemic administration of fluoxetine reduces nociception in inflammatory and neuropathic pain models (Nayebi et al., 2001; Singh et al., 2001; Pedersen et al., 2005; LaBuda and Little, 2005; Leventhal et al., 2007; Sikka et al., 2011). Furthermore, it has been reported that local peripheral injection of fluoxetine reduces formalin-induced nociceptive behavior while it increases paw volume (Sawynok et al., 1999). Fluoxetine exhibits high affinity for the 5-HT transporter (Owens et al., 1997; Tatsumi et al., 1997) and it blocks 5-HT reuptake, release and synthesis as well as neuronal discharge (Hjorth and Auerbach, 1994; Barton and Hutson, 1999). The antinociceptive effect of fluoxetine has been attributed to these actions prolonging the inhibitory actions of 5-HT on the spinal cord neurons involved in transmitting/modulating pain (Basbaum and Fields, 1978; Yaksh and Wilson, 1979). However, systemic fluoxetine can increase 5-HT levels in central (Beyer and Cremers, 2008; Nagayasu et al., 2010) as well as in peripheral sites (Weihe and Eiden, 2000; Bianchi et al., 2002; Vega and Rudolph, 2002; O'Connell et al., 2006; Mercado and Kilic, 2010). Since accumulation of 5-HT at the periphery and spinal sites may lead to different effects by activating several 5-HT receptors, the present study investigated the participation of peripheral and spinal 5-HT receptors in the effects of fluoxetine in formalin-induced acute nociception and long-lasting allodynia and hyperalgesia.

EXPERIMENTAL PROCEDURES

Animals

Female Wistar rats aged 8–10 weeks (weight range 180–220 g) from our own breeding facilities were used in this study. Animals were housed in a controlled environment with temperature maintained at 22 °C and a 12-h light/dark cycle. They had free access to food and drinking water before experiments. All experiments were in compliance with the Guidelines on Ethical Standards for Investigation of Experimental Pain in Animals (Zimmermann, 1983) and the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 8023, revised 1996). Protocol was approved by our Institutional Animal Care and Use Committee (Cinvestav, México City, Protocol 455-09). Efforts were made to minimize the number of animals used and their suffering.

Spinal surgery

Rats underwent surgery for insertion of a spinal catheter for drug administration 5 days prior to formalin injection. Animals were anesthetized with a ketamine/xylazine mixture (45/12 mg/kg, i.p.), placed in a stereotaxic head holder, and the atlanto-occipital membrane was exposed (LoPachin et al., 1981). The membrane was pierced, and a PE-10 catheter (8 cm) was introduced intrathecally to the level of the thoracolumbar junction after which the wound was sutured. Rats were allowed to recover from surgery for at least five days in individualized cages before use. Animals showing any sign of motor impairment were euthanized in a CO_2 chamber.

Measurement of acute nociceptive activity

Antinociception was assessed using the formalin test described by Dubuisson and Dennis, 1977 with some modifications (Rocha-González et al., 2005). Briefly, rats were placed in open clear acrylic cylinders for 30 min to allow them to acclimate to their surroundings. Then, they were removed for formalin injection. Rats were gently restrained while the dorsum of the hind paw was injected with 50 μL of diluted formalin (0.5%) into the dorsal surface of the right hind paw with a 30-gauge needle. The animals were returned to the chambers and nociceptive behavior was observed immediately after formalin injection. Mirrors were placed in each cylinder to enable unhindered observation. Nociceptive behavior was quantified as the number of flinches of the injected paw/hindquarters during 1-min periods every 5 min, up to 60 min after injection (Wheeler-Aceto et al., 1990). Flinching was readily discriminated and was characterized as rapid and brief withdrawal, or as flexing of the injected paw/hindquarters. We decided to evaluate flinching because it is a simple and reliable parameter of pain behavior and one producing high scores (Wheeler-Aceto et al., 1990; Abbott et al., 1995). Formalin-induced flinching behavior was biphasic (Wheeler-Aceto et al., 1990; Rocha-González et al., 2005). The initial acute phase (0–10 min) was followed by a relatively short quiescent period, which was then followed by a prolonged tonic response (15–60 min). At the end of the experiment the rats were sacrificed in a CO_2 chamber.

Measurement of secondary allodynia and hyperalgesia

Rats were briefly immobilized to get open access to the right hind limb. Then, they received a s.c. injection of