Pharmacology, Biochemistry and Behavior 122 (2014) 1–6

Contents lists available at ScienceDirect

Pharmacology, Biochemistry and Behavior

journal homepage: www.elsevier.com/locate/pharmbiochembeh

The antinociceptive effect of intravenous imipramine in colorectal distension-induced visceral pain in rats: The role of serotonergic and noradrenergic receptors☆,☆☆

Fatih İlkaya [a,*,1], S. Sırrı Bilge [a,2], Ayhan Bozkurt [b,3], Duygu B. Baş [c,4], Arzu Erdal [a,5], Engin Çiftçioğlu [d,6], Yüksel Kesim [a,7]

[a] Department of Pharmacology, School of Medicine, Ondokuz Mayis University, Samsun, Turkey
[b] Department of Physiology, School of Medicine, Ondokuz Mayis University, Samsun, Turkey
[c] Department of Physiology and Pharmacology, Karolinska Institutet, Stockholm, Sweden
[d] Department of Anatomy, School of Medicine, Ondokuz Mayis University, Samsun, Turkey

ARTICLE INFO

Article history:
Received 18 December 2012
Received in revised form 19 February 2014
Accepted 20 February 2014
Available online 26 February 2014

Keywords:
Imipramine
Serotonergic receptors
Adrenergic receptors
Visceral pain
Colorectal distension
Antinociception

ABSTRACT

It has been shown that imipramine, a tricyclic antidepressant (TCA), is a potent analgesic agent. However, the effect of imipramine on visceral pain has not been extensively investigated. In the current study, our aim was to characterise the putative analgesic effect of intravenous imipramine on visceral pain in rats. Our second aim was to assess the involvement of serotonergic (5-$HT_{2,3,4}$) and noradrenergic ($\alpha_{2A,2B,2C}$) receptor subtypes in this putative antinociceptive effect of imipramine. Male Sprague Dawley rats (250–300 g) were implanted with venous catheters for drug administration and implanted with enamelled nichrome electrodes for electromyography of the external oblique muscles. Noxious visceral stimulation was applied via by colorectal distension (CRD). The visceromotor responses (VMRs) to CRD were quantified electromyographically before and after imipramine administration at 5, 15, 30, 60, 90 and 120 min. In the antagonist groups, the agents were administered 10 min before imipramine. The administration of imipramine (5–40 mg/kg) produced a dose-dependent reduction in VMR. The administration of yohimbine (a nonselective α_2-adrenoceptor antagonist, 1 mg/kg), BRL-44408 (an α_{2A}-adrenoceptor antagonist, 1 mg/kg) or MK-912 (an α_{2C}-adrenoceptor antagonist, 300 μg/kg) but not imiloxan (an α_{2B}-adrenoceptor antagonist, 1 mg/kg) inhibited the antinociceptive effect of imipramine (20 mg/kg). Additionally, ketanserin (a 5-HT_2 receptor antagonist, 0.5, 1, and 2 mg/kg) and GR113808 (a 5-HT_4 receptor antagonist, 1 mg/kg) enhanced, and ondansetron (a 5-HT_3 receptor antagonist, 0.5, 1, and 2 mg/kg) failed to alter the imipramine-induced antinociceptive effect. Our data demonstrated that, in the CDR-induced rat visceral pain model, intravenous imipramine appeared to have antinociceptive potential and that α_{2A}-/α_{2C}-adrenoceptors and 5-HT_2/5-HT_4 receptors may be responsible for the antinociceptive effect of imipramine on visceral pain in rats.

1. Introduction

Tricyclic antidepressants (TCAs) have been used as potent analgesic agents for the treatment of pain for many years. TCAs are used for both neuropathic and other pain disorders that include diabetic nerve pain, postherpetic neuralgia, irritable bowel syndrome, atypical facial pain, fibromyalgia, migraine, and tension-type headache (Bourel and Sabouraud, 1962; Broadhead et al., 1991; Fishbain et al., 2000). Although these antidepressants are widely used for various painful conditions, the precise analgesic mechanism(s) have not been identified.

TCAs block the reuptake of serotonin (5-HT) and noradrenaline, and it is well-known that these neurotransmitters are involved in the perception and modulation of pain (Spiegel et al., 1983; Tura and Tura, 1990; Millan, 2002; Micó et al., 2006). Thus, it has been suggested that

☆ Funding statement: This study was supported by the Ondokuz Mayıs University Project Management Office, grant number T.533.
☆☆ Conflict of interest: None of the authors have any conflicts of interest in connection with this study.
* Corresponding author at: Ondokuz Mayis University, School of Medicine, Atakum, Samsun, Turkey. Tel.: +90 362 3121919/3453; fax: +90 362 4576041.
E-mail address: fatihilkaya@gmail.com (F. İlkaya).
[1] Fatih İlkaya designed the study, conducted the study, collected the data and prepared the manuscript.
[2] S. Sırrı Bilge analysed the data and helped in the preparation of the manuscript.
[3] Ayhan Bozkurt analysed the data and helped in the preparation of the manuscript.
[4] Duygu Belkıs Bas collected the data.
[5] Arzu Erdal collected the data.
[6] Engin Çiftçioğlu collected the data.
[7] Yüksel Kesim conducted the study and helped in the preparation of the manuscript.

http://dx.doi.org/10.1016/j.pbb.2014.02.017
0091-3057/

classic TCAs exert antinociceptive effects that are more potent than those of selective 5-HT reuptake inhibitors (Otsuka et al., 2001; Micó et al., 2006). Imipramine is a classic TCA that inhibits the reuptake of both noradrenaline and 5-HT. Previous studies have reported antinociceptive effects of imipramine in various pain models (Valverde et al., 1994; Ghelardini et al., 2000; Otsuka et al., 2001; Yokogawa et al., 2002). However, the effect of imipramine on visceral pain is not known and requires elucidation.

Pain is the most common reason that people consult physicians. It has been asserted that the majority of visits are for pain of visceral origin (Kamp et al., 2003). Because of the clinical importance of visceral pain, the mechanisms and pharmacologic control of visceral pain need to be clarified. However, most of the current knowledge regarding the mechanisms and pharmacological control of pain has been obtained from somatic pain studies, and there is growing evidence that the mechanisms, characteristics and stimulus types of somatic pain are distinct from those of visceral pain (Cervero, 1994; Cervero and Laird, 1999; Raj, 2004).

Distension of the descending colon and rectum produces a noxious and easily measured visceral stimulus that mimics visceral pain in humans (Ness and Gebhart, 1988). Colorectal distension (CRD) is known to increase blood pressure and to contract the abdominal muscles (a visceromotor response), and these effects provide measurable and recordable changes. The antinociceptive effect of a drug can be demonstrated by the suppression of these responses (Ness and Gebhart, 1988).

The goals of the current study were as follows: (1) to elucidate the antinociceptive effect of intravenous imipramine on the visceral pain induced by CRD in rats; and (2) to assess the involvement of serotonergic and noradrenergic receptor subtypes (5-$HT_{2,\ 3,\ 4}$ and $\alpha_{2A,\ 2B,\ 2C}$, respectively) in the putative antinociceptive effect of this antidepressant.

2. Materials and methods

2.1. Animals and surgical preparation

Adult male Sprague Dawley rats weighing 250 to 300 g were obtained from the Ondokuz Mayis University vivarium. The rats were maintained in a vivarium at 22 ± 1 °C on a 12-h alternating light–dark cycle. All experiments were approved by the Institutional Animal Care and Use Committee of the Ondokuz Mayis University and adhered to the guidelines of the Committee for Research and Ethical Issues of the International Association for the Study of Pain.

2.2. Surgical procedures

Under anaesthesia (100 mg/kg ketamine and 0.75 mg/kg chlorpromazine i.p.), enamelled nichrome electrodes (diameter: 80 μm; Driver-Harris, Cedex, France) were implanted into the external oblique muscles just above the inguinal ligament for electromyographic (EMG) recordings. For the administration of drugs, a jugular vein was catheterized. Both the EMG electrodes and the venous catheter were tunnelled subcutaneously and externalised at the nape of the neck. During the 6-day recovery period prior to the commencement of the experiments, the rats were adapted to light restraint in Bollman cages and to manipulation to reduce motion artefacts and confounding effects due to stress-related responses (Ulger et al., 2009). Between sessions in the Bollman cages, the rats were caged individually with free access to food and water.

2.3. Experimental protocol

CRD was used to establish a model of visceral nociception in rats (Ness and Gebhart, 1988; Ulger et al., 2009). Flexible Tygon plastic tubing was inserted into a 6-cm latex balloon, and the end of the balloon was securely tied to the tube. The balloon, which was lubricated with ultrasound gel (Norm Co., Turkey), was inserted through the anus into the descending colon until the end of the balloon extended approximately 1–1.5 cm into the rectum. To prevent displacement, the tubing was taped to the base of the tail. The rats were fully awake and placed inside the Bollman cages during testing. To produce CRD, the intracolonic balloon was inflated with air. The catheter was attached to a bridge amplifier (ML221, ADInstruments, Australia) via a pressure transducer (MLT380, ADInstruments, Australia). The intracolonic pressure was monitored and recorded by a data acquisition system (ML870/P, PowerLab 8/30, ADInstruments, Australia) connected to the bridge amplifier.

The visceromotor response (VMR), which originated from the contraction of the external oblique musculature (Ness and Gebhart, 1988), was quantified based on the EMG activity recorded from the electrodes implanted in the external oblique musculature. The EMG signal was amplified using a Bio Amp (ML132, ADInstruments, Australia) connected to the data acquisition system and integrated offline using the Chart programme (version 5.2). Distension was induced in a staircase manner to establish CRD (Ulger et al., 2009; Bilge et al., 2012). Briefly, beginning at 0 mm Hg, intracolonic pressure was increased in steps (10–20 mm Hg) over approximately 80 s to a final pressure of 80 mm Hg. As the maximum VMR usually occurred in response to the 80-mm Hg stimuli, the EMG activity recorded over the first 5 s of the application of this pressure was used for data analysis. On the day of testing, five staircase distensions were administered at 5-min intervals to obtain the baseline response before drug administration. Staircase distension was repeated 5, 15, 30, 60, 90, and 120 min after the administration of imipramine.

2.4. Drugs

All drugs used in the study were prepared daily in saline and administered intravenously (i.v.) in a volume of 0.5 ml. The nonselective α_2-adrenoceptor antagonist yohimbine hydrochloride (1 mg/kg) (Tocris Cookson, St. Louis, MO, USA), the α_{2A}-adrenoceptor antagonist BRL-44408 maleate (1 mg/kg) (Sigma, St. Louis, MO, USA), the α_{2B}-adrenoceptor antagonist imiloxan hydrochloride (1 mg/kg) (Sigma, St. Louis, MO, USA), the α_{2C}-adrenoceptor antagonist MK-912 (300 μg/kg) (Sigma, St. Louis, MO, USA), the 5-HT_2 receptor antagonist ketanserin (0.5, 1 and 2 mg/kg) (Sigma, St. Louis, MO, USA), the 5-HT_3 receptor antagonist ondansetron (0.5, 1 and 2 mg/kg) (Sigma, St. Louis, MO, USA), and the 5-HT_4 receptor antagonist GR113808 (1 mg/kg) (Sigma, St. Louis, MO, USA) were administered 10 min prior to imipramine (20 mg/kg) (Abdi İbrahim, Istanbul, Turkey).

2.5. Data analyses

All data are expressed as the means ± the S.E.M.s of 7–12 rats per group. The VMRs to CRD are represented as percentages of the control values (% control); i.e., the mean of the baseline responses prior to drug administration at 80 mm Hg was defined as 100%. The overall effect of each treatment was also determined as the area under the curve (AUC) of the time–response function using an Excel computer programme. The AUC was calculated from the time plot of the post drug administration responses normalised to the baseline response (100%) and plotted against time using the trapezoidal rule (AUC = Σ response × 120 min).

Statistical analyses were performed using the GraphPad InStat (version 3.06) software (GraphPad Software, San Diego, CA, USA). After verifying that the data were normally distributed, one-way analyses of variance (ANOVAs) followed by Tukey–Kramer post-hoc tests for multiple comparisons were performed. A value of $p < 0.05$ was considered statistically significant.