INVOLVEMENT OF THE SEROTONIN SYSTEM IN SSRI-INDUCED ANTINOCICEPTION

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ABSTRACT

Serotonin specific reuptake inhibitors (SSRI) may induce antinociception; however, the mechanism of this effect is not clear. SSRIs increase 5-HT levels in neuronal synapses and facilitate serotonergic activity. In this study, therefore, the activity of para-chlorophenylalanine (pCPA), which reduces 5-HT release, and 5-hydroxytryptophan (5-HTP), a precursor of 5-HT, were examined on the antinociceptive activity of six SSRIs, in the abdominal constriction test. The compounds studied included fluoxetine, fluvoxamine, sertraline, paroxetine, citalopram and zimelidine. The effects of pCPA and 5-HTP were also evaluated on morphine analgesia as a standard compound. All antidepressants tested demonstrated dose-inhibition of acetic acid-induced abdominal constrictions. The antinociceptive activities of morphine, fluoxetine, fluvoxamine and sertraline, but not paroxetine, citalopram and zimelidine were significantly reduced by pCPA. Subsequently, 5-HTP restored the reduced antinociception of morphine, fluoxetine and fluvoxamine caused by pCPA. Furthermore, 5-HTP increased morphine, fluoxetine, fluvoxamine and sertraline-induced antinociception. Opioid receptor antagonists have been shown to reduce the antinociception induced by morphine, fluoxetine, fluvoxamine and sertraline but not by paroxetine, citalopram and zimelidine. It can be concluded, therefore, that the serotonin system is only involved in antinociception produced by antidepressants, and their antinociception is opioid antagonist reversible.

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INTRODUCTION

It has been reported that peripherally administered 5-HT evokes hyperalgesic activity,1 whereas intrathecal (i.t.) injection of 5-HT has been shown to have analgesic properties.2 Serotonin specific reuptake inhibitors (SSRI) may induce antinociception;3 however, the mechanism of this effect is not clear. SSRIs increase 5-HT levels in neuronal synapses and therefore facilitate serotonergic activity.4 It has been suggested, therefore, that SSRIs might induce antinociception through inhibition of 5-HT reuptake.5 It should be noted that this hypothesis has not been examined in detail.

In the current study therefore the activity of 5-hydroxytryptophan (5-HTP), a precursor of 5-HT, and para-chlorophenylalanine (pCPA), which specifically reduces 5-HT release,6,7 were examined on the antinociceptive activity of six SSRIs in the abdominal
Serotonin and SSRI-Induced Antinociception

Fig. 1. Dose response antinociceptive activity of SSRI antidepressants in the abdominal constriction assay. All SSRIs were administered subcutaneously 30 min before the test. Values are mean±S.E.M. (n=8-10).

Constrictions test in order to probe their action on 5-HT generating systems. The compounds studied included fluoxetine (FLX), fluvoxamine (FLV), sertraline (Sert), paroxetine (Par), citalopram (Cit) and zimelidine (Zim). The effect of pCPA and 5-HTP were also evaluated on morphine analgesia as a standard compound.

MATERIALS AND METHODS

Male ICI-WSP derived mice (22-25g) were employed throughout. Animals were allowed food and water *ad libitum* and were maintained at a temperature of 22.0±1.0°C on a 12/12h light/dark cycle, the experiments being performed between 9.00 and 17.00 h.

Antinociceptive activity in mice was assessed using the abdominal constriction assay. Briefly, abdominal constrictions were elicited by intraperitoneal (ip) injection of 1% acetic acid and their incidence recorded over a 20 min period. Any decrease in abdominal constrictions compared to vehicle controls was calculated as a percentage of control values and designated % protection (as a criterion for antinociceptive activity of the compound).

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\% \text{Protection} = 100 \times \frac{\text{Writhes in drug group}}{\text{Writhes in control group}}
\]

Acetic acid (BDH, UK) was administered i.p. 5 min before the test. Morphine (AAH Pharmaceuticals, UK) was dissolved in normal saline, and SSRIs including fluoxetine (Lilly), fluvoxamine (Duphar), sertraline (Pfizer), paroxetine (SmithKline Beecham), citalopram (Lundbeck) and zimelidine (Astra) were all dissolved in Tween 80 (5% Tween 20).

Fig. 2. Antinociceptive activity of pCPA (300 mg/kg) and 5-HTP (100 mg/kg) in the acetic acid abdominal constriction assay. pCPA was administered i.p. 72 h and 5-HTP 30 min before the test. Values are mean±S.E.M. (n>8). *p>0.05 compared with the vehicle control group.

Fig. 3. Effect of pCPA (300 mg/kg) or 5-HTP (100 mg/kg) on morphine-induced antinociception, in the acetic acid abdominal constriction assay. pCPA was administered i.p. 72 h and 5-HTP 30 min before the test (n>8). Morphine (0.5 mg/kg s.c.) was administered with vehicle as a 30 min pre-treatment before acetic acid challenge. Values are mean±S.E.M. (n>8). **p<0.01 compared with the morphine+vehicle group. ***p<0.001 compared with the morphine+vehicle group.
Effect of pCPA (300 mg/kg) or 5-HTP (100 mg/kg) on fluoxetine-induced antinociception in the acetic acid abdominal constriction assay. pCPA was administered i.p. 72 h and 5-HTP 30 min before the test. Fluoxetine (27 mg/kg) was administered s.c. with vehicle as a 30 min pre-treatment before the test. Values are mean±S.E.M. (n>8).

*p<0.05 compared with the fluoxetine+vehicle group.

***p<0.001 compared with the fluoxetine+vehicle group.

Analgesic results were analyzed employing one way analysis of variance (ANOVA) and post hoc Tukey's tests. ED₅₀ values of drugs to induce antinociception and their 95% confidence intervals were determined using linear regression. In all cases p<0.05 was considered as significantly different.

**RESULTS**

**Antinociceptive activity of SSRIs and morphine in the abdominal constriction test**

Citalopram (10-50 mg/kg), fluoxetine (10-50 mg/kg), zimelidine (10-50 mg/kg), fluvoxamine (35-75 mg/kg), paroxetine (20-80 mg/kg), and sertraline (35-90 mg/kg) all produced dose-related inhibition of acetic acid-induced abdominal constriction in mice (Fig. 1). Their log dose-antinociceptive relationships possessed positive linear trends with respective correlation coefficients of 0.98, 0.93, 0.97, 0.99, 0.99, and 0.92. Their ED₅₀ values (with confidence intervals) were 21.74 (14.4-27.3), 27 (19.7-36.1), 38.65 (27-75), 60.46 (54-68), 71 (64.7-80), and 87.7 (81.3-97.3) mg/kg. Morphine also induced antinociception in a similar fashion as SSRIs with an ED₅₀ value of 0.49 (0.35-0.66) mg/kg.

**Evaluation of antinociceptive activities of pCPA and 5-HTP in the abdominal constriction assay**

As seen in Fig. 2, neither pCPA (300 mg/kg, i.p. 72 h pre-treatment), nor 5-HTP (100 mg/kg, i.p. 30 min pre-treatment) produced significant alterations in nociceptive threshold, compared to the vehicle control group. Also, the protection against constrictions displayed in animals receiving pCPA was not significantly different from that
serotonin and ssri-induced antinociception

on the recruitment of intrinsic systems, which mediate their effects by opiate and non-opiate systems. Emphasis has been on the recruitment of intrinsic systems, which mediate their effects by opiate and non-opiate systems. Emphasis has been on the recruitment of intrinsic systems, which mediate their effects by opiate and non-opiate systems. Emphasis has been on the recruitment of intrinsic systems, which mediate their effects by opiate and non-opiate systems. Emphasis has been on the recruitment of intrinsic systems, which mediate their effects by opiate and non-opiate systems. Emphasis has been on the recruitment of intrinsic systems, which mediate their effects by opiate and non-opiate systems. Emphasis has been on the recruitment of intrinsic systems, which mediate their effects by opiate and non-opiate systems. Emphasis has been on the recruitment of intrinsic systems, which mediate their effects by opiate and non-opiate systems. 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been put on the role played by opioids and the monoamines 5-hydroxytryptamine (5-HT), and noradrenaline (NA), in mediating the effects of intrinsic antinociceptive systems.

Reuptake of monoamines is considered to be a major known component of the pharmacology of antidepressants.⁴ Therefore, attempts to explain the direct antinociceptive effect of antidepressants in pharmacological terms center mostly on how these compounds interact with neurotransmitter systems in the brain and spinal cord.¹⁰,¹²

The results obtained in the present study showed that pCPA had no inherent effect on antinociceptive threshold. However, the antinociceptive activities of morphine, fluoxetine, fluvoxamine and sertraline but not paroxetine, citalopram and zimelidine were significantly reduced by pCPA. Subsequently, 5-HTP restored the reduced antinociception of morphine, fluoxetine and fluvoxamine caused by pCPA. It was noteworthy that 5-HTP increased morphine, fluoxetine, fluvoxamine and sertraline-induced antinociception, although the possibility cannot be excluded that this may have been an additive effect. On the other hand, administration of this 5-HT precursor had no effect on paroxetine, citalopram and zimelidine antinociception, which might tend to negate the idea of an additive effect for all SSRIs in the presence of 5-HTP.

Significant involvement of 5-HT in antidepressant activity and antinociception, particularly in the specific serotonin reuptake inhibitor types, has been considered.⁵ This consideration has arisen from various pieces of evidence. Firstly, all SSRIs inhibit serotonin reuptake, thus increasing intra-synaptic 5-HT levels in the CNS,⁴ and most compounds of this type have been shown to elicit antinociceptive activities.⁶ Secondly, the involvement of 5-HT in regulating the transmission of nociceptive information at various levels of the peripheral and central nervous system is undisputed.¹² Moreover, neuronal systems containing serotonin appear not only to be involved in regulation of segmental nociceptive input at a spinal and supraspinal level, but also in mediating the antinociceptive action of morphine and that resulting from electrical stimulation of certain brain stem nuclei.²

Attenuation of fluoxetine, fluvoxamine and sertraline antinociception by pCPA and enhancement of this action by 5-HTP tends to confirm the involvement of 5-HT in SSRI antinociception. However, the current data demonstrates that paroxetine, citalopram and zimelidine antinociception are not modified by these two compounds, suggesting that involvement of 5-HT in SSRI antinociception may not be universal. It should be noted that other neurotransmitters such as acetylcholine, dopamine, and GABA are also involved in antinociception.¹⁶ However, it is not clear to what extent these parameters have interfered in the results of SSRI induced antinociception.

Fluoxetine, fluvoxamine and sertraline-induced antinociception were significantly reduced by naloxone, a general opioid antagonist and also Mr2266BS, a K⁺ receptor preferring antagonist.¹³ In contrast, paroxetine, citalopram and zimelidine antinociception were not modified by these two opioid receptor antagonists. These data demonstrate an opioid-like activity for fluoxetine, fluvoxamine and sertraline.

From the results here, therefore, it could be concluded that 5-HT activity is probably implicated only in visceral antinociception produced by those SSRIs which are naloxone sensitive. In this regard, opioid antinociception has also been established as being serotonin-dependent.¹³ This would be in accordance with the dependency of an opioid-like SSRI involving 5-HT in its analgesic effects.

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involved in antinociceptive activity of antidepressants?


