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European Journal of Pharmacology 538 (2006) 80–84
www.elsevier.com/locate/ejphar

Possible involvement of cholinergic and opioid receptor mechanisms in fluoxetine mediated antinociception response in streptozotocin-induced diabetic mice

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Received 10 March 2006; received in revised form 20 March 2006; accepted 28 March 2006
Available online 4 April 2006

Abstract

Clinical and experimental studies have been reported that antidepressant drugs can be used as co-analgesics in the management of neuropathic pain. However, the mechanism through which they alleviate pain still remains unclear. The aim of the present study was to investigate the possible mechanism of action of fluoxetine-induced antinociceptive effect in streptozotocin-induced diabetic mice, especially the involvement of non-serotonergic neurotransmitters and their receptors. Diabetes was induced in male Laka mice with a single intraperitoneal injection of streptozotocin (200 mg/kg). Four weeks after streptozotocin, diabetic mice were tested for pain responses in the tail-immersion and hot-plate assays. Diabetic mice exhibited significant hyperalgesia as compared with control mice. Fluoxetine (10 and 20 mg/kg, i.p) injected into diabetic mice produced an antinociceptive effect in both tail-immersion and hot-plate assays. The antinociceptive effect of fluoxetine in diabetic mice was significantly lower as compared with that in control mice. Pretreatment with a muscarinic receptor antagonist, atropine (2 and 5 mg/kg, i.p) and an opioid receptor antagonist, naloxone (2 and 5 mg/kg, i.p), but not the α_2-adrenoreceptor antagonist, yohimbine (2 and 5 mg/kg, i.p) reversed the antinociceptive effect of fluoxetine (20 mg/kg). These results suggest that apart from serotonin pathway, muscarinic and opioid receptors also participate in fluoxetine-induced antinociception in diabetic neuropathic pain.

Keywords: Atropine; Hot-plate; Fluoxetine; Naloxone; Neuropathic pain; Streptozotocin; Tail-immersion

1. Introduction

Neuropathic pain is generally considered to be one of the most common complications of diabetes, affecting both types of diabetes equally (Watkins, 1990; Vinik et al., 1992; Clark and Lee, 1995). It is mostly characterized by pain which can occur spontaneously as a result of exposure to normally mildly painful stimuli, i.e. hyperalgesia (Brown and Asbury, 1984). Although hyperglycaemia (Greene et al., 1987), neuronal loss (Dyck et al., 1985; Said et al., 1992) or neurotransmitter changes (Bitar et al., 1985; Chu et al., 1986; Bellush and Reid, 1991) have been reported to be responsible for the change in pain perception, the exact aetiological factors involved are still under investigation.

The behavioural reaction to hyperalgesia has been described in animal models of diabetes (Kamei et al., 1991; Courteix et al., 1993; Anjaneyulu and Chopra, 2003). Streptozotocin-induced diabetic mice have been widely used as a model of diabetes mellitus, and a number of anomalies in pain perception have been demonstrated in this model (Kamei et al., 2000). Chemical-induced flinching, thermal hyperalgesia and allodynia have been observed in streptozotocin-treated mice (Ohsawa and Kamei, 1999; Kamei et al., 2001).

The neurotransmitter 5-hydroxytryptamine (5-HT) is widely accepted as an important participant in the brain and spinal inhibition of nociceptive transmission (Bardin et al., 2000; Zhang and Wu, 2000). Behavioural studies have demonstrated that 5-HT is implicated in the control exerted by the brain on nociception either by afferent fibre hyperpolarization or through a presynaptic action. It has been reported that destruction of serotonergic projection is known to greatly affect nociception. Serotonergic deficiency is a common factor in both mental depression and chronic pain (Vogel et al., 2003). It has been reported that destruction of serotonergic projections greatly

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doi:10.1016/j.ejphar.2006.03.067

affects nociception. In contrast, increasing the availability of 5-HT at the synapse is reported to inhibit nociception by acting at spinal cord, brainstem or thalamic levels. Several lines of evidence suggest that 5-HT may regulate acetylcholine and noradrenaline release in the central nervous system (Sawynok and Reid, 1991; Fuerstein et al., 1996; Ramirez et al., 1997). 5-HT mediated spinal antinociception has also been shown to involve μ-opioid receptors (Goodchild et al., 1997). Recently, we reported the involvement of 5-HT_1 and 5-HT_2 receptors in fluoxetine-induced antinociception in diabetic mice (Anjaneyulu and Chopra, 2004), but the mechanism of indirect modulation of fluoxetine analgesia by other neurotransmitters needs to be evaluated in the diabetic pain.

Against this background, the present study aimed to investigate the possible involvement of cholinergic, adrenergic and opioid receptors with respect to the antinociceptive action of fluoxetine in streptozotocin-induced diabetic mice.

2. Material and methods

2.1. Animals

Male albino mice of Laka strain (20–30 g) bred in Central Animal House facility of Panjab University were used in the present study. The animals were housed under optimal laboratory conditions, maintained on a natural light and dark cycle, and had free access to food and water ad libitum. Animals were acclimatized to laboratory conditions before the test. All experiments were carried out blindly between 09:00 and 17:00 h. The experimental protocols were approved by the Institutional Animal Ethics Committee and conducted according to the Indian National Science Academy Guidelines for the use and care of animals (licence number: 388/Ethic/2002).

2.2. Drugs and reagents

Streptozotocin, atropine, naloxone and yohimbine were purchased from Sigma Chemicals (St. Louis, MO, USA). Fluoxetine (gift sample from Divis Pharma, India), atropine, naloxone and yohimbine were dissolved in distilled water. The glucose oxidase peroxidase enzyme kit was purchased from Span Diagnostics, India.

2.3. Induction and assessment of diabetes

Streptozotocin was prepared in citrate buffer (pH 4.4, 0.1 M) (Banisinath et al., 1988) and injected intraperitoneally in a single dose of 200 mg/kg (Ramabadran et al., 1989). The age-matched control mice received an equivalent volume of citrate buffer. At 2 days after streptozotocin injection, plasma glucose levels were estimated with the glucose oxidase peroxidase enzyme kit method (Schmidt, 1971). Plasma glucose levels were also measured at the time of 4 weeks. About 90% of streptozotocin-injected mice developed diabetes and mice with plasma glucose levels of more than 250 mg/dl (Anjaneyulu and Ramarao, 2002) were considered as diabetic and used for the present study after 4 weeks.

2.4. Treatment schedule

Preliminary threshold to tail-immersion and hot-plate responses (the means of two consecutive stable values which do not differ more than 10%) were determined before the drug administration. At the end of 4 weeks, control and diabetic mice were randomly divided into different groups consisting of 6–7 animals. Fluoxetine (10 and 20 mg/kg) was administered intraperitoneally in two groups of diabetic mice after measurement of baseline response. To other groups of diabetic mice, atropine (2 and 5 mg/kg), yohimbine (2 and 5 mg/kg) and naloxone (2 and 5 mg/kg) were injected intraperitoneally 5 min before the fluoxetine (20 mg/kg) injection. In both tail-immersion and hot-plate assays, nociceptive latency was measured at 15, 30, 60 and 180 min after fluoxetine injection and expressed as % of the maximum possible effect (% MPE), where % MPE = (post-drug threshold − pre-drug threshold) × 100/(cut-off time − pre-drug threshold). All drug solutions were freshly prepared and injected intraperitoneally in a constant volume of 1 ml/100 g of body weight.

2.5. Assessment of thermal hyperalgesia

2.5.1. Tail-immersion (warm water) test

The tail was immersed in a warm water bath (52.5 ± 0.5 °C) until tail withdrawal (flicking response) or signs of struggle were observed (cut-off 12 s). The hyperalgesic response in the tail-withdrawal test is generally attributed to central mechanisms (Ramabadran et al., 1989; Kannan et al., 1996).

2.5.2. Hot-plate test

The hyperalgesic response on the hot-plate is considered to result from a combination of central and peripheral mechanisms (Kannan et al., 1996). In this test, animals were individually placed on a hot-plate (Eddy's Hot-Plate) with the temperature adjusted to 55 ± 1 °C. The latency to the first sign of paw licking or jump response to avoid the heat was taken as an index of the pain threshold; the cut-off time was 10 s in order to avoid damage to the paw.

2.6. Statistical analysis

The nociceptive threshold, i.e., the latency (in seconds) to thermal noxious stimulus was measured and % MPE was calculated. The data are expressed as means ± S.E.M. The hyperalgesic response was analysed by analysis of variance followed by Tukey's *t*-test. Student's unpaired *t*-test was used to compare the values from two groups. $P < 0.05$ was considered as significant.

3. Results

3.1. Effect of streptozotocin injection in mice on blood glucose and body weight

Four weeks after streptozotocin injection, the diabetic mice had significantly higher blood glucose levels (486.44 ± 25.58 mg/dl) than the control mice (106.92 ± 18.42 mg/dl; $P < 0.001$). There