Chronic administration of imipramine but not agomelatine and moclobemide affects the nitrergic relaxation of rabbit corpus cavernosum smooth muscle

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ARTICLE INFO

Article history:
Received 9 January 2013
Received in revised form 10 June 2013
Accepted 1 July 2013
Available online 9 July 2013

Keywords:
Corpus cavernosum
Imipramine
Agomelatine
Moclobemide
Erectile dysfunction

ABSTRACT

Sexual dysfunction is a common and underestimated effect of antidepressants. However, the mechanism by which these drugs cause erectile dysfunction is unclear. We investigated the reactivity of the corpus cavernosum of rabbits that were treated with either chronic imipramine, which is a tricyclic agent; agomelatine, which is a melatonergic agonist and serotonin 5HT2c antagonist; or moclobemide, which is a reversible inhibitor of monoamine-oxidase A. Twenty rabbits were randomly divided into four groups: the control group (n=5), the imipramine-treated group (n=5), which received i.p. injections of 30 mg/kg/day of imipramine, the moclobemide-treated group (n=5), which received i.p. injections of 20 mg/kg/day of moclobemide, and the agomelatine-treated group (n=5), which was orally administered 10 mg/kg/day of agomelatine. The reactivities of corpus cavernosum tissue obtained from the antidepressant-treated and the control groups were studied in organ chambers after the animals were subjected to 21 days of drug administration. The acetylcholine-induced endothelium-dependent and the electrical field stimulation (EFS)-induced neurogenic relaxation of the corpus cavernosum of the imipramine-treated group was significantly decreased compared with the control group. However, neither the acetylcholine- nor EFS-induced relaxation was changed in the moclobemide- or agomelatine-treated groups. There were no change in the relaxant response to the nitric oxide (NO) donor sodium nitroprusside and contractile response to KCl between the groups. This study suggests that chronic imipramine treatment but not agomelatine and moclobemide treatments causes significant functional changes in the penile erectile tissue of rabbits and that these changes may contribute to the development of impotence.

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1. Introduction

The nitric oxide (NO)-cyclic GMP cascade is an important signalling pathway involved in the regulation of erectile function (Ignarro et al., 1990; Andersson, 2001; Kim et al., 1991). Experimental and clinical evidence supports the possible role of impaired NO functioning in erectile dysfunction (Saenz de Tejada et al., 1989; Kim et al., 1995; Gocmez et al., 2005; Utkan et al., 2002; Ozdemirci et al., 2001; Yazir et al., 2012).

Most of the currently available antidepressants induce sexual dysfunction in humans (Montejo et al., 2001; Clayton et al., 2002, 2007; Delgado et al., 2005; Kennedy et al., 2006). The incidence of sexual dysfunction is strongly dependent on the antidepressants used, such as selective serotonin reuptake inhibitors (SSRIs), monoamine oxidase inhibitors, and tricyclic agents (Montejo et al., 1999; Clayton et al., 2002; Clayton and Montejo, 2006). We recently reported that chronic fluoxetine, which is an SSRI antidepressant, and venlafaxine, which is an SNRI antidepressant, affect the contractile responses of rat vas deferens smooth muscle and that this impaired motility can lead to ejaculatory dysfunction (Gocmez et al., 2010). In addition to the vas deferens, fluoxetine inhibited the NO-mediated relaxant responses in corpus cavernosum smooth muscle, which suggests this impairment leads to erectile dysfunction (Gocmez et al., 2011). These findings are consistent with a study that found that treatment with both acute and chronic paroxetine, which is an SSRI antidepressant, inhibits erectile responses due to reduced NO production and neuronal NO synthase (nNOS) expression in rats (Angulo et al., 2001). Furthermore, it has been reported that the in vivo administration of fluoxetine reduces the erectile responses of rats to cavernous stimulation, likely due to interference with the NO production (Jun et al., 2005). Additionally, clinical studies have shown a high incidence of sexual dysfunction with
imipramine treatment (Hsu and Shen, 1995). Similarly to SSRIs, imipramine was found to inhibit NO synthesis in the brain, which suggests the involvement of the NO mechanism in the antidepressant effect of imipramine (Crespi, 2010; Krass et al., 2011).

In contrast to serotonergic antidepressants, drugs that predominantly increase noradrenaline or dopamine uptake and serotonin 5-HT2 receptor blockers appear to have few negative effects on sexual functioning (Montejo et al., 1999; Clayton et al., 2002). Agomelatine is an antidepressant with melatonergic agonist and serotonin 5-HT2C antagonist properties (Olie and Kasper, 2007) that has exhibited an efficacy that is similar to those of antidepressants used for the treatment of patients with major depressive disorder (Kennedy and Emsley, 2006; Olie and Kasper, 2007). In addition, a substantially lower risk of sexual dysfunction was found in patients subjected to agomelatine treatment compared with venlafaxine treatment (Kennedy et al., 2008). Moclobemide, which is a reversible inhibitor of monoamine oxidase A, has also been extensively evaluated in the treatment of depressive disorders, and it was found that moclobemide treatment resulted in sexual dysfunction much less frequently compared with treatment with SSRIs (Philipp et al., 2000).

Therefore, the aim of the present study was to investigate the effects of 21-day chronic imipramine, moclobemide, and agomelatine treatments on the nitricergic-mediated relaxant responses of the rabbit corpus cavernosum.

2. Materials and methods

2.1. Treatment schedule

The experiments were performed on mature male albino white rabbits (2.5–3 kg) obtained from the Experimental Medical Research and Application Centre (DETAB, Kocaeli University, Kocaeli, Turkey). The experiments reported in this study were performed in accordance with the Regulation of Animal Research Ethics Committee of Turkey (6 July 2006, Number 26220). Ethical approval was granted by the Kocaeli University Animal Research Ethics Committee (Kocaeli, Turkey). The control group (n = 5) received saline through intraperitoneal (i.p.) injections, and the agomelatine-treated group (n = 5) was orally administered 10 mg/kg/day of agomelatine. In addition, the imipramine-treated group (n = 5) received i.p. injections of 10 mg/kg/day of imipramine, and the moclobemide-treated group (n = 5) received i.p. injections of 20 mg/kg/day of moclobemide. After 21 days of drug administration, the following studies were conducted.

2.2. Strip preparation and organ bath studies

After the rabbits were sacrificed with an overdose of pentobarbital, their penises were excised, and the corpora cavernosa erectile tissues were harvested, as previously described (Yildirim et al., 1997). Briefly, the penis was dissected and removed from its crural attachments to the puboischial bones. The specimen was immediately placed in Krebs bicarbonate buffer composed of 118 mM NaCl, 4.7 mM KCl, 2.5 mM CaCl2, 25 mM NaHCO3, 1.2 mM MgSO4, 1.2 mM KH2PO4, and 11 mM glucose. The tunica albuginea was cleared of any overlying tissue and opened. The proximal half of the corporal body was dissected from the tunica and harvested en bloc, as previously described (Utkan et al., 1999, 2002, 2010). Each corporal body was cut to obtain two longitudinal strips. Each strip, which measured approximately 2 x 2 x 15 mm3, was mounted on a 20 ml organ bath containing Krebs bicarbonate buffer equilibrated with a gas mixture of 95% O2 and 5% CO2 and maintained at 37 °C for isometric tension measurements. The tissue strips were connected to a force-displacement transducer (May–Com, FDT 10-A, COMMAT Iletisim Co., Ankara, Turkey). The strips were stretched to a resting tension of 2 g (this value was previously found to be optimal for the measurement of changes in the tension of rabbit corpus cavernosal tissue preparations; Utkan et al., 1999, 2002) and allowed to equilibrate for 60 min. Any changes in the isometric force were recorded using a four-channel transducer data acquisition system (TDA 94, COMMAT Iletisim Co., Ankara, Turkey). After equilibration, the contractile responses to phenylephrine (10–9–10–4 M) were obtained cumulatively. Following completion of phenylephrine dose-response curve, tissues were washed for a further 30 min, the strips were contracted with phenylephrine (10–5 M; it was previously found that this concentration induces 70–80% of the maximal response to phenylephrine). Acetylcholine (3 x 10–9–10–4 M) or sodium nitroprusside (10–9–10–4 M) was then added to the bath in a cumulative manner. The drugs were dissolved such that each concentration was added in a total volume of 100 μl to the chamber. The transmural electrical field stimulations (EFSs) were studied after the phenylephrine-induced contraction had reached a plateau.

The EFS was provided by a stimulator (ST 95 P, COMMAT Iletisim Co., Ankara, Turkey) and applied through two platinum wire electrodes that were set vertically within the organ bath at opposite sides of the suspended tissue. Prior to the electrical stimulation, the tissue was treated with 5 μM guanethidine, which is an adrenergic nerve blocker, and 1 μM atropine, which is a muscarinic receptor blocker, for 30 min. Square-wave pulses of 10 V with a 0.5 ms duration in 10 s trains with various frequencies (2–32 Hz) were applied at 5 min intervals. The strips were allowed to return to baseline precontractile tension at each frequency.

2.3. Analysis of data and statistics

The experimental results are expressed as the mean ± the standard error of the mean (S.E.M.). The relaxant effects of the agonists are expressed as a percentage of the precontraction response to phenylephrine. To evaluate the effects of the agonists, the maximum responses (Em) and pD2 values (i.e., the negative logarithm of the concentration required to obtain the half-maximal response: –log EC50) were calculated. The agonist pD2 values were calculated from the linear portion of the agonist dose-response curves and are considered a measure of the sensitivity of the tissues to the agonist.

Statistical comparisons between the groups were performed using ANOVA, followed by a pos hoc comparison of the means with Tukey’s test. Differences with a probability of less than 5% (p < 0.05) were considered statistically significant.

2.4. Drugs

The following drugs were obtained from Sigma Chemical Co. (St. Louis, MO, USA): acetylcholine chloride, phenylephrine hydrochloride, sodium nitroprusside, imipramine, atropine sulphate, and guanethidine sulphate. Moclobemide was a gift from Roche (Turkey). Agomelatine (Valdoxan) was obtained from Servier in Turkey. All of the drugs except for agomelatine were dissolved in distilled water and were freshly prepared on the day of the experiment. Agomelatine was suspended in distilled water.

3. Results

There was no significant difference in the cumulative phenylephrine concentration–response curves (10–9–10–4 M; Fig. 1). The contractions elicited by 124 mM KCl in three groups were similar (Table 1). Acetylcholine (3 x 10–9–10–4 M; Figs. 2 and 3) and sodium nitroprusside (10–9–10–4 M; Figs. 4 and 5) produced...
induced by 124 mM KCl and is given as the mean ± standard error of the mean (S.E.M.). Number of rabbits in each group is shown in parentheses.

Values are arithmetic means ± S.E.M., n refers to the number of preparations used.

Table 1

<table>
<thead>
<tr>
<th>Phenylephrine</th>
<th>Control (n=5)</th>
<th>Imipramine (n=5)</th>
<th>Moclobemide (n=5)</th>
<th>Agomelatine (n=5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>E_{max} (mg)</td>
<td>54.4 ± 9.0</td>
<td>26.4 ± 4.0*</td>
<td>30.2 ± 2.6</td>
<td>42.0 ± 9.2</td>
</tr>
<tr>
<td>pD2</td>
<td>6.22 ± 0.05</td>
<td>5.70 ± 0.06*</td>
<td>6.52 ± 0.05</td>
<td>6.15 ± 0.07</td>
</tr>
<tr>
<td>SNP E_{max}</td>
<td>75.3 ± 4.9</td>
<td>81.4 ± 3.7</td>
<td>82.2 ± 3.0</td>
<td>84.0 ± 5.0</td>
</tr>
<tr>
<td>pD2</td>
<td>5.36 ± 0.08</td>
<td>5.60 ± 0.06</td>
<td>5.46 ± 0.07</td>
<td>5.49 ± 0.05</td>
</tr>
<tr>
<td>EFS KCl E_{max}</td>
<td>64.8 ± 10.9</td>
<td>21.4 ± 6.6*</td>
<td>62.9 ± 2.5</td>
<td>60.0 ± 3.0</td>
</tr>
<tr>
<td>E_{max}</td>
<td>3119.8 ± 412.7</td>
<td>3271.4 ± 307.97</td>
<td>2712.5 ± 370.9</td>
<td>2852.0 ± 140.1</td>
</tr>
</tbody>
</table>

Values are arithmetic means ± S.E.M., n = the number of preparations used.

*p < 0.05, statistically different from the response of strips from control rabbits.

![Fig. 1. Phenytoin concentration–response curves in isolated rabbit corpus cavernosum strips.](image1)

![Fig. 2. The relaxation responses of isolated rabbit corpus cavernosum tissue precontracted with 10^{-5} M phenylephrine in response to acetylcholine (10^{-8}–10^{-4} M (A, B, C, D)) in control (A), imipramine- (B), mocllobemide - (C) and agomelatine-treated (D) rabbits.](image2)

![Fig. 3. Acetylcholine concentration–response curves in isolated rabbit corpus cavernosum strips precontracted with 10^{-5} M phenylephrine. Imipramine but not other drugs attenuated acetylcholine-induced relaxation. Each point represents the mean ± the standard error of the mean (S.E.M.) and indicates a percentage of the contraction induced by phenylephrine. The numbers in parentheses indicate the number of preparations used. *p < 0.05 compared with the response obtained from tissue strips from control rabbits.](image3)
In contrast to imipramine, agomelatine and moclobemide did not affect either the acetylcholine- or the EFS-induced relaxant responses. Similarly, the sodium nitroprusside-induced relaxation did not differ between these groups (Figs. 3, 5, and 7, Table 1). The tensions induced by the contraction of the tissues with phenylephrine (10^{-5} M) to assess the responses to relaxation-inducing agonists in all of the groups were similar. These tensions were 2540.8 ± 90.9 mg, 2597.6 ± 133.2 mg, 2387.8 ± 353.9 mg, and 2639.0 ± 148.6 mg (mean ± S.E.M) in the imipramine-treated, the moclobemide-treated and the control groups, respectively.

4. Discussion

This study demonstrated that chronic imipramine treatment but not agomelatine or moclobemide treatment impairs both neurogenic and endothelium-dependent NO-mediated relaxant responses in the rabbit corpus cavernosum.

In this study, transmural electrical stimulation produced frequency-dependent relaxation in isolated rabbit corpus cavernosum pretreated with guanethidine and atropine. According to previous studies, the stimulation-induced relaxation of the rabbit corpus cavernosum is abolished by pretreatment with 10^{-6} M tetrodotoxin, and the response is thus thought to result from nerve stimulation (Bagcivan et al., 2003; Bozkurt et al., 2007). In addition, we and others have confirmed the nitricergic nature of both the EFS-induced and the endothelium-dependent responses by studying the influence of NOS inhibitors (Ignarro et al., 1990; Utkan et al., 1999; Yildirim et al., 1999; Bagcivan et al., 2003; Bozkurt et al., 2007 Gocmez et al., 2011). The relaxation of the trabecular smooth muscle is necessary to achieve and maintain penile erection (Saenz de Tejada et al., 1991). Moreover, previous in vitro studies have indicated that NO-mediated corporal smooth muscle relaxation may be impaired in a large proportion of impotent men (Saenz de Tejada et al., 1989; Kim et al., 1995) and in experimental animal models of erectile dysfunction (Utkan et al., 1999, 2002, 2010; Ozdemirci et al., 2001; Gocmez et al., 2005). In particular, in vitro studies have shown that the chronic administration of paroxetine, which is an SSRI antidepressant, impairs erectile responses in rats (Angulo et al., 2001). This finding was recently supported by our observation that chronic fluoxetine treatment resulted in decreased EFS-induced neurogenic and endothelium-dependent relaxation of smooth muscle in response to acetylcholine (Gocmez et al., 2011). It is well known that paroxetine inhibits NO production (Finkel et al., 1996) and that fluoxetine reduces the amount of NO released into the media by synovial cells (Yaron et al., 1999). Therefore, it has been speculated that an impairment of the NO production may be responsible for the erectile dysfunction observed in response to antidepressant treatment. We consistently found impaired endothelial dependent and neurogenic relaxations in imipramine-treated corporal smooth muscle. These data suggest a possible common pathophysiological mechanism through which imipramine treatment or alteration of the NO/cGMP pathway and other pathways may impair the relaxation of trabecular smooth muscle or diminish...
its sensitivity to NO. However, these possibilities are unlikely because the corporal strips relaxed in response to sodium nitroprusside, which is metabolised by smooth muscle into NO. The normal responses to sodium nitroprusside in the imipramine-treated rabbits indicate a normal cGMP-dependent relaxation of corporal smooth muscle. Moreover, the tensions that developed in response to the concentrations of phenylephrine used were similar for the trabecular strips obtained from both groups, which ensures that any difference in the relaxations between the imipramine-treated and the control preparations was not due to differences in the degree of precontraction. In addition, there were no differences in the KCl-induced contractile responses between the two groups. Thus, the contractile and relaxant mechanisms in the cavernosal smooth muscle were intact. Therefore, we can speculate that chronic imipramine treatment impairs the synthesis or availability of nitric oxide in corpus cavernosum tissue. This hypothesis is in agreement with previous studies that described experimental data that demonstrate the suppression of NO synthesis in imipramine-treated rat brains (Kraus et al., 2011). However, moclobemide and agomelatine did not affect either endothelium-dependent or neurogenic relaxant responses of corpus cavernosum smooth muscle. We can therefore speculate that moclobemide and agomelatine induce less erectile dysfunction than imipramine. Additionally, we did not use a washout period, therefore, the effect we have observed could be due to direct effect of the drugs that remained in the tissue. This point is a limitation our study. However, in previous studies when two weeks paroxetine and citalopram or three weeks fluoxetine administration inhibited erectile responses without withdrawn in rats (Angulo et al., 2001; Gocmez et al., 2011). Csoka et al. (2008) also reported that SSRIs can cause long-term effects on all aspects of the sexual response cycle that may persist after they are discontinued. Further studies are needed to explain if the in vitro observed effect is due to structural-functional changes induced by antidepressant treatment.

In addition to NO, it is well established that various complex hormonal and neurochemical changes in the central and peripheral nervous systems occur during antidepressant usage. It is generally believed that serotonin is an inhibitory neurotransmitter involved in the sexual behaviour of male rats (Ahlenius et al., 1980). Agomelatine, which is a naphthalene analogue of melatonin, is a newly developed selective agonist of melatonin MT1 and MT2 receptors and shows serotonin 5-HT2 receptor antagonist activity (Olie and Kasper, 2007). Furthermore, in vivo data indicate that agomelatine enhances the levels of dopamine and noradrenaline in the brain (this effect is likely secondary to the blockade of serotonin 5-HT2C receptors) but does not exhibit significant affinity for the muscarinic, adrenergic, noradrenergic, and serotonin 5-HT1A receptors (Papp et al., 2003; Dubocovich, 2006). It has been proposed that agomelatine treatment induces a low level of sexual dysfunctions that may be related to its serotonin 5-HT2C antagonist action (Montejo et al., 2010). This hypothesis is

Fig. 6. The relaxation responses of isolated rabbit corpus cavernosum tissue precontracted with 10^{-5} M phenylephrine in response to electrical field stimulation (EFS) (2–32 Hz (A, B, C, D)) in control (A), imipramine- (B), moclobemide- (C) and agomelatine-treated (D) rabbits.

Fig. 7. The relaxation responses evoked by electrical field stimulation (EFS) of isolated rabbit corpus cavernosum strips precontracted with 10^{-5} M phenylephrine. Imipramine, but not other drugs attenuated EFS-induced relaxation. Each point represents the mean ± the standard error of the mean (S.E.M.) and indicates a percentage of the contraction induced by phenylephrine. The numbers in parentheses indicate the number of preparations used. *p < 0.05 compared with the response obtained from tissue strips from control rabbits.
consistent with a report that serotonin 5-HT2c receptor blockers, such as mirtazapine, exhibit few negative effects on sexual function (Waldinger et al., 2003).

5. Conclusions

Taken together, our results support the hypothesis that alterations in the neurogenic and/or endothelium-mediated relaxation of corporal smooth muscle in rats through the use of chronic imipramine but not amoxapine or moclobemide, treatment may, at least in part, contribute to the development of erectile dysfunction. The development of this animal model will facilitate further investigation of the alterations of penile smooth muscle reactivity caused by chronic imipramine, agomelatine, and moclobemide treatments.

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References