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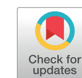
Research article

Desipramine, commonly used as a noradrenergic neuroprotectant in 6-OHDA-lesions, leads to local functional changes in the urinary bladder and gastrointestinal tract in healthy rats



Maria del Pilar Murillo, Patrik Aronsson, Michael Winder, Thomas Carlsson *

Department of Pharmacology, Institute of Neuroscience and Physiology, The Sahlgrenska Academy, University of Gothenburg, Box 431, SE-40530 Gothenburg, Sweden

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ABSTRACT

The 6-hydroxydopamine (6-OHDA) rat model is one of the most common animal models of Parkinson's disease. When experimentally inducing dopaminergic neurodegeneration in the nigrostriatal pathway using 6-OHDA, the noradrenergic reuptake inhibitor desipramine is often systemically injected in order to protect against damages to the noradrenergic system in the brain. An increasing number of studies are focusing on understanding the pathophysiological changes underlying autonomic non-motor symptoms, in particular urinary bladder and gastrointestinal dysfunctions, of the disease. Several of these studies have investigated the contractile properties and the activation of smooth muscle in the 6-OHDA rat model. Since the injection of desipramine is commonly placed in close proximity to the urinary bladder and gastrointestinal tract, in the current study we wanted to understand if the drug alone has an effect. For this, we have injected a single dose (25 mg/kg) of desipramine either intraperitonially or subcutaneously and investigated smooth muscle contractility in vitro in the urinary bladder, proximal colon and distal ileum four weeks post injection. Our data show that desipramine significantly alters smooth muscle contractility of the urinary bladder and proximal colon in healthy rats. Conclusively, we suggest, based on our data, that desipramine should be omitted when using the 6-OHDA rat model to investigate smooth muscle function in Parkinson's disease research.

1. Introduction

One of the most commonly used models in preclinical research in Parkinson's disease (PD) is the 6-hydroxydopamine (6-OHDA) rat model, which was established in the late 1960′s by Urban Ungerstedt (1968). This model is based on the dopamine (DA) analog and neurotoxin 6-OHDA, which is injected into the nigrostriatal pathway, i.e. in the substantia nigra (SN), the medial forebrain bundle (MFB) or the striatum, to create a substantial DA loss in the brain similar to that in PD patients (Kirik et al., 1998). In addition to its selectivity for dopamine cells, 6-OHDA is also neurotoxic for noradrenergic neurons (Bell et al., 1970; Reader and Gauthier, 1984). In order to protect these latter neurons, we and others have used the tricyclic antidepressant, noradrenergic reuptake inhibitor, desipramine in studies investigating motor and non-motor symptoms as well as molecular changes in the 6-OHDA rodent model (Alzoubi et al., 2018; Ermine et al., 2018; Kostrzewa and Kostrzewa, 2020; Lindgren et al., 2014; Mitra et al., 2015; Taghzouti et al., 1991; Takahashi et al., 1984; Zhang et al., 2007). Importantly, desipramine is often used in 6-OHDA animals, but not always in the control groups. An increasing number of animal studies are focused on investigating non-motor symptoms in PD, including the troublesome urinary bladder symptoms and gastrointestinal dysfunction often seen in PD patients, using in particular the 6-OHDA rat model (Blandini et al., 2009; Colucci et al., 2012; Fornai et al., 2016; Mitra et al., 2015; Pellegrini et al., 2020; Soler et al., 2011; Yoshimura et al., 2003; Zhang et al., 2015). More specifically, many studies have investigated the contractility of isolated smooth muscle in gut (Fornai et al., 2016; Levandis et al., 2015; Pellegrini et al., 2016, 2017; Zhang et al., 2015) and urinary bladder tissues (Mitra et al., 2015). In the 6-OHDA model, desipramine is normally injected as a systemic intraperitoneal (i.p.) injection, in close proximity to the urinary bladder as well as the colon and ileum, which are extensively innervated by not only noradrenergic and dopaminergic fibers but also serotonin and histamine fibers. Desipramine, in fact, has been shown to have significant effects on these latter neurotransmitter systems (Esteban et al., 1999; Owens et al., 1997; Rehavi et al., 1987). Moreover, toxic effects of desipramine have been demonstrated in *in vitro* studies, including

* Corresponding author.
E-mail address: thomas.carlsson.2@gu.se (T. Carlsson).

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prostate and colon cancer cells (Arimochi and Morita, 2008; Chang et al., 2008).

This raises the hypothesis that the noradrenergic reuptake inhibitor desipramine alone can lead to local effects on gastrointestinal and urogenital smooth muscle. In the current study we have therefore investigated if desipramine could affect the smooth muscle contractile function of the urinary bladder, proximal colon and distal ileum, using an *in vitro* organ bath setup. The effect was evaluated four weeks after administrating a single, systemic, i.p. or subcutaneous (s.c.) dose of 25 mg/kg desipramine, a dose which is commonly used in the 6-OHDA lesion rat model of PD (Alzoubi et al., 2018; Mitra et al., 2015; Taghzouti et al., 1991; Zhang et al., 2007).

Our data show that both the urinary bladder and the colon smooth muscle contractility is altered after a single injection of desipramine. These long-term changes could be observed both following presynaptic stimulation by electrical field stimulation (EFS) and after direct postsynaptic receptor stimulation of the cholinergic system. The distal ileum contractility was, however, unaffected by the desipramine injection. These findings clearly indicate that desipramine as pre-treatment to 6-OHDA injection, should be avoided, in particular when investigating smooth muscle function in animal models of PD.

2. Materials and methods

2.1. Animals

In this study a total of 26 adult male Sprague–Dawley rats (Charles-River GmbH, Germany or Charles-River SRL, Calco, Italy), housed under standard laboratory environment conditions with free access to food and water and under standard 12h light - 12h dark conditions, were used. The animals weighed 230–570 g at the beginning of the experiment. All procedures and animal care were in accordance with Directive 2010/63/EU of the European Parliament and of the Council of the European Union (Document 32010L0063), and were approved by the local ethical committee at the University of Gothenburg, Sweden (permit #145-15).

2.2. Study design

First, the rats were divided into four groups where: I. received a single i.p. injection of physiological saline solution (1 ml/kg; *Saline i.p.*, n = 8); II. received a single i.p. injection of desipramine hydrochloride (25 mg/kg dissolved in saline, pH:4.95; *Desipramine i.p.*, n = 7; Sigma-Aldrich, St Louis, MO, USA); III. received a single s.c. injection of physiological saline (1 ml/kg; *Saline s.c.*, n = 5); and IV. received a single s.c. injection of desipramine hydrochloride (25 mg/kg, dissolved in saline, pH:4.95; *Desipramine s.c.,* n = 6). Following the injection, the animals were placed in their home cages. At 23–30 days (26.9 ± 0.8 and 25.4 ± 0.6 days for *Saline i.p. and Desipramine i.p.,* respectively) following the injection the i.p. injected animals were placed individually in metabolic cages, with free access to water only, to collect urine and feces during 8 h (dark cycle, from 9 PM to 5 AM). For the s.c.-treated animals the metabolic cage experiment was not performed, and the tissue was dissected out at 27.8 ± 0.4 and 25.5 ± 0.4 days for *Saline s.c* and *Desipramine s.c.*, respectively, following the injections.

At 4–6 h after the metabolic cage experiment, the animals were sacrificed by an overdose of pentobarbital sodium (100–150 mg/kg, APL, Stockholm, Sweden), and the urinary bladder, distal ileum and proximal colon were dissected out and placed in room temperature Krebs solution (NaCl, 118 mM; KCl, 4.6 mM; KH_2PO_4, 1.15 mM; $MgSO_4$, 1.15 mM; $NaHCO_3$, 25 mM; $CaCl_2$, 1.25 mM; and glucose, 5.5 mM; gassed by 95% O_2 and 5% CO_2). The intestinal segments were prepared as 1 cm long tubular segments, while the bladder was prepared as two equal size full-thickness tissue strips (approx. 2 × 6 mm). Following the preparation, the tissues were mounted in organ baths (Linton Instrumentation, Norfolk, UK) to measure muscle contractions (see *In vitro organ baths* section). First, the viability (responsiveness) of the smooth muscle tissue was tested using a high K^+ Krebs solution (containing 124 mM KCl; by *exchanging NaCl for equimolar amounts of KCl*). A contractile muscle response of >8 mN was set as a criterion for a viable urinary bladder strip (Mitra et al., 2015), while for the intestinal segment a >7 mN response was utilized. Following the viability test, the tissues were first evaluated using electric field stimulation (EFS) at 1, 2, 5, 10, 20, and 40 Hz, and thereafter challenged with a cumulative administration of the muscarinic agonist methacholine (10^{-8} M - 10^{-3} M). Finally, the tissues were once again challenged with a high K^+ Krebs solution, then removed from the organ bath, briefly dried and weighed.

2.3. Metabolic cage experiment

Four weeks following injections, *i.e.* on the night before sacrifice, the animals in the i.p. injected groups (*Saline i.p.* and *Desipramine i.p.*) were individually housed for 8 h (dark cycle, from 9 PM to 5 AM) with free access to water, but no food, in metabolic cages fitted with a doppler sensor (SICK, Stockholm, Sweden) connected to a MP100 data acquisition system (Biopac Systems Inc., Goleta, CA). The bladder function was monitored and parameters including total number of micturitions, intermicturition intervals and number of drops per micturition were registered using Acknowledge Software v 3.8 (Biopac Systems Inc.). The total urine volume, number and weight of feces pellets and total water consumed was also registered.

2.4. Tissue processing

Following an overdose with pentobarbital sodium (100–150 mg/kg, APL), the rats' urinary bladder, distal ileum and proximal colon were dissected out. For the distal ileum and proximal colon, 3–4 cm long tissue pieces were collected approx. 3 cm from each direction of the cecum. All collected tissues were then placed in room temperature Krebs solution for transport.

For the urinary bladder, medially to the orifices of the ureters, two full-thickness detrusor strips (approx. 2 × 6 mm) were dissected out from each animal, as previous described (Tobin and Sjogren, 1995). A silk thread (Vömel, Kronberg, Germany) was then tied to each end of the strips, with one end forming a loop.

For the colon and ileum tissue preparations, they were first carefully rinsed with Krebs solution to remove any remaining feces without using any force. Following this, one or two approx. 1 cm long intestinal segments were cut out from the middle of the collected colon and ileum tissues. Silk threads (Vömel) were attached at each end (one end forming a loop) of the gut tissue tubular segments, closing the luminal space.

2.5. In vitro organ bath experiments

Following the preparation, the tissues were mounted vertically in an isolated tissue bath (Linton Instrumentation) with one end attached to a fixed hook in the bath and the other to a force transducer (TSD125C, Biopac Systems Inc.), submerged in 25 mL of Krebs solution kept at 37 °C and oxygenated with a mixture of 95% O_2 and 5% CO_2. The tissues were then pre-stretched to a basal tension of 5–8 mN for urinary bladder strips and 8–12 mN for intestinal preparations, and then let to equilibrate for 45 min. Following the equilibration, the muscle tissues were challenged with high K^+ Krebs; once for urinary bladder strips and three consecutive times for intestinal segments, 10 min apart, in order to evaluate the viability and, in the latter, to activate the tissue. The tissues were then first stimulated with EFS at 1, 2, 5, 10, 20, and 40 Hz, at supra-maximal voltage delivered as square waves pulses with the duration of 0.8 ms (Stimulator STM100C, Linton Instrumentation), until peak contraction could be observed. This was followed by a recovery period of at least 10 min after changing to fresh Krebs solution in the organ bath. The tissues were then challenged with increasing cumulative concentrations of methacholine; 10^{-8}, 10^{-7}, 10^{-6}, 10^{-5}, 10^{-4} and 10^{-3} M (Sigma-Aldrich, St Louis, MO, USA). Following the EFS- and methacholine-evoked

contractions, and after a resting period of 10 min, the tissues were once again challenged with high K^+ Krebs solution (124mM), to test the viability, and then removed from the organ baths, briefly dried and weighed.

2.6. Data acquisition and analysis

All contractile responses of the bladder and intestinal tissues were recorded and analyzed using the MP100WSW data acquisition system with Acknowledge Software v 3.8 (Biopac Systems Inc.). For each contraction analysis of the urinary bladder tissue, the basal tension measurement was averaged over 5–10 s prior to each high K^+ Krebs, EFS stimulation or first concentration of methacholine. For the intestinal tissue, the basal tension was averaged over 20–60 s prior to stimulation. The maximum contractions were measured at the absolute peak for all analyses.

Further, the response ratio of the contractions to EFS and methacholine per mg tissue (i.e. tissue weight) were also calculated. In addition, the high K^+ Krebs response per mg tissue was also calculated using the high K^+ response obtained before the experiment.

2.7. Included and excluded tissue samples

2.7.1. Urinary bladder

Four tissue strips in the *Saline i.p.*, 4 tissue strips in the *Desipramine i.p.* group and 3 tissue strips in the *Desipramine s.c.* group were excluded due to low high K^+ Krebs responses (<8mN). One additional strip was excluded in the *Saline i.p.* group due to being an outlier (Grubbs test; as described in the statistical analysis section). This generated a total of 11 strips (from 6 rats) in the *Saline i.p.* group, 8 strips (from 5 rats) in the *Desipramine i.p.* group, 10 strips (from 5 rats) in the *Saline s.c.* group and 9 strips (from 6 rats) in the *Desipramine s.c.* group. These tissues were included in the data analysis.

2.7.2. Proximal colon

One tissue segment in the *Desipramine i.p.* group was excluded due to being an outlier (Grubbs test; as described in the statistical analysis section). Further, the EFS responses of two animals in the *Desipramine s.c.* group were excluded due to malfunction of the electrical stimulator. This generated a total of 8 segments (from 8 rats) in the *Saline i.p.* group, 6 segments (from 6 rats) in the *Desipramine i.p.* group, 5 segments (from 5 rats) in the *Saline s.c.* group and 7–9 segments (from 4-6 rats) in the *Desipramine s.c.* group. These tissues were included in the data analysis.

2.7.3. Distal ileum

Two tissue segments in the *Saline i.p.* group, two in the *Desipramine i.p.* group and one in the *Saline s.c.* group were excluded due to low high K^+ Krebs responses (<7mN; and no response either to EFS nor methacholine). Further, the EFS responses of two segments in the *Desipramine i.p.* group were excluded due to malfunction of the electrical stimulator. This generated a total of 6 segments (from 6 rats) in the *Saline i.p.* group, 3–5 segments (from 3-5 rats) in the *Desipramine i.p.* group, 4 segments (from 4 rats) in the *Saline s.c.* group and 9 segments (from 6 rats) in the *Desipramine s.c.* group. These tissues were included in the data analysis.

2.8. Statistical analysis

The EFS and methacholine data are presented as frequency-respective concentration-response curves using two-way repeated measure ANOVAs, followed by Sidak posthoc test for statistical comparisons. Here the statistics are presented as group comparisons if not stated otherwise. For the rat weight, tissue weight, high K^+ Krebs response and all metabolic cage data unpaired t-tests with equal standard deviation where used. In order to identify possible outliers in each data set, Grubbs test with alpha set to 0.05 was used. All statistical comparisons were performed using the GraphPad Prism for Mac OS X (GraphPad Software Inc, San Diego, CA, USA), and the data are presented as the mean ± S.E.M., with significance set to $p < 0.05$.

3. Results

3.1. Effect of i.p. injections of desipramine

At sacrifice the rat body weight in the *Saline i.p.* and *Desipramine i.p.* groups were 500 ± 18g and 422 ± 34g, respectively (unpaired t-test p = 0.054). The weights of the dissected urinary bladder strip preparations were not significantly different between the groups (*Saline i.p.:* 5.2 ± 0.3 mg vs. *Desipramine i.p.:* 4.5 ± 0.5 mg; unpaired t-test: p = 0.26). The weights of the tissue preparations of the distal ileum (*Saline i.p.:* 153 ± 13mg vs. *Desipramine i.p.:* 165 ± 13 mg; unpaired t-test: p = 0.54) and proximal colon (*Saline i.p.:* 226 ± 12 mg vs. *Desipramine i.p.:* 244 ± 19 mg; unpaired t-test: p = 0.41) also showed no significant difference between groups.

3.1.1. Urinary bladder

The viability tests using high K^+ Krebs in the urinary bladder tissue showed no difference between the *Saline i.p.* group (17.2 ± 1.8 mN) and the *Desipramine i.p.* group (22.0 ± 2.6 mN) at the beginning of the experiment (Table 1). However, a significant difference in high K^+ response per mg tissue was observed between the groups (3.4 ± 0.4 mN/mg vs. 5.0 ± 0.5 mN/mg in the *Saline i.p.* and *Desipramine i.p.* groups, respectively; Table 1).

3.1.1.1. EFS-induced response. Following EFS, the urinary bladder smooth muscle tissue responded significantly higher in the *Desipramine i.p*-treated group as compared to the *Saline i.p.*-treated group (Figure 1A, B, Table 1). This was specifically pronounced at the higher frequencies reaching 23.5 ± 2.8 mN following *Desipramine i.p.* treatment as compared to 16.7 ± 1.8 mN in the *Saline i.p.*-treated group, at 40 Hz. This was also observed when normalizing the EFS contractions with the tissue weight as seen by an increased ratio (mN/mg tissue) in the *Desipramine i.p.* group (Table 1).

3.1.1.2. Methacholine-induced response. Cumulative administration of the muscarinic agonist methacholine showed also a significantly, although less pronounced, increased muscle contraction in the *Desipramine i.p.*-treated rats with a maximum response at 10^{-4} - 10^{-3} M (Figure 1C, D, Table 1). At 10^{-3} M the contractions were 27.5 ± 2.7 mN following *Desipramine i.p.* treatment as compared to 20.6 ± 1.6 mN in the *Saline i.p.* group. Normalizing the methacholine response to the tissue weight confirmed this significant increased contraction in the *Desipramine i.p.* group (Table 1).

3.1.2. Micturition pattern in metabolic cage

The micturition pattern was not affected following i.p. injection of desipramine. The frequency was 1.4 ± 0.4 micturitions/h in the *Desipramine i.p.* group (n = 7) as compared to 1.2 ± 0.3 micturitions/h in the *Saline i.p.* group (n = 6; unpaired t-tests: p = 0.75). Similarly, the volume/micturition were 0.7 ± 0.1 ml vs. 0.9 ± 0.1 ml in the *Desipramine i.p.*-treated and *Saline i.p.*-treated groups respectively, and was not significantly different (Unpaired t-tests: p = 0.21). The water consumption was not significantly different between the groups (*Saline i.p.*: 23.3 ± 8.0 ml vs *Desipramine i.p.:* 14.6 ± 4.1 ml; unpaired t-tests: p = 0.33).

3.1.3. Proximal colon

No significant difference between the groups could be observed in the high K^+ response before the experimental setup (15.0 ± 1.3 mN and 12.9 ± 1.5 mN in the *Saline i.p.* and *Desipramine i.p.* groups, respectively; Table 1). In addition, no difference in high K^+ response per mg tissue was observed between the groups (0.068 ± 0.0072 mN/mg vs. 0.055 ±

Table 1. Summary of absolute and tissue weight corrected responses to high K^+ Krebs, EFS and methacholine following intraperitoneal (i.p.) and subcutaneous (s.c.) injection of desipramine.

i.p.				
Urinary Bladder	*High K^+*	Absolute response	↔	p = 0.14
		Corr. tissue weight	↑	**p = 0.031** *
	EFS	Absolute response	↑	**p = 0.039** *
		Corr. tissue weight	↑	**p = 0.0049** *
	Methacholine	Absolute response	↑	p = 0.061/**p = 0.014** *
		Corr. tissue weight	↑	**p = 0.023** *
Colon	*High K^+*	Absolute response	↔	p = 0.31
		Corr. tissue weight	↔	p = 0.31
	EFS	Absolute response	↔	p = 0.14
		Corr. tissue weight	↔	p = 0.14
	Methacholine	Absolute response	↓	**p = 0.0498** *
		Corr. tissue weight	↓	**p = 0.048** *
Ileum	*High K^+*	Absolute response	↔	p = 0.87
		Corr. tissue weight	↔	p = 0.63
	EFS	Absolute response	↔	p = 0.30
		Corr. tissue weight	↔	p = 0.97
	Methacholine	Absolute response	↔	p = 0.68
		Corr. tissue weight	↔	p = 0.37
s.c.				
Urinary bladder	*High K^+*	Absolute response	↓	p = 0.092
		Corr. tissue weight	↓	p = 0.096
	EFS	Absolute response	↓	p = 0.052/**p = 0.028** *
		Corr. tissue weight	↓	**p = 0.0498** *
	Methacholine	Absolute response	↓	p = 0.074/**p = 0.017**
		Corr. tissue weight	↔	p = 0.11/p = 0.050
Colon	*High K^+*	Absolute response	↔	p = 0.74
		Corr. tissue weight	↔	p = 0.41
	EFS	Absolute response	↔	p = 0.12/p = 0.073
		Corr. tissue weight	↔	p = 0.28
	Methacholine	Absolute response	↑	**p = 0.014** *
		Corr. tissue weight	↑	p = 0.087
Ileum	*High K^+*	Absolute response	↑	**p = 0.0034** *
		Corr. tissue weight	↑	**p = 0.039** *
	EFS	Absolute response	↔	p = 0.76
		Corr. tissue weight	↓	p = 0.058
	Methacholine	Absolute response	↑	**p = 0.021** *
		Corr. tissue weight	↔	p = 0.92

* = significant difference between respective Saline and desipramine group; ↔ indicates no significant change; ↑/↓ indicates significant increase/decrease in contraction or contraction/mg tissue; ↑/↓ indicates a non-significant trend to increase/decrease in contraction or contraction/mg tissue ratio in the desipramine-treated animals. The high K^+ was statistically analysed using unpaired t-tests, while EFS and methacholine were analysed by two-way ANOVAs. The p-values represent group comparisons. Where a second p-value is presented, the latter represents concentration-group interaction. Significant p-values are indicated in bold.

0.0095 mN/mg in the *Saline i.p.* and *Desipramine i.p.* groups, respectively; Table 1).

3.1.3.1. EFS-induced response. The electrical stimulation of the colon tissue following desipramine i.p. treatment showed no change in smooth muscle contraction (Figure 2A, B; Table 1). A trend could however be observed at lower frequencies 5–10 Hz (at 5 Hz: 11.2 ± 2.5 mN and 5.9 ± 1.1 mN in the *Saline i.p.* and *Desipramine i.p.* group, respectively; unpaired t-test, p = 0.11). The tissue weight normalization showed no significant differences between the groups (Table 1).

3.1.3.2. Methacholine-induced response. The methacholine response showed, in contrast to the EFS responses, a significantly decreased smooth muscle contraction in the *Desipramine i.p.*-treated group as compared to the *Saline i.p.* group (Figure 2C, D, Table 1), which was in particular observed at the concentration of 10^{-4} where the *Saline i.p.* group contracted 26.5 ± 4.1 mN as compared to 13.7 ± 2.8 mN in the *Desipramine i.p.* group. Similar significant changes were evident following normalization to tissue weight (Table 1).

3.1.4. Distal ileum

In the ileum tissue, the high K^+ Krebs response was not significantly altered between the groups at the beginning of the experiment (*Saline i.p.:* 13.6 ± 2.0 mN vs. *Desipramine i.p.:* 14.2 ± 3.2 mN; Table 1). Similarly, no difference in high K^+ response per mg tissue was observed following desipramine treatment (0.094 ± 0.017 mN/mg vs. 0.082 ± 0.017 mN/mg in the *Saline i.p.* and *Desipramine i.p.* groups, respectively; Table 1).

3.1.4.1. EFS-induced response. The EFS-induced response did not show any significant change in smooth muscle contraction between the groups (Figure 3A, B, Table 1). This finding was further confirmed when the contraction was normalized to tissue weight (Table 1).

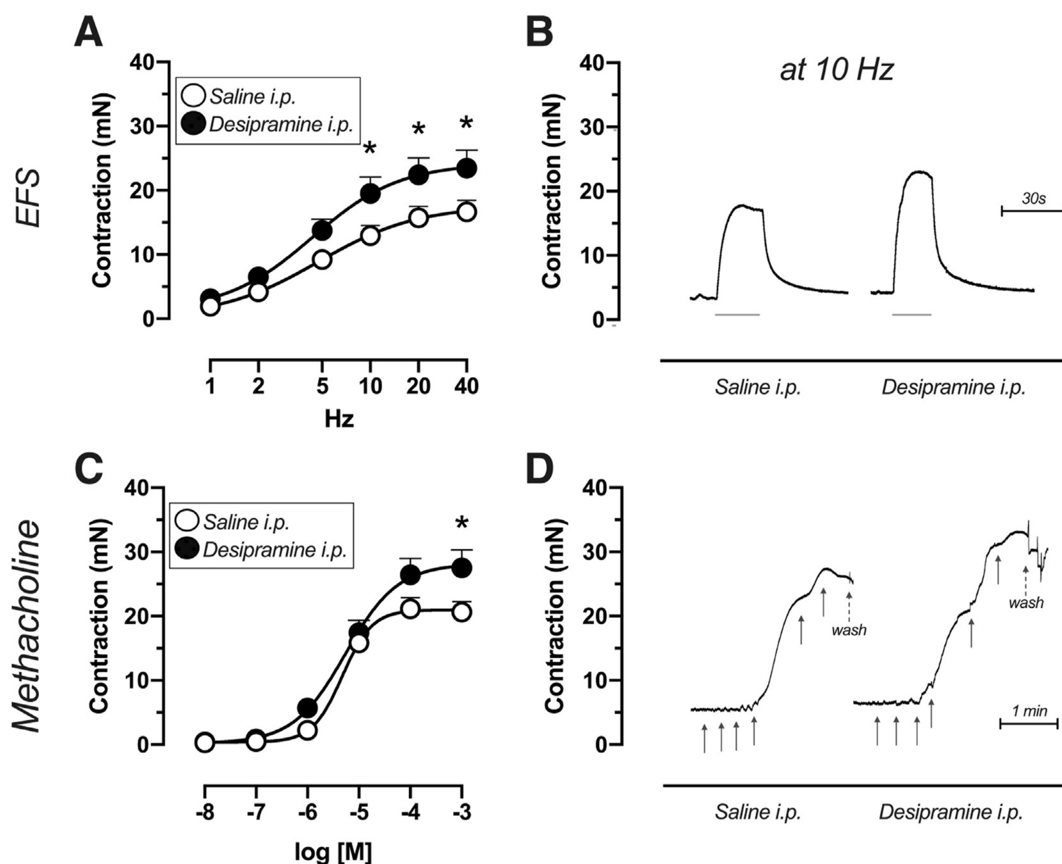

Figure 1. Urinary bladder smooth muscle contractions following electrical (EFS)- and methacholine-induced stimulations in intraperitoneal (i.p.)-treated animals. Significantly higher smooth muscle strip contractions were observed in the *Desipramine i.p.*-treated animals (n =11), as compared to *Saline i.p.*-treated animals (n = 8), following EFS (A) and methacholine stimulations (C). These changes are illustrated by original registrations at 10 Hz EFS (B) and cumulative methacholine curves (D) in representative animals in respective group. * = different from *Saline i.p.* group; Two-way ANOVAs A: F (1,17) = 5.02, p = 0.039; C: Two-way ANOVAs group F (1,17 = 4.03), p = 0.061; concentration-group interaction F (5,85 = 3.07), p = 0.014; followed by Sidak posthoc test. Grey lines in B indicated the stimulation durations and grey arrows in D indicated the administration of methacholine from 10^{-8} – 10^{-3} M. Data are presented as mean ± SEM.

3.1.4.2. Methacholine-induced response. Similar to the EFS-induced responses, the methacholine response did not change following *Desipramine i.p.* treatment as compared to *Saline i.p.* treatment; reaching a maximum at 10^{-5} M; 21.9 ± 1.5 mN in the *Saline i.p.* group and 19.8 ± 2.8 mN in the *Desipramine i.p.* group (Figure 3C, D, Table 1). Likewise, no changes were seen after normalizing the response to tissue weight (Table 1).

3.1.5. Defecation in metabolic cage

No difference could be observed between the *Saline i.p. (n = 7)* and *Desipramine i.p. (n = 7)* groups in amount of feces (2.1 ± 0.6 g vs. 3.2 ± 1.0 g respectively; unpaired t-test: p = 0.34) or number of pellets collected (7.0 ± 2.0 pellets vs. 9.9 ± 2.3 pellets respectively; unpaired t-test: p = 0.36).

3.2. Effect of s.c. injection of desipramine

The rat weight, at sacrifice, in the *Saline s.c.* (428 ± 19g) and *Desipramine s.c.* (463 ± 19g) groups was not significantly different from each other (unpaired t.-test p = 0.23). The urinary bladder tissue weight was also not different between the groups. (*Saline s.c.:* 6.8 ± 0.3 mg vs. *Desipramine s.c.:* 6.2 ± 0.6 mg; unpaired t-test: p = 0.38). However, both the proximal colon and the distal ileum tissue weights were significantly higher in the desipramine-treated group as compared to the saline-treated group. (Proximal colon: *Saline s.c.:* 157.3 ± 13.5 mg vs. *Desipramine s.c.:* 198.6 ± 10.5 mg, unpaired t-tests p = 0.034; Distal ileum: *Saline i.p.:* 95.5 ± 9.5 mg vs. *Desipramine s.c.:* 142.8 ± 9.5 mg, unpaired t-test 0.0071).

3.2.1. Urinary bladder

High K^+ Krebs responses of the urinary bladder tissue showed a non-significant difference between the groups (*Saline s.c.*: 43.4 ± 3.8 mN vs *Desipramine s.c.*: 31.0 ± 6.0 mN; Table 1). A similar, but non-significant, trend in high K^+ response per mg tissue was observed between the groups (6.5 ± 0.49 mN/mg vs. 4.9 ± 0.79 mN/mg in the *Saline s.c.* and *Desipramine s.c.* groups, respectively; Table 1).

3.2.1.1. EFS-induced response. In contrast to the i.p. injected animals, in the s.c.-injected rats following EFS stimulation the urinary bladder smooth muscle tissue responded significantly lower in the desipramine-treated group as compared to the saline group (Figure 4A; Table 1). The significance was also confirmed after normalizing the EFS-induced contractions by tissue weight (Table 1).

3.2.1.2. Methacholine-induced response. Similar to the EFS-induced contractions, a significant decrease in contraction was observed following administration of the muscarinic agonist methacholine in the *Desipramine s.c.*-treated rats (Figure 4D; Table 1). The decrease was in particular observed at 10^{-4} M with contractions of 33.1 ± 5.7 mN following *Desipramine s.c.* treatment as compared to 44.8 ± 3.2 mN in the *Saline s.c.* group. Normalizing the response to the tissue weight showed only a non-significant trend (Table 1).

3.2.2. Proximal colon

No significant difference in the high K^+ response between the *Saline s.c.* and *Desipramine s.c.* groups was seen at the beginning of the organ

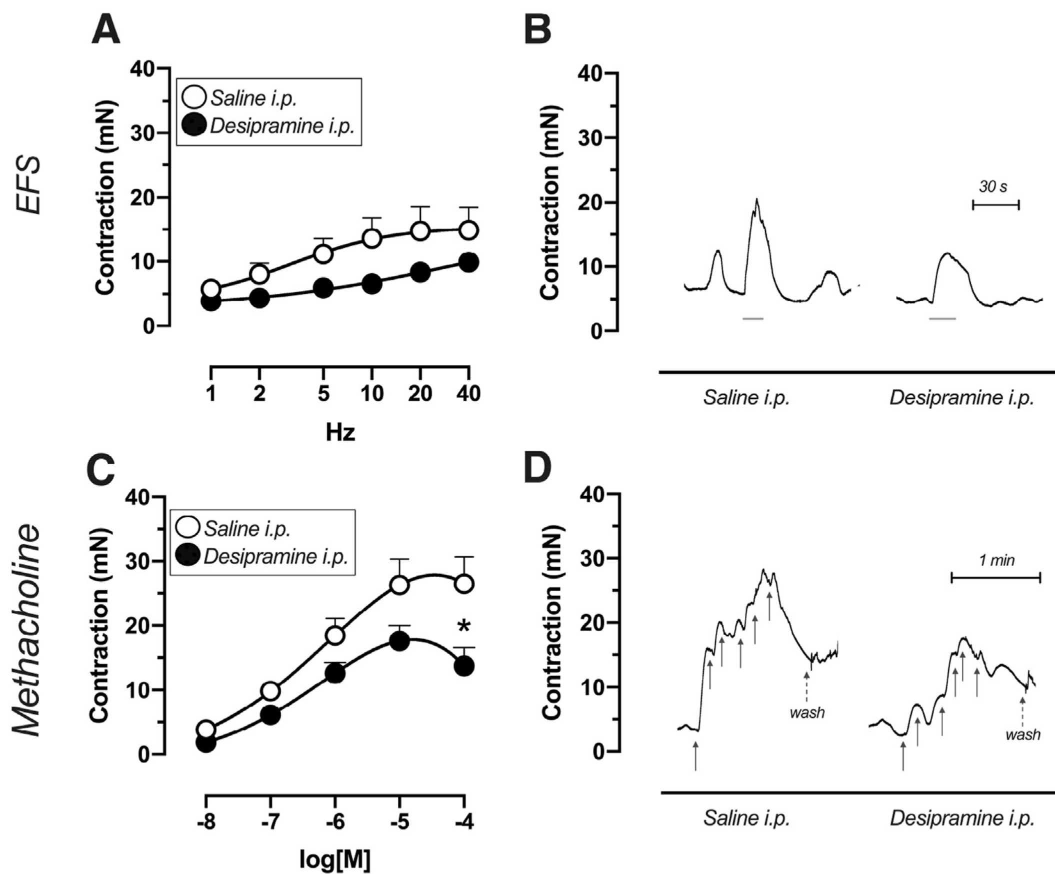

Figure 2. Proximal colon smooth muscle contractions following electrical (EFS)- and methacholine-induced stimulations in intraperitoneal (i.p.)-treated animals. A tendency of decreased smooth muscle segment contraction was observed in the *Desipramine i.p.*-treated animals (n = 6), as compared to *Saline i.p.*-treated animals (n = 8), following EFS stimulation (A). This decrease was significantly evident following methacholine stimulations, in particular at contraction of 10^{-4} M (C). These changes are illustrated by original registrations at 10 Hz EFS (B) and cumulative methacholine curves (D) in representative animals in respective group. * = different from *Saline i.p.* group; Two-way ANOVAs A: F (1,12 = 2.53), p = 0.14); C: F (1,12 = 4.76), p = 0.0498); followed by Sidak posthoc test. Grey lines in B indicated the stimulation durations and grey arrows in D indicated the administration of methacholine from 10^{-8} – 10^{-4} M. Data are presented as mean ± SEM.

bath experiment (28.0 ± 3.2 mN and 29.5 ± 3.0 mN, respectively; Table 1). Moreover, the high K^+ response per mg tissue showed no significant difference between the groups (0.18 ± 0.020 mN/mg vs. 0.15 ± 0.021 mN/mg in the *Saline s.c.* and *Desipramine s.c.* groups, respectively; Table 1).

3.2.2.1. EFS-induced responses. A non-significant trend in EFS response was evident in the *Desipramine s.c.*-treated group reaching 31.9 ± 7.1 mN as compared to 20.2 ± 3.7 in the *Saline s.c.* group at 20 Hz (Figure 4B; Table 1). This could however not be observed following normalization against the tissue weight (Table 1).

3.2.2.2. Methacholine-induced response. The response to the muscarinic agonist methacholine showed a significant increase in smooth muscle contraction in the *Desipramine s.c.*-treated group as compared to the *Saline s.c.* group (Figure 4E; Table 1), with contractions at 10^{-3} M of 28.8 ± 1.0 mN and 48.7 ± 6.4 mN in the *Saline s.c.* and *Desipramine s.c.* groups, respectively. This alteration was, however, only seen as a non-significant trend following normalization to the tissue weight (Table 1).

3.2.3. Distal ileum

In the ileum tissue, the K^+ Krebs response was significantly different between the groups (*Saline s.c.*: 11.8 ± 1.1 mN vs. *Desipramine s.c.*: 26.5 ± 2.5 mN; Table 1). Interestingly, a significant difference between the groups was also observed in the high K^+ response per mg tissue (0.12 ± 0.0093 mN/mg vs. 0.19 ± 0.017 mN/mg in the *Saline s.c.* and *Desipramine s.c.* groups, respectively; Table 1).

3.2.3.1. EFS-induced respons. No change in absolute EFS-induced responses could be observed in ileum following s.c. injections of desipramine (Figure 4C; Table 1). However, a non-significant trend was observed when the response was normalized to the tissue weight (Table 1).

3.2.3.2. Methacholine-induced response. Different to the EFS-induced response, the methacholine-induced response showed an increased contractility in the *Desipramine s.c.* group compared to *Saline s.c.* treatment group reaching contractions of 21.3 ± 2.3 mN and 28.4 ± 2.2 mN in the *Saline s.c.* and *Desipramine s.c.* groups, respectively, at 10^{-5} M (Figure 4F; Table 1). Interestingly, this could however not be observed after normalizing the data to the tissue weight (Table 1).

4. Discussion

In the current study we have analyzed the effects of a single i.p. or s.c. injection of the noradrenergic reuptake inhibitor desipramine hydrochloride on smooth muscle contractions in the gut and urinary bladder. This drug is often injected systemically as an i.p. injection in order to protect the noradrenergic neurons in the brain following a 6-OHDA-induced DA lesion, which is the most commonly used animal model of PD. The s.c. injection routine was included to evaluate whether or not the route of administration was important for the observed effect of desipramine seen after i.p. injection. Further, we investigated the effects of the single injections four weeks post treatment, a time period ensuring complete nigrostriatal DA lesions with established plastic changes in the

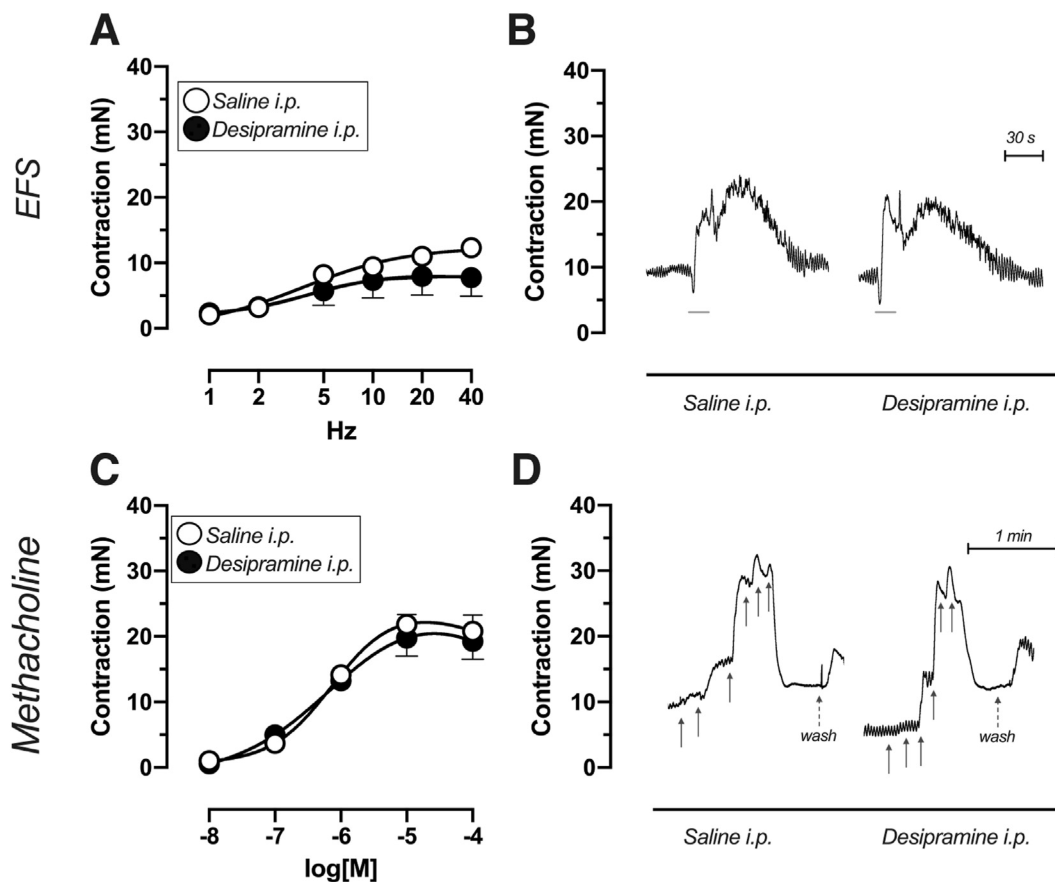

Figure 3. Distal ileum smooth muscle contractions following electrical (EFS)- and methacholine-induced stimulations in intraperitoneal (i.p.)-treated animals. No difference in smooth muscle segment contractions were observed between the *Desipramine i.p.* and *Saline i.p.* groups following neither EFS (A: n = 6 and n = 3, respectively) nor methacholine stimulations (C: n = 6 and n = 5, respectively). Original registrations at 10 Hz EFS (B) and cumulative methacholine curves (D) are illustrated in representative animals in each group. Two-way ANOVAs A: F (1,7 = 1.28), p = 0.30); C: F (1,9 = 0.184), p = 0.68); followed by Sidak posthoc test. Grey lines in B indicated the stimulation durations and grey arrows in D indicated the administration of methacholine from 10^{-8} – 10^{-4} M. Data are presented as mean ± SEM.

brain of 6-OHDA-lesioned rats, used in previous studies to investigate smooth muscle function in this PD rat model (Blandini et al., 2009; Colucci et al., 2012; Mitra et al., 2015; Soler et al., 2011).

Our data clearly show that desipramine can significantly change the local muscle contractility in the urinary bladder as well as the gastrointestinal tract up to at least four weeks after injection. Interestingly, desipramine seems to alter the contractility differently depending on the route of administration, where i.p. desipramine injections significantly increase the contraction in the urinary bladder, while s.c. injections decrease the same. The changes were observed both following EFS and/or direct cholinergic agonist (methacholine) stimulation. Similar, but inverse, patterns were observed in the proximal colon where an i.p. injection decreased and s.c. injection increased the muscle contractility, even though this was only observed after direct cholinergic stimulation. The distal ileum smooth muscle function, in contrast, was barely affected by the desipramine administration. It is important to stress that these changes were not associated with a significant dysfunction in either micturition or fecal output, at least not following i.p. injection. This could indicate that the CNS and/or the ENS may compensate for these local changes in smooth muscle responses seen *in vitro*. This further suggests that increased smooth muscle contractility alone is not sufficient to induce alterations in the micturition parameters or fecal output as evaluated in this study.

The significant increase in high K^+ response per mg tissue in the urinary bladder following i.p. injection suggests that a direct muscle alteration has occurred. However, the greater changes which are observed following EFS, compared to the direct cholinergic receptor stimulation, also indicate that changes in local innervation may be important. In the colon, however, no general change in contractility could be observed. A significant change, *i.e.* decrease, could only be seen for the methacholine-induced response, but not following EFS. This indicates that the direct muscarinic receptor response has been altered, but local innervation, including serotonin, dopamine and/or noradrenaline systems, may have compensated for these changes. Importantly, these changes were not weight-dependent since normalization showed the same significant alterations. On the other hand, the urinary bladder, following s.c. injections of desipramine, showed a trend of less contractility, as seen in high K^+ response per mg tissue, which coincides with a significantly reduced response following both EFS and methacholine stimulations. This could indicate that both the muscle innervation as well as cholinergic receptor expression may have been altered. These changes were also not tissue weight-dependent as the same was observed following normalization. Following s.c. injection of desipramine, the colon showed a significantly increased muscarinic receptor response, and a non-significant increase during EFS. However, in this case the tissue weight was significantly different between the groups, which may partly explain these changes. This is further strengthened by the fact that the significant difference was abolished after normalization to tissue weight and by the lack of difference in high K^+ response per mg tissue.

Desipramine has several effects on peripheral organs and the brain. Studies have shown that desipramine, although considered to have a high selectivity for noradrenaline, also interferes with serotonin metabolism as well as exerts inhibitory effects on cholinergic (muscarinic), histaminergic and adrenergic receptors in the brain of several species

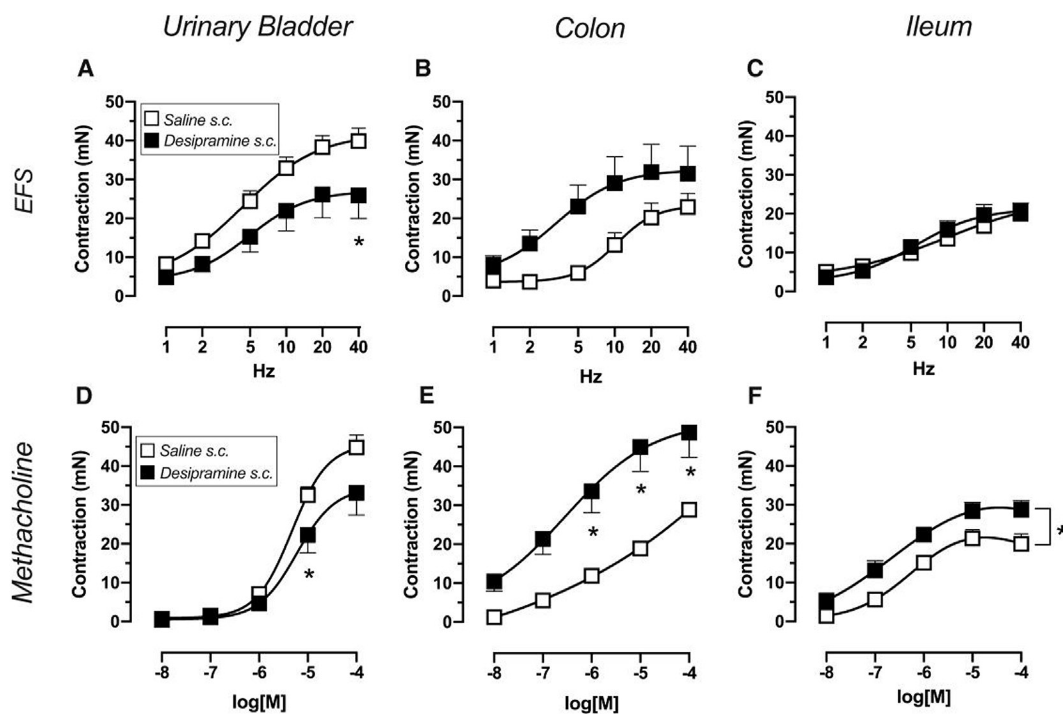

Figure 4. Smooth muscle contractions following electrical (EFS)- and methacholine-induced stimulations in subcutaneous (s.c.)-treated animals. Urinary bladder muscle contractions were significantly lower in *Desipramine s.c.*-treated rats (n = 9), as compared to *Saline s.c.*-treated rats (n = 10), following both EFS (A) and methacholine stimulations (D). Increased response could in turn be observed in the proximal colon, where a non-significant trend was evident in EFS-induced contraction (B; *Saline s.c.*, n = 5 and *Desipramine s.c*, n = 7) and a significant increase in the methacholine-induced response (E; *Saline s.c.*, n = 5 and *Desipramine s.c.*, n = 9). In distal ileum, no changes in response between the groups were seen in response to EFS (C), but an overall significant increase in the response to methacholine (F) was observed in the *Desipramine s.c.*-treated group (n = 4) as compared to the Saline s.c.-treated group (n = 9). * = different from *Saline i.p.* group; Two-way ANOVAs A: $F(1,17) = 4.36$, $p = 0.052$ for group and $F(1,17) = 2.65$, $p = 0.028$ for concentration-group interaction; B: $F(1,10 = 2.96)$, $p = 0.12$ for group and $F(5,50 = 2.16$, $p = 0.073$ for concentration-group interaction C: $F(1,11 = 0.098)$, $p = 0.76$; D: $F(1,17 = 3.62)$, $p = 0.074$ for group and $F(5,85 = 2.96)$, $p = 0.017$ for concentration-group interaction; E: $F(1,12 = 8.18)$, $p = 0.014$, and F: $F(1,11 = 7.32)$, $p = 0.021$; followed by Sidak posthoc test. Data are presented as mean ± SEM.

including the rat (Esteban et al., 1999; Owens et al., 1997; Rehavi et al., 1987). Acute effects of desipramine, through inhibition of neuronal uptake, result in activation of brain presynaptic adrenergic α_{2A}-as well as 5-HT_{1A} autoreceptors that regulate the synthesis and release of neurotransmitters. Moreover, when chronically administrated, desipramine can cause desensitization and/or down-regulation of certain receptors, for instance α_2-adrenoceptors (Esteban et al., 1999; Mateo et al., 2001). More specifically desipramine has been demonstrated to have a direct effect on the smooth muscle of human and guinea-pig urinary bladder, urethra, intestine and coronary artery in rabbits (Marino et al., 1994; Obara et al., 2019; Rehavi et al., 1987; Shin et al., 2018). This may partly be related to desipramine's anticholinergic effects which are clearly observed in patients taking desipramine as an antidepressant, where side effects including dry mouth, urinary dysfunction and constipation are common (Maan and Saadabadi, 2020).

It is also important to stress that the acute dose presently used during 6-OHDA lesion experiments is 25–30 mg/kg, which is approx. 2.5–10 fold higher than acute doses which cause significant effects on brain adrenergic and serotonergic systems (Esteban et al., 1999). With a half-life of desipramine of 12–54 h in man (Nagy and Johansson, 1975), together with that the concentration of its active metabolite DDMI (active in rats but not man) is stable for around 12 h before initial decrease in rats (Kozisek et al., 2007), it is likely that the high concentration used during 6-OHDA lesions may mimic a more sub-chronic dosing rather than acute. With this in mind it is plausible to believe that a single dose of desipramine, used in the 6-OHDA rat lesion model of PD, could have a longer-lasting effect on the expression and function of muscarinic receptors in the urinary bladder and gastrointestinal tract.

Desipramine's contrasting effects on smooth muscle contractility depending on the route of administration indicates that the changes may also be related to an indirect mechanical or toxic effect in proximity to the injection, *i.e.* the urinary bladder and colon. An unselective toxic effect may be caused by the acidity of the desipramine solution, which was measured to pH 4.95. We observed a significant immunological reaction to the desipramine hydrochloride at the site of injection when administered s.c.. In all animals severe itching was observed and in a significant number of rats necrotic skin tissue around the injection site was seen. However, a similar reaction in the peritoneum could not be seen following i.p. injection, during postmortem ocular examination of the inner organs. This indicates that this phenomenon cannot explain the altered smooth muscle function in the i.p. group.

Interestingly, desipramine has been shown to cause significant chromosomal damage in the bone marrow of mice following a single i.p. dose at the same dose range as used in the 6-OHDA model (Madrigal--Bujaidar et al., 2010). Moreover, desipramine has consistently been shown to concentration-dependently cause apoptosis in different cell lines, including colon carcinoma cells *in vitro* (Arimochi and Morita, 2008; Chang et al., 2008; Ho et al., 2005). Taken together, this may contribute to the differential contractile changes following i.p. vs. s.c. administration as the concentration of the bolus dose i.p. may lead to a significantly higher local concentration close to the urinary bladder and colon.

Contrarily to the colon, the ileum was not substantially affected by desipramine. This may be explained, at least following the i.p. injection, by local toxicity. Specifically, by that the colon is closer to the injection site than the ileum. However, the colon, but not ileum, is also

significantly affected by the s.c. injection. This may be explained by differences in ENS and CNS innervation and the composition of the receptor signaling in between the two intestinal tissues. However, this is not within the scope of the current study, but may be interesting to further investigate in future studies.

Finally, it is important to note that in this study we only used male adult rats, which limits the interpretation. It is unknown if the same phenomena can also be seen in female rats. It has been shown that i.p. injections of desipramine leads to 2–4 times higher concentrations of the drug in female (compared to male) rat brain (Biegon and Samuel, 1979). The concentrations are also dependent on the hormone cycle, where lower concentrations are observed at proestrus and higher at estrus, which may also influence desipramine's noradrenergic effect in rats (Biegon and Samuel, 1979; Shah and Frazer, 2014).

5. Conclusion

The data in the current study conclude that desipramine, which is frequently used in order to protect the noradrenergic neurons following 6-OHDA lesions, could lead to long-term functional changes in smooth muscle tissue, specifically in the urinary bladder and the proximal colon in the rat. We therefore suggest that desipramine pre-treatment should be omitted in the 6-OHDA lesion rodent model of PD, in particular when investigating the effect of central DA degeneration on peripheral organ function.

Declarations

Author contribution statement

M.P. Murillo: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

P. Aronsson: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

M. Winder: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

T. Carlsson: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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Declaration of interests statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

References

Alzoubi, K.H., Mokhemer, E., Abuirmeileh, A.N., 2018. Beneficial effect of etazolate on depression-like behavior and, learning, and memory impairment in a model of Parkinson's disease. Behav. Brain Res. 350, 109–115.

Arimochi, H., Morita, K., 2008. Desipramine induces apoptotic cell death through nonmitochondrial and mitochondrial pathways in different types of human colon carcinoma cells. Pharmacology 81, 164–172.

Bell, L.J., Iversen, L.L., Uretsky, N.J., 1970. Time course of the effects of 6-hydroxydopamine on catecholamine-containing neurones in rat hypothalamus and striatum. Br. J. Pharmacol. 40, 790–799.

Biegon, A., Samuel, D., 1979. The in vivo distribution of an antidepressant drug (DMI) in male and female rats. Psychopharmacology (Berl) 65, 259–263.

Blandini, F., Balestra, B., Levandis, G., Cervio, M., Greco, R., Tassorelli, C., Colucci, M., Faniglione, M., Bazzini, E., Nappi, G., Clavenzani, P., Vigneri, S., De Giorgio, R., Tonini, M., 2009. Functional and neurochemical changes of the gastrointestinal tract in a rodent model of Parkinson's disease. Neurosci. Lett. 467, 203–207.

Chang, H.C., Huang, C.C., Huang, C.J., Cheng, J.S., Liu, S.I., Tsai, J.Y., Chang, H.T., Huang, J.K., Chou, C.T., Jan, C.R., 2008. Desipramine-induced apoptosis in human PC3 prostate cancer cells: activation of JNK kinase and caspase-3 pathways and a protective role of [Ca2+]i elevation. Toxicology 250, 9–14.

Colucci, M., Cervio, M., Faniglione, M., De Angelis, S., Pajoro, M., Levandis, G., Tassorelli, C., Blandini, F., Feletti, F., De Giorgio, R., Dellabianca, A., Tonini, S., Tonini, M., 2012. Intestinal dysmotility and enteric neurochemical changes in a Parkinson's disease rat model. Auton. Neurosci. 169, 77–86.

Ermine, C.M., Wright, J.L., Frausin, S., Kauhausen, J.A., Parish, C.L., Stanic, D., Thompson, L.H., 2018. Modelling the dopamine and noradrenergic cell loss that occurs in Parkinson's disease and the impact on hippocampal neurogenesis. Hippocampus 28, 327–337.

Esteban, S., Llado, J., Sastre-Coll, A., Garcia-Sevilla, J.A., 1999. Activation and desensitization by cyclic antidepressant drugs of alpha2-autoreceptors, alpha2-heteroreceptors and 5-HT1A-autoreceptors regulating monamine synthesis in the rat brain in vivo. Naunyn-Schmiedeberg's Arch. Pharmacol. 360, 135–143.

Fornai, M., Pellegrini, C., Antonioli, L., Segnani, C., Ippolito, C., Barocelli, E., Ballabeni, V., Vegezzi, G., Al Harraq, Z., Blandini, F., Levandis, G., Cerri, S., Blandizzi, C., Bernardini, N., Colucci, R., 2016. Enteric dysfunctions in experimental Parkinson's disease: alterations of excitatory cholinergic neurotransmission regulating colonic motility in rats. J. Pharmacol. Exp. Therapeut. 356, 434–444.

Ho, C.M., Kuo, S.Y., Chen, C.H., Huang, J.K., Jan, C.R., 2005. Effect of desipramine on Ca2+ levels and growth in renal tubular cells. Cell. Signal. 17, 837–845.

Kirik, D., Rosenblad, C., Bjorklund, A., 1998. Characterization of behavioral and neurodegenerative changes following partial lesions of the nigrostriatal dopamine system induced by intrastriatal 6-hydroxydopamine in the rat. Exp. Neurol. 152, 259–277.

Kostrzewa, J.P., Kostrzewa, R.M., 2020. p-Chloroamphetamine-Enhanced neostriatal dopamine exocytosis in rats neonatally Co-lesioned with 6-OHDA and 5,7-DHT: relevance to Parkinson's disease. Neurotox. Res. 37, 543–552.

Kozisek, M.E., Deupree, J.D., Burke, W.J., Bylund, D.B., 2007. Appropriate dosing regimens for treating juvenile rats with desipramine for neuropharmacological and behavioral studies. J. Neurosci. Methods 163, 83–91.

Levandis, G., Balestra, B., Siani, F., Rizzo, V., Ghezzi, C., Ambrosi, G., Cerri, S., Bonizzi, A., Vicini, R., Vairetti, M., Ferrigno, A., Pastoris, O., Blandini, F., 2015. Response of colonic motility to dopaminergic stimulation is subverted in rats with nigrostriatal lesion: relevance to gastrointestinal dysfunctions in Parkinson's disease. Neuro Gastroenterol. Motil. 27, 1783–1795.

Lindgren, H.S., Demirbugen, M., Bergqvist, F., Lane, E.L., Dunnett, S.B., 2014. The effect of additional noradrenergic and serotonergic depletion on a lateralised choice reaction time task in rats with nigral 6-OHDA lesions. Exp. Neurol. 253, 52–62.

Maan, J.S., Saadabadi, A., 2020. Desipramine. StatPearls: Treasure Island (FL).

Madrigal-Bujaidar, E., Cardenas Garcia, Y., Alvarez-Gonzalez, I., 2010. Chromosomal aberrations induced by imipramine and desipramine in mouse. Hum. Exp. Toxicol. 29, 297–302.

Marino, F., Marcoli, M., De Ponti, F., Cosentino, M., Lecchini, S., Frigo, G.M., 1994. Effect of desipramine-induced blockade of neuronal uptake mechanisms on adrenoceptor-mediated responses in the Guinea-pig colon. Naunyn-Schmiedeberg's Arch. Pharmacol. 350, 499–506.

Mateo, Y., Fernández-Pastor, B., Meana, J.J., 2001. Acute and chronic effects of desipramine and clorgyline on alpha(2)-adrenoceptors regulating noradrenergic transmission in the rat brain: a dual-probe microdialysis study. Br. J. Pharmacol. 133, 1362–1370.

Mitra, R., Aronsson, P., Winder, M., Tobin, G., Bergquist, F., Carlsson, T., 2015. Local change in urinary bladder contractility following CNS dopamine denervation in the 6-OHDA rat model of Parkinson's disease. J. Parkinsons Dis. 5, 301–311.

Nagy, A., Johansson, R., 1975. Plasma levels of imipramine and desipramine in man after different routes of administration. Naunyn-Schmiedeberg's Arch. Pharmacol. 290, 145–160.

Obara, K., Imanaka, S., Fukuhara, H., Yamaki, F., Matsuo, K., Yoshio, T., Tanaka, Y., 2019. Evaluation of the potentiating effects of antidepressants on the contractile response to noradrenaline in Guinea pig urethra smooth muscles. Clin. Exp. Pharmacol. Physiol. 46, 444–455.

Owens, M.J., Morgan, W.N., Plott, S.J., Nemeroff, C.B., 1997. Neurotransmitter receptor and transporter binding profile of antidepressants and their metabolites. J. Pharmacol. Exp. Therapeut. 283, 1305–1322.

Pellegrini, C., Antonioli, L., Colucci, R., Tirotta, E., Gentile, D., Ippolito, C., Segnani, C., Levandis, G., Cerri, S., Blandini, F., Barocelli, E., Ballabeni, V., Bernardini, N., Blandizzi, C., Fornai, M., 2017. Effects of L-DOPA/benserazide co-treatment on colonic excitatory cholinergic motility and enteric inflammation following dopaminergic nigrostriatal neurodegeneration. Neuropharmacology 123, 22–33.

Pellegrini, C., Fornai, M., Colucci, R., Tirotta, E., Blandini, F., Levandis, G., Cerri, S., Segnani, C., Ippolito, C., Bernardini, N., Cseri, K., Blandizzi, C., Hasko, G., Antonioli, L., 2016. Alteration of colonic excitatory tachykininergic motility and enteric inflammation following dopaminergic nigrostriatal neurodegeneration. J. Neuroinflammation 13, 146.

Pellegrini, C., Ippolito, C., Segnani, C., Dolfi, A., Errede, M., Virgintino, D., Fornai, M., Antonioli, L., Garelli, F., Nericcio, A., Colucci, R., Cerri, S., Blandini, F., Blandizzi, C., Bernardini, N., 2020. Pathological remodelling of colonic wall following dopaminergic nigrostriatal neurodegeneration. Neurobiol. Dis. 139, 104821.

Reader, T.A., Gauthier, P., 1984. Catecholamines and serotonin in the rat central nervous system after 6-OHDA, 5-7-DHT and p-CPA. J. Neural. Transm. 59, 207–227.

Rehavi, M., Weiss, H., Nissenkorn, I., Rubinstein, R., Cohen, S., 1987. A comparative study of the affinities of some tricyclic antidepressants for the muscarinic cholinergic receptor in human and Guinea-pig bladder, ileum and brain in relation to differential drug potency. Life Sci. 40, 1819–1827.
Shah, A., Frazer, A., 2014. Influence of acute or chronic administration of ovarian hormones on the effects of desipramine in the forced swim test in female rats. Psychopharmacology (Berl) 231, 3685–3694.
Shin, S.E., Li, H., An, J.R., Seo, M.S., Na, S.H., Jung, W.K., Firth, A.L., Ha, K.S., Han, E.T., Hong, S.H., Choi, I.W., Park, W.S., 2018. Inhibition of the voltage-dependent K(+) current by the tricyclic antidepressant desipramine in rabbit coronary arterial smooth muscle cells. Cardiovasc. Toxicol. 18, 252–260.
Soler, R., Fullhase, C., Santos, C., Andersson, K.E., 2011. Development of bladder dysfunction in a rat model of dopaminergic brain lesion. Neurourol. Urodyn. 30, 188–193.
Taghzouti, K., Le Moal, M., Simon, H., 1991. Suppression of noradrenergic innervation compensates for behavioral deficits induced by lesion of dopaminergic terminals in the lateral septum. Brain Res. 552, 124–128.
Takahashi, H., Inoue, A., Takeda, K., Okajima, H., Sasaki, S., Yoshimura, M., Nakagawa, M., Ijichi, H., 1984. Augmented central cholinergic mechanisms in spontaneously hypertensive rats. Involvement of deranged noradrenergic mechanisms in the brain. Jpn. Heart J. 25, 397–410.
Tobin, G., Sjogren, C., 1995. In vivo and in vitro effects of muscarinic receptor antagonists on contractions and release of [3H]acetylcholine in the rabbit urinary bladder. Eur. J. Pharmacol. 281, 1–8.
Ungerstedt, U., 1968. 6-Hydroxy-dopamine induced degeneration of central monoamine neurons. Eur. J. Pharmacol. 5, 107–110.
Yoshimura, N., Kuno, S., Chancellor, M.B., De Groat, W.C., Seki, S., 2003. Dopaminergic mechanisms underlying bladder hyperactivity in rats with a unilateral 6-hydroxy-dopamine (6-OHDA) lesion of the nigrostriatal pathway. Br. J. Pharmacol. 139, 1425–1432.
Zhang, X., Andren, P.E., Svenningsson, P., 2007. Changes on 5-HT2 receptor mRNAs in striatum and subthalamic nucleus in Parkinson's disease model. Physiol. Behav. 92, 29–33.
Zhang, X., Li, Y., Liu, C., Fan, R., Wang, P., Zheng, L., Hong, F., Feng, X., Zhang, Y., Li, L., Zhu, J., 2015. Alteration of enteric monoamines with monoamine receptors and colonic dysmotility in 6-hydroxydopamine-induced Parkinson's disease rats. Transl. Res. 166, 152–162.