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Spinal 5-HT_2 receptor-mediated facilitation of pudendal nerve reflexes in the anaesthetized cat

*,†Hansjorg Danuser & [1],*Karl B. Thor

*Division of CNS Research, Lilly Research Laboratories and Dept. of Urology, Indiana University School of Medicine, Indianapolis, IN, U.S.A. and †Dept. of Urology, Bern University, Bern, Switzerland

1 5-Hydroxytryptamine (5-HT) is intimately associated with central sympathetic and somatic control of the lower urinary tract. The sympathetic and somatic innervation of the lower urinary tract is conveyed through efferent axons of the hypogastric and pudendal nerves, respectively.

2 The present study examined the effects of 2,5-dimethoxy-4-iodophenylisopropylamine (DOI), a 5-HT_2 receptor subtype-selective agonist, on evoked potentials recorded from the central ends of the hypogastric and pudendal nerves in response to electrical stimulation of afferent fibres in the pelvic and pudendal nerves, respectively. Various spinalization paradigms were employed to localize the site of action. All cats were pretreated with xylamidine (1 mg kg^{-1}), a peripherally-restricted 5-HT_2 receptor antagonist.

3 In acute spinal cats, DOI (0.01–3 mg kg^{-1}, i.v.) reliably produced dose-dependent increases in the pudendal nerve reflex (to 228±31% of control). These increases were reversed by the 5-HT_2 receptor-selective antagonist, LY53857 (0.3–3 mg kg^{-1}, i.v.). On the other hand, in spinally-intact cats, DOI produced no significant changes in the pudendal reflex. However, within minutes of spinalization of DOI-pretreated cats, a marked increase (to 221±16% of control) in the pudendal reflex was observed which could be reversed by LY53857. No significant effects were observed on hypogastric reflexes in either acute spinal or spinally-intact cats following DOI administration. No effects were seen in either spinally-intact or acute spinal animals when LY53857 was administered as the initial drug.

4 These results indicate that activation of spinal 5-HT_2 receptors facilitates pudendal reflexes. In spinally-intact cats, it is hypothesized that DOI activates supraspinal pathways that mediate inhibition of the pudendal reflexes and counteracts the facilitatory effects of spinal 5-HT_2 receptor activation.

Keywords: 5-HT receptors; pudendal nerve; hypogastric nerve; sympathetic nervous system; motor neurones; external urethral sphincter; spinal cord; LY53857; DOI; urinary bladder

Introduction

Urine storage and micturition are complex physiological functions that require coordination between all three divisions of the efferent peripheral nervous system (*i.e.* parasympathetic, sympathetic, and somatic) to control the bladder smooth muscle and the smooth and striated muscle of the urethra. Micturition is mediated by sacral parasympathetic activation of bladder smooth muscle *via* axons contained in the pelvic nerve, while urine storage is maintained by somatic and sympathetic activation of the striated and smooth muscle of the external and internal urethral sphincters, respectively. The present study focuses on somatic and sympathetic outflow to the lower urinary tract.

Somatic and sympathetic innervation of the lower urinary tract arises from somatic motor neurones in Onuf's nucleus (Ueyama *et al.*, 1984; Thor *et al.*, 1989b) and sympathetic preganglionic neurones in the intermediolateral cell column (Morgan *et al.*, 1986), respectively. The somatic and sympathetic innervation is conveyed from the spinal cord to the lower urinary tract *via* the pudendal and hypogastric nerves, respectively. Various studies have shown that these somatic and sympathetic pathways to the lower urinary tract are intimately associated with central 5-hydroxytryptamine (5-HT) pathways.

Immunohistochemical studies have identified a dense plexus of 5-HT-immunoreactive terminals surrounding neurones in both Onuf's nucleus and the intermediolateral cell column (Mizukawa, 1980; Kojima *et al.*, 1982; 1983; Hosoya *et al.*, 1991; Rajaofetra *et al.*, 1992). Autoradiography studies demonstrated that various 5-HT receptor subtypes are intimately associated with these two groups of neurones (Thor *et al.*, 1993).

Previous pharmacological studies have shown that the non-selective 5-HT receptor agonist, 5-methoxy-N,N-dimethyltryptamine (5MeODMT) produces an increase in external urethral sphincter electromyographic (EMG) activity in both spinally-intact and chronic spinal cats (Thor *et al.*, 1990). Furthermore, the 5-HT and noradrenaline re-uptake inhibitor, duloxetine, has been shown to produce increases in external urethral sphincter EMG activity that were reversed by the non-selective 5-HT receptor antagonist, methiothepin and by the 5-HT_2 receptor subtype selective antagonist, 4-isopropyl-7-methyl-9-(2-hydroxy-1-methylpropoxycarbonyl)-4,6,6A,7,8,9,10,10A-octahydroindolo-[4,3-fg] quinoline maleate, (LY53857; Thor & Katofiasc, 1995). An involvement of 5-hydroxytryptaminergic systems in regulation of sympathetic neural activity is also well-established (deGroat & Ryall, 1967; McCall, 1984; Lewis & Coote, 1990; Helke *et al.*, 1991), but the specific receptor subtypes mediating various effects are not well-defined.

The present study was designed to examine the effects of a selective 5-HT_2 receptor agonist, 2,5-dimethoxy-4-iodophenylisopropylamine (DOI; Dabire *et al.*, 1989; Hoyer *et al.*, 1994), and the 5-HT_2-selective antagonist, LY53857 (Cohen *et al.*, 1983), on electrically-evoked reflex potentials recorded from the pudendal and hypogastric nerves in acute spinal and spinally-intact cats. All animals were pretreated with xylamidine, a peripherally-restricted 5HT_2 receptor antagonist (Mawson & Whittington, 1970; Fuller *et al.*, 1986), to prevent

[1] Author for correspondence.

indirect effects (bronchoconstriction, bladder smooth muscle contraction, etc.; Ramage *et al.*, 1993) from influencing the central reflexes.

Methods

General preparation

Three male and 31 female cats (2.5–3.5 kg) were anesthetized with α-chloralose (70 mg kg^{-1}, i.v.) following induction with isoflurane 5% and surgically-prepared for recording of mean arterial pressure (MAP), heart rate, bladder pressure, body temperature, and expired CO_2 levels. The ureters were cannulated and drained externally to prevent bladder filling during the experiment. Bladder pressure was measured to verify that drug administration did not produce direct effects on the bladder smooth muscle that might indirectly influence the sympathetic or somatic activity. Body temperature and CO_2 levels were monitored to be certain that they remained within physiological levels.

Electrophysiology

Detailed methods of nerve stimulation and recording can be found in previous publications (deGroat & Lalley, 1972; Rampal & Mignard, 1975a,b; Downie & Bialik, 1988; Thor *et al.*, 1989a; Downie *et al.*, 1991; Danuser & Thor, 1995a,b). Briefly, in 9 cats, the pelvic and hypogastric nerves were prepared for electrical stimulation and recording following a laparotomy and removal of the intestines. In 25 cats, the ipsilateral and contralateral pudendal nerves were isolated and prepared for electrical stimulation and recording in the ischiorectal fossa. The pelvic or pudendal nerve was electrically stimulated with square-wave pulses of 0.05 ms duration, 0.5–10 V, 0.5 Hz. Although complete intensity-response curves were examined, data used in construction of the dose-response curves were collected at an empirically-determined working voltage (which remained constant throughout the course of an individual experiment and was *ca.* 50–80% of the voltage used to evoke the maximal response) at a frequency of 0.5 Hz. This is the highest frequency of stimulation that the evoked reflexes can follow with no 'run-down' and which is commonly used (deGroat & Lalley, 1972; Rampal & Mignard, 1975a,b; Downie & Bialik, 1988; Thor *et al.*, 1989a; Downie *et al.*, 1991; Danuser & Thor, 1995a,b). Evoked potentials were signal averaged for 10–20 consecutive potentials, recorded, and stored at 1 min intervals. All evoked potentials were visually examined for onset of drug effects, time to maximal drug effects, and to ensure stable responses during both control periods and following drug administration. The 3 evoked potentials occurring at 14, 15 and 16 min post-drug administration were quantified by measuring the area under the potential beginning at the time of stimulus application and concluding at 40 ms post-stimulus for the pudendal-to-pudendal reflex and concluding at 240 ms for the pelvic-to-hypogastric reflex (i.e. after evoked potentials had returned to their respective baselines).

Spinally-intact cats In 4 cats (pelvic-to-hypogastric reflex preparation) and 10 cats (pudendal-to-pudendal reflex preparation), after establishing control evoked response amplitude, saline vehicle was administered and evoked potentials averaged for 15 min. The area under the evoked potential was measured and expressed as a percentage of the control response. Xylamadine tosylate, a peripherally-restricted 5HT$_2$ receptor antagonist (Mawson & Whittington, 1970; Fuller *et al.*, 1986), was then administered, and after 15 min the area under the evoked potentials was again measured. Increasing cumulative doses of DOI were then administered with about 15 min elapsing between each dose. Fifteen min after the final dose of DOI, LY53857 was administered intravenously. In 3 cats, LY53857 administration preceded DOI administration by 15 min.

Spinalization paradigms In 5 cats (pelvic-to-hypogastric reflex preparation) and 10 cats (pudendal-to-pudendal reflex preparation), the spinal cords were transected at the T 13 level during surgical preparation. Approximately 2 h elapsed between the time of transection and recording of control evoked potentials. Then saline, xylamidine, DOI and LY53857 were administered as described above. In 3 cats, LY53857 was administered prior to DOI.

In 9 cats (pudendal-to-pudendal reflex preparation) a T 13 laminectomy was performed at the time of surgical preparation, but the cord was left intact until after control reflexes were recorded (3 cats) or until after DOI effects on the reflex were recorded (3 cats), after which time the cord was transected, and the effects of the transection on the evoked potentials recorded.

Analysis

Differences in the areas of the evoked potentials between vehicle administration, administration of DOI, or administration of LY53857 were expressed as a percentage of the pre-vehicle control (mean ± s.e.mean). One-way ANOVA and Dunnet's test were used to compare mean responses after vehicle administration with mean responses after DOI administration, which were subsequently compared with mean responses after LY53857 administration. Separate analysis were done for acute spinal animals and spinally-intact animals. Differences were considered significant if $P \leqslant 0.05$.

Drugs

DOI, (2,5-dimethoxy-4-iodophenylisopropylamine, RBI, Natick, MA, U.S.A.), was dissolved in saline. LY53857 (4-isopropyl-7-methyl-9-(2-hydroxy-1-methylpropoxycarbonyl)-4,6,6A,7,8,9,10,10A-octahydroindolo-[4,3-fg] quinoline maleate, synthesized at Lilly Research Labs, Indianapolis, IN, U.S.A. but available from RBI, Natick, MA, U.S.A.) was dissolved in saline with gentle warming. Xylamidine tosylate was a gift from Burroughs-Wellcome (Beckenham, Kent, England).

Results

Electrical stimulation of the contralateral pudendal nerve resulted in a large amplitude evoked reflex potential recorded from the ipsilateral pudendal nerve at a latency of 10 ms in either spinally-intact (Figure 1b) or acute spinal cats (Figure 1a). These reflexes remain stable for 8 h or longer (deGroat & Lalley, 1972; Rampal & Mignard, 1975a,b; Downie & Bialik, 1988; Thor *et al.*, 1989a; Downie *et al.*, 1991; Danuser & Thor, 1995a,b). Prior to drug administration, averaged reflex recordings (10–20 sweeps/average) were made at 1 min intervals until the amplitude of the reflexes remained stable for a period of 10 min (approximately 30 min were required from the initial recordings for fluctuations in baseline to stabilize). The records taken during this 10 min period provided the baseline control response. Administration of saline vehicle produced no changes in the baseline values recorded over the subsequent 5 min period (Figure 2, $n=25$). Administration of xylamidine (1 mg kg^{-1}, i.v., $n=25$) likewise produced no effect on the pudendal-to-pudendal reflex (spinal cord intact cat 95 ± 3% of control, $n=18$, and spinal cat 98 ± 2% of control, $n=7$).

Administration of DOI produced no effect on the pudendal-to-pudendal reflex in the spinally-intact cats (Figures 1b and 2, $n=6$) but did produce dose-dependent (0.03–3 mg kg^{-1}) increases in the magnitude of the reflex in acute spinal cats (Figures 1a and 2, $n=7$). LY53857 antagonized the facilitatory effects of DOI in acute spinal cats (Figures 1a and 2, $n=7$). The increases in the reflex in acute spinal cats occurred within 2 min of DOI administration, and maximal effects were noted

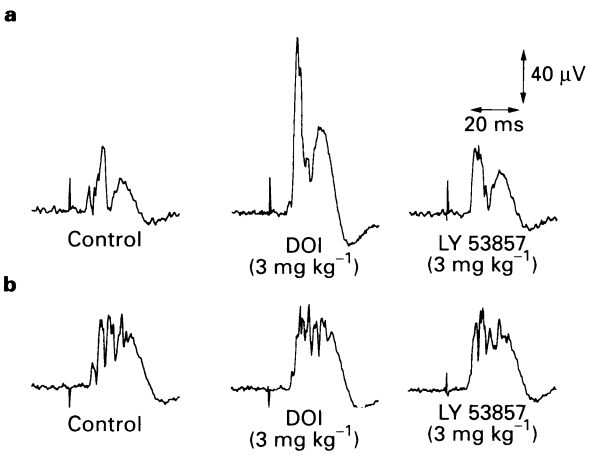

Figure 1 Examples of the effect of DOI and LY53857 on computer averaged (10 sweeps each) evoked potentials recorded from the pudendal nerve in response to electrical stimulation of the contralateral pudendal nerve in (a) an acute spinal cat and (b) a spinally-intact cat. All records in (a) are from one acute spinal cat with the same stimulation parameters in all records showing evoked reflex during control period (left record), after 3 mg kg^{-1} duloxetine (centre record), and after 3 mg kg^{-1} i.v. LY53857 (right record). Similarly, all records in (b) are from one spinally-intact cat in which the same stimulation parameters were used in all records showing evoked reflex during control period (left record), after 3 mg kg^{-1} duloxetine (centre record), and after 3 mg kg^{-1} i.v. LY53857 (right record). Notice in acute spinal cats that DOI administration markedly potentiates the amplitude of the evoked reflex (indicating recruitment of pudendal motor neurones) and that subsequent LY53857 administration reverses the potentiation. Also note that neither DOI administration, nor the subsequent administration of LY53857, had any effect on the evoked reflex in cats with an intact spinal cord.

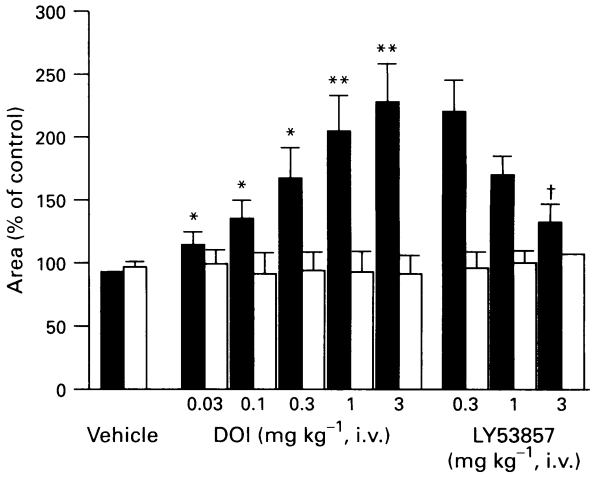

Figure 2 Cumulative dose-response graph for effects of DOI and LY53857 on the pudendal nerve reflex in acute spinal cats ($n=7$, solid columns) and spinally-intact cats ($n=9$, open columns). Histograms represent mean value (±s.e.mean). *Significantly different from control ($P\leqslant0.05$); **significantly different from control ($P\leqslant0.01$); †significantly different from the 3 mg kg^{-1} dose of DOI ($P\leqslant0.05$).

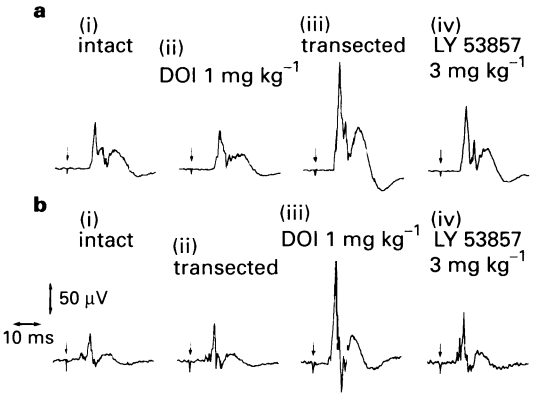

Figure 3 Examples of effects of DOI on computer averaged evoked pudendal reflexes when administered prior to (a(ii)) or following (b(iii)) spinal cord transection. All panels ((i)–(iv)) in (a) and in (b) are each from individual animals that received a laminectomy prior to recording (i.e. all four records in (a) are from one cat and all four records in (b) are from one cat). The laminectomy allowed access to the spinal cord for transection during the recording session (i.e. between panels a(ii) and a(iii) and between panels b(i) and b(ii)), but the spinal cord remained intact during the initial recording period (panels a(i) and b(i)). (a) Panel (i) shows the reflex under control conditions. In panel (ii) the spinal cord (SC) remained intact, and DOI administration produced no effect. Between records in panels (ii) and (iii) the spinal cord was transected; note the marked facilitation of the reflex subsequent to DOI administration and spinalization (a(iii)). Panel (iv) shows that this facilitation was antagonized by LY53857. (b) Panel (i) shows the reflex under control conditions. Between records in panel (i) and panel (ii) the spinal cord was transected. Note that spinalization *per se* did not change the magnitude of the reflex (b(ii)). Panel (iii) shows that subsequent to spinalization and DOI administration that the reflex is markedly facilitated. Panel (iv) shows that this facilitation was antagonized by LY53857.

within 5 min. Although not quantified for all experiments, qualitatively DOI reduced the threshold for activation of the reflex and produced increases in the magnitude of the reflex across all voltages.

In experiments in which a single, large dose of DOI (1 mg kg^{-1}, i.v.) was administered to acute spinal cats ($n=6$), increases in reflex amplitude (to 275±17% of control) were significantly greater ($P\leqslant0.05$) than the increases following a dose of 1 mg kg^{-1} administered after smaller incremental doses during construction of cumulative dose-response curves (205±29% of control), indicating some tachyphylaxis may have occurred during cumulative dosing. Facilitation of the pudendal reflex by the single dose of 1 mg kg^{-1}, however, remained stable for as long as 2 h, at which time LY53857 (1 mg kg^{-1}, i.v.) was administered and reduced the reflex nearly to control values (135±10% of control) within 4 min of its administration ($n=6$). Administration of the antagonist, LY53857 (0.1 to 3.0 mg kg^{-1}, i.v., $n=3$), as the initial drug to acute spinal cats (*i.e.* prior to DOI administration) produced no effects on the reflex itself, but it did block the facilitatory effect of a subsequent 1 mg kg^{-1} dose of DOI. Furthermore, administration of LY53857 (0.1 to 3.0 mg kg^{-1}, i.v., $n=3$) as the initial drug did not have any effects on the reflex in the spinally-intact cats (data not shown).

As shown in Figure 3a (panels (i) and (ii)), in spinally-intact cats, DOI (1 mg kg^{-1}, i.v.) produced no significant effect on the pudendal reflex. However, following administration of DOI with the spinal cord intact, subsequent transection of the spinal cord facilitated the pudendal reflex (221±16% of control, Figure 3a, panel (iii)) within 2 min of spinalization ($n=3$). This facilitation remained stable for at least 30 min, at which time LY53857 was administered ($n=3$), and the reflex amplitude diminished within 4 min of antagonist administration (Figure 3a, panel (iv)). Spinal transection *per se* (*i.e.* without prior administration of DOI) produced no facilitation of the pudendal reflex (94±3% of control, $n=3$) at any time following transection (Figure 3b, panels (i) and (ii)). However, following spinal cord transection, subsequent administration of DOI (Figure 3b, panel (iii)) produced facilitatory responses that were reversed by LY53857 (Figure 3b, panel (iv)), similar

to responses seen in animals that had their spinal cords transected during surgical preparation, i.e. prior to recording (*e.g.* Figure 2).

As previously described (de Groat & Lalley, 1975; Krier *et al.*, 1979; Danuser & Thor, 1995a,b), electrical stimulation of the pelvic nerve produced an evoked reflex potential recorded from the hypogastric nerve at a latency of 60 ms. DOI, administered as a single large bolus of 1 mg kg^{-1}, i.v. or incrementally in the construction of dose-response curve (up to 3 mg kg^{-1}) produced no significant effects on the pelvic-to-hypogastric reflex in either spinally intact (85±10% of control, $n=4$) or acute spinal cats (97±3% of control at 3 mg kg^{-1}, i.v., $n=5$, data not shown).

Although not analyzed in depth, it was noted in both intact and spinal animals that DOI up to a dose of 1 mg kg^{-1} consistently (about 75% of the animals) produced a pressor response, raising MAP 15–25% above baseline, and less consistently (about 50% of the animals) produced a 5–15% increase in heart rate. It was also consistently noted that the 3 mg kg^{-1} dose of DOI did not produce a pressor response or tachycardia, but instead produced a depressor response (about 5% below the control baseline) or bradycardia (about 5% below the control baseline) in about 50% of the animals. These effects were independent of whether or not the animal was spinalized at T 13. Importantly, the effects (or lack of effects) of DOI on the pudendal and hypogastric reflexes in intact or spinal cats were independent of the effects on blood pressure and heart rate, in accordance with our previous studies (Danuser & Thor, 1995a).

Discussion

From the results of these experiments, one can conclude that spinal 5-HT$_2$ receptor activation facilitates pudendal nerve reflexes. Both the agonist, DOI, and the antagonist, LY53857, at the doses used in the present study are selective for 5-HT$_2$ receptors compared to other receptor subtypes (Cohen *et al.*, 1983; Alper & Snider, 1987; Pergola & Alper, 1992; Hoyer *et al.*, 1994). Since the animals were pretreated with a peripherally-restricted 5-HT$_2$ receptor antagonist, xylamidine (Mawson & Whittington, 1970; Fuller *et al.*, 1986), we can conclude that the effects were mediated centrally and were not due to indirect effects on smooth muscle. Since the facilitatory effects were seen in the acute spinal animal, it is logical to conclude that the 5-HT$_2$ receptors mediating the facilitatory effect are located in the spinal cord. Based upon previous studies showing a 5-HT$_2$ receptor-mediated facilitation of motoneurones (McCall & Aghajanian, 1979; White & Neuman, 1980; Roberts *et al.*, 1988; Rasmussen & Aghajanian, 1990), a probable site of action for this facilitatory effect is directly on the pudendal motoneurone.

The finding of facilitation of pudendal nerve activity by a 5-HT$_2$ receptor agonist is supported by other studies in which it was shown that external urethral sphincter EMG activity was facilitated by moderately high doses of 5MeODMT (100–500 μg kg^{-1}, i.v.) in both spinally-intact and chronic spinal cats (Thor *et al.*, 1990). Although lower doses of 5MeODMT preferentially activate 5-HT$_{1A}$ receptors, the higher doses of 5MeODMT required to produce sphincter activation would be sufficient to stimulate 5-HT$_2$ as well as 5-HT$_{1A}$ receptors (McCall & Aghajanian, 1979; Hoyer *et al.*, 1994). Since 5MeODMT also facilitated sphincter activity in chronic spinal cats, a spinal site of action was proposed (Thor *et al.*, 1990). The presence of facilitatory 5-HT$_2$ receptors along central pudendal nerve reflex pathways is also supported by experiments in which LY53857, the 5-HT$_2$ receptor antagonist, reversed increases in sphincter activity produced by duloxetine, a combined 5-HT and noradrenaline re-uptake inhibitor (Thor & Katofiasc, 1995).

Previous autoradiographic studies of [^{125}I]-DOI binding have demonstrated that 5-HT$_2$ receptors are preferentially located in motor nuclei of the rat spinal cord, including those regions that contain sphincter motor neurones (Thor *et al.*, 1993). Recently, three subtypes of 5-HT$_2$ receptors (5-HT$_{2A}$, 5-HT$_{2B}$ and 5-HT$_{2C}$) have been described, and DOI does not discriminate between them (Hoyer *et al.*, 1994). Molecular studies have localized both 5-HT$_{2A}$ and 5-HT$_{2B}$ receptor mRNA in the spinal cords of cats (Helton *et al.*, 1995). Although 5-HT$_{2C}$ receptor mRNA was identified in the spinal cords of rat, monkey, and human, as well as in the brain of cats, this receptor mRNA was not detected in the spinal cords of cat (Helton *et al.*, 1995). These molecular studies suggest that the facilitatory effects of DOI in acute spinal cats seen in the present study could be mediated by either 5-HT$_{2A}$ or 5-HT$_{2B}$ receptor subtypes but not the 5-HT$_{2C}$ receptor subtype.

Since DOI facilitated the pudendal reflex in acute spinal cats, but not spinally-intact cats, it is hypothesized that in spinally-intact cats DOI was not only stimulating facilitatory 5-HT$_2$ receptors in the spinal cord but was also stimulating supraspinal 5-HT$_2$ receptors that activate inhibitory controls on the reflex, which negated the spinal facilitatory effects. This hypothesis was supported by experiments in which the spinal cord was transected after administration of DOI (which produced no effect while the spinal cord was intact), and a robust increase in the reflex was seen that was sensitive to LY53857. Careful examination of the amplitude of the pudendal reflex before and after spinalization in control animals indicated that spinalization *per se* had no influence on the reflex. Subsequent administration of DOI to these control spinalized animals produced a typical increase in the reflex, which was again sensitive to LY53857.

The finding that DOI produced no effect on hypogastric nerve reflexes was unexpected. In previous studies, LY53857 produced a reduction in 'spontaneous' hypogastric nerve activity when administered after duloxetine, a combined 5-HT and noradrenaline re-uptake inhibitor (Thor & Katofiasc, 1995, *cf.* Figure 7c). Although duloxetine had no net effect on the hypogastric nerve activity, the inhibitory effect of LY53857 suggested that 5-HT$_2$ receptors were producing a tonic facilitation of the activity. However, the present study could find no evidence for a facilitatory role of 5-HT$_2$ receptors on the hypogastric reflex. This discrepancy might be explained by differences in the end points measured in the two series of experiments, *i.e.* 'spontaneous' activity (Thor & Katofiasc, 1995) *versus* pelvic nerve-evoked reflex activity (present study). (In the present study, an increase in 'spontaneous' hypogastric nerve activity was seen following DOI [1 mg kg^{-1}, i.v.] in 1 of 4 spinal cord intact and 2 of 5 spinal cord transected experiments. However, the inconsistency and the variable magnitude of the increase precluded significant quantitative analysis.)

The lack of facilitation of the hypogastric reflex cannot be attributed to the experimental protocol, since, using an identical protocol, facilitation of the hypogastric reflex was seen following administration of a noradrenaline re-uptake inhibitor combined with an α_2-adrenoceptor antagonist (Danuser & Thor, 1995b). Since this drug treatment paradigm also increased spontaneous hypogastric nerve activity (more so than DOI), one can discount occlusion of the evoked reflex activity by high levels of spontaneous activity as a reason for not seeing facilitation of the hypogastric reflex in the present experiments.

In summary, the present experiments indicate that DOI facilitates pudendal nerve reflexes *via* activation of spinal 5-HT$_2$ receptors. The facilitation of pudendal nerve reflexes by DOI supports the hypothesis that the 5-hydroxytryptaminergic system can contribute to the maintenance of urinary continence through augmentation of external urethral sphincter function (Thor & Katofiasc, 1995).

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