Contractile Effects of Serotonin (5-HT) in the Rat Cauda Epididymis: Expression and Functional Characterization of 5-HT Receptors

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ABSTRACT
Serotonin [5-hydroxytryptamine (5-HT)] exerts multiple central and peripheral functions. High concentrations of 5-HT have been found in the epididymis, a ductal organ that plays pivotal roles in sperm transport and maturation. The contraction of the epididymal smooth muscle is essential for sperm transport and emission during ejaculation. The contributions of the epididymal 5-HT system to these events are poorly understood. Here, we assessed the contractile function of 5-HT in the rat cauda epididymis (CE), pharmacologically targeting the receptor(s) and the reuptake mechanism involved in this system. Segments of CE duct from adult Wistar rats were set up in an organ bath system for isometric tension recordings, and concentration-response curves to 5-HT and norepinephrine were obtained. 5-HT elicited concentration-dependent contractions of the CE duct (pEC\textsubscript{50} = 6.5 ± 0.1) that were potentiated with high potency by the norepinephrine transporter (NET) inhibitor desipramine and with low potency by the highly selective serotonin transporter inhibitor paroxetine, indicating that the NET is the major mediator of 5-HT reuptake in vitro. CE contractions to 5-HT were antagonized by the \(\alpha_1\)-adrenoceptor (\(\alpha_1\)-AR) antagonist prazosin (pA\textsubscript{2} = 8.9), 5-HT\textsubscript{2A}/2C antagonists ketanserin (pA\textsubscript{2} = 9.4) and fluoxetine (pA\textsubscript{2} = 7.4), and 5-HT\textsubscript{1A} ligands WAY 100635 (pA\textsubscript{2} = 8.9) and buspirone (pA\textsubscript{2} = 7.3). Reverse transcriptase polymerase chain reaction analysis demonstrated that 5-HT\textsubscript{1A} and 5-HT\textsubscript{2C} transcripts are highly abundant in the cauda epididymis, whereas 5-HT\textsubscript{2C} transcript was not found. Altogether, our results reveal that contractions of the CE duct to 5-HT encompasses at least activation of \(\alpha_1\)-ARs and 5-HT\textsubscript{1A} and 5-HT\textsubscript{2A} receptors, providing new insights into the roles of 5-HT on the epididymal function.

Introduction
Serotonin [5-hydroxytryptamine (5-HT)] is a largely distributed biogenic monoamine exerting several effects in both neuronal and non-neuronal tissues. In the central nervous system 5-HT controls behavioral processes and other functions such as emesis, body temperature, ejaculation, and circadian rhythms (Berger et al., 2009). Outside the central nervous system 5-HT is involved in a number of functions, such as vasoconstriction, nociception, intestinal motility and secretion, platelet aggregation, uterine smooth muscle contraction, among others (Hoyer et al., 1994; Berger et al., 2009). This myriad of actions of 5-HT are mediated by interactions with 14 different 5-HT receptors, which are classified into seven families (5-HT\textsubscript{1–7}). All 5-HT receptors are metabotropic seven transmembrane domain receptors (or G protein-coupled receptors) (Alexander et al., 2017a), with the exception of 5-HT\textsubscript{3}, which is an ionotropic (cation-permeable) receptor (Alexander et al., 2017b). The variety of 5-HT receptor subtypes reflects the extraordinary biologic relevance of 5-HT as a neurotransmitter, as well as a paracrine and autocrine signaling molecule.

Concerning the metabolotropic 5-HT receptors, the 5-HT\textsubscript{1} and 5-HT\textsubscript{2} classes are comprised of five (5-HT\textsubscript{1A}, 1B, 1D, 1E, 1F) and three (5-HT\textsubscript{2A}, 2B, 2C) subtypes, respectively (Hannon and Hoyer, 2008; Alexander et al., 2017a). In agreement with the widespread distribution of 5-HT receptors, the 5-HT\textsubscript{1A}, 5-HT\textsubscript{1B}, and 5-HT\textsubscript{2A} subtypes have been described in male reproductive tissues. 5-HT is known to regulate testicular blood flow and testosterone secretion (Kinson et al., 1973), and to contract the smooth muscle of male reproductive organs, such as the seminal vesicle (Kim and Paick, 2004), vas deferens (Hay and Wadsworth, 1982; Campos et al., 1999; Pedroso et al., 2017), and prostate (Steidle et al., 1989), through activation of the 5-HT\textsubscript{1} and/or 5-HT\textsubscript{2} receptor subtypes. Interestingly, high concentrations of 5-HT have been found in the epididymis, a ductal organ that plays a crucial role in sperm transport, concentration, maturation, and storage until ejaculation (Korman and Penttilä, 1968; Anderson et al., 1979). Jiménez-Trejo et al. (2007) revealed that the rat caput

ABBREVIATIONS: AR, adrenoceptor; CE, cauda epididymis; CR, concentration ratio; CRC, concentration-response curve; \(E_{\text{max}}\), maximal response; 5-HT, serotonin [5-hydroxytryptamine]; NET, norepinephrine transporter; PCR, polymerase chain reaction; RT-PCR, reverse transcriptase polymerase chain reaction; SERT, serotonin transporter; SSRI, selective serotonin reuptake inhibitor.
epididymis possesses a local serotonergic system that includes the expression of 5-HT biosynthetic enzymes, 5-HT receptors (5-HT1A, 5-HT2A, etc.), and serotonin transporters by epididymal epithelial and non-epithelial cells. Notwithstanding, the contribution of 5-HT to epididymal physiology is still poorly understood.

It has been reported that 5-HT stimulates anion secretion via 5-HT1B and 5-HT2B receptors in rat cauda epididymal cell primary cultures (Leung et al., 1999), suggesting that 5-HT plays a role in the regulation of epididymal luminal content and sperm quiescence during storage in the epididymis. Furthermore, administration of exogenous 5-HT in rats resulted in fluid accumulation in the epididymis (Singh et al., 1987). In men, high blood 5-HT levels were associated with poor sperm count and motility (Gonzales et al., 1992), and treatment with the antidepressant paroxetine, a selective serotonin reuptake inhibitor (SSRI), caused ejaculatory difficulties and increased sperm DNA fragmentation (Tanrikut et al., 2010). Despite these negative outcomes, SSRIs, such as dapoxetine, can be used in the pharmacotherapy of premature ejaculation (Giuliano and Clément, 2005; Rowland et al., 2010; Abu El-Hamd and Abdelhamid, 2018). It is thought that the effects of SSRIs on the regulation of ejaculatory function are mediated by increasing 5-HT signaling in the central nervous system. Nevertheless, peripheral mechanisms in the male reproductive organs could also be involved in these clinical outcomes (Jannini et al., 2015).

Epididymal epithelial cells are surrounded by a smooth muscle layer, in which the thickness increases from the proximal (caput) to distal (cauda) epididymis. In the cauda epididymis (CE), the thicker smooth muscle layer is densely innervated by postganglionic sympathetic nerve fibers that release norepinephrine (Ricker, 1998; Silva et al., 2010). These sympathetic nerve fibers play a crucial role in the contraction of the CE smooth muscle, which is paramount for sperm transport during the emission phase of ejaculation and hence for reproduction (Ricker et al., 1997; Pacini et al., 2018). Likewise, the presence of a dense network of serotonergic nerve fibers has been detected in the CE (Leung et al., 1999), although the role of 5-HT in the contraction of the epididymal smooth muscle remains elusive.

Taking this into account and considering: 1) the ability of 5-HT to contract smooth muscle in reproductive organs, 2) the existence of a local serotonergic system in the epididymis, and 3) the reproductive outcomes of SSRI drugs, we hypothesized that 5-HT contracts CE smooth muscle. Thus, we systematically assessed the contractions induced by 5-HT in the rat CE smooth muscle in vitro, and investigated the roles of 5-HT receptor subtypes and reuptake systems in the pharmacological events mediated by 5-HT in this tissue.

Materials and Methods

Animals and Drugs. Adult male Wistar rats (90–150 days old) were maintained under controlled conditions (12-hour/12-hour light/dark cycle, 25 ± 2°C, and 40%–70% humidity) with food and water ad libitum. All animal procedures were approved by the Ethics Committee for the Use of Experimental Animals from the Institute of Biosciences of Botucatu, São Paulo State University (Process No. 749/15) and were in agreement with the Guide for the Care and Use of Laboratory Animals (National Institutes of Health) and Animal in Research: Reporting In Vivo Experiments guidelines (Kilkenny et al., 2010). The drugs used in the experiments were obtained from the following sources: L-(−)-norepinephrine bitartrate salt monohydrate, serotonin HCl, ketanserin tartrate, WAY 100635, busiprine, and corticosterone were purchased from Sigma; fluoxetine HCl was purchased from Fagron; paroxetine was purchased from Tocris; and desipramine HCl, prazosin HCl, yohimbine HCl, and (±)-propranolol HCl were purchased from Research Biochemicals Inc.

In Vitro Contraction Studies. Contraction experiments were conducted following the procedure detailed in Pacini et al. (2018). Rats were killed by decapitation and the epididymis was carefully excised. The CE was uncoiled, cleaned of adherent tissues, and freed from intraluminal content by flushing 1 ml of nutrient solution through a blunt-ended 30 gauge needle. For digital recording of isometric contractions, segments of the distal CE (~1.0 cm in length), corresponding to region 19 according to Jelinsky et al. (2007), were mounted in organ baths under 1.0 g of resting tension in a modified Tyrode’s solution (138 mM NaCl, 5.7 mM KCl, 1.8 mM CaCl2, 0.36 mM NaH2PO4, 15 mM NaHCO3, and 5.5 mM dextrose) prepared in glass-distilled deionized water, maintained at 30°C and pH 7.4, and continuously bubbled with 95% O2/5% CO2. After a 30-minute stabilization period, CE segments were challenged with 80 mM KCl until reproducible contractions were obtained. Then, cumulative concentration-response curves (CRCs) to norepinephrine or 5-HT were obtained and taken as control curves. All curves were conducted in the presence of a cocktail of inhibitors containing corticosterone (10 μM), yohimbine (0.1 μM), and propranolol (0.1 μM) to block the monoamine uptake not mediated by the norepinephrine transporter (NET), serotonin transporter (SERT), and α2- and β-adrenoceptors (ARs), respectively.

To investigate the role of the NET and/or SERT, CRCs to 5-HT and norepinephrine were built up in the presence of fluoxetine, paroxetine (selective SERT inhibitors), and desipramine (selective NET inhibitor). The involvement of α2-ARs was investigated using prazosin (α1-AR antagonist) and that of 5-HT receptors was investigated using ketanserin (5-HT2A antagonist), WAY 100635 (5-HT1A antagonist), busiprine (5-HT1A partial agonist), or flutamide (SERT inhibitor with high affinity for 5-HT2A). All inhibitors/antagonists were previously incubated for 45 minutes to allow equilibration, and then a new CRC to either norepinephrine or 5-HT was obtained. Contractions were expressed as percentage of maximal contraction of the control curves. The potencies of norepinephrine and 5-HT in the absence and presence of antagonists are presented as pEC50 values, i.e., the negative logarithm of the concentration of 5-HT or norepinephrine producing 50% of its maximal effect (Emax). The effects of each concentration of norepinephrine or 5-HT were measured as the peak of the respective induced contraction of agonist.

Schild Analysis. Antagonist potencies and affinities were evaluated by Schild analysis (Arunlakshana and Schild, 1959). The parallel rightward displacements induced by each antagonist concentration on 5-HT or norepinephrine CRCs were quantified and used to calculate the concentration ratio (CR), which is the ratio between equeffective norepinephrine or 5-HT concentrations in the presence and absence of antagonist. The CR was plotted as log (CR − 1) versus the respective antagonist concentration in a Schild plot and analyzed by linear regression. The slopes of linear regressions were calculated, and when not different from unity the intercept in abscissa (pA2) was taken as the antagonist dissociation constant (pKd). However, as in most assays, since the slopes in the Schild plots were much lower than 1.0, the pA2 values were taken as estimates of antagonist potencies calculated from the formula pA2 = log (CR − 1) − log [B], where [B] is the lowest effective antagonist concentration.

Reverse Transcriptase Polymerase Chain Reaction. Reverse transcriptase polymerase chain reaction (RT-PCR) was performed to detect each specific gene target. Total RNA from epididymal regions (initial segment, caput, corpus, and cauda), testis, and brain was extracted using TRIzol Reagent (Thermo Fisher) according to the manufacturer’s instruction, followed by DNase treatment (DNase I Amplification Grade; Invitrogen) and first strand cDNA synthesis.
Primers and conditions for RT-PCR

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primer Sequence</th>
<th>Amplicon Size</th>
<th>Accession Number</th>
<th>Initial Denaturation</th>
<th>35 Cycles</th>
<th>Final Elongation</th>
</tr>
</thead>
<tbody>
<tr>
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<td>bp</td>
<td></td>
<td>Denaturation</td>
<td>Annealing</td>
<td>Elongation</td>
</tr>
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<td>Htr1a</td>
<td>Forward: 5'-GTCACCTGCCGACCTGTATTAT-3'</td>
<td>286</td>
<td>NM_012585.1</td>
<td>95/2</td>
<td>95/60</td>
<td>58/60</td>
</tr>
<tr>
<td></td>
<td>Reverse: 5’-CGGAAGTGGAGTAGATGGTGTT-3'</td>
<td>286</td>
<td>NM_012585.1</td>
<td>95/2</td>
<td>95/60</td>
<td>58/60</td>
</tr>
<tr>
<td>Htr1b</td>
<td>Forward: 5'-CACTGATCGCGTGGAGCTAT-3'</td>
<td>217</td>
<td>NM_022225.1</td>
<td>95/2</td>
<td>95/60</td>
<td>58/60</td>
</tr>
<tr>
<td></td>
<td>Reverse: 5’-GAGCAGGTGGTGAATAGAA-3'</td>
<td>217</td>
<td>NM_022225.1</td>
<td>95/2</td>
<td>95/60</td>
<td>58/60</td>
</tr>
<tr>
<td>Htr2a</td>
<td>Forward: 5’-AGCTCCAGAATGCGACACAACT-3'</td>
<td>322</td>
<td>NM_017254.1</td>
<td>95/2</td>
<td>95/60</td>
<td>60/60</td>
</tr>
<tr>
<td></td>
<td>Reverse: 5’-GGTATTGGCATGGATATACCTAC-3'</td>
<td>322</td>
<td>NM_017254.1</td>
<td>95/2</td>
<td>95/60</td>
<td>60/60</td>
</tr>
<tr>
<td>Htr2b</td>
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<td>224</td>
<td>NM_017250.1</td>
<td>95/2</td>
<td>95/60</td>
<td>58/60</td>
</tr>
<tr>
<td></td>
<td>Reverse: 5’-TGACACATACACGGCTCTAC-3'</td>
<td>224</td>
<td>NM_017250.1</td>
<td>95/2</td>
<td>95/60</td>
<td>58/60</td>
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<td>Htr2c</td>
<td>Forward: 5’-GCTACCCGTGTTCTCAACTAT-3'</td>
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<td>95/2</td>
<td>95/60</td>
<td>60/60</td>
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<td></td>
<td>Reverse: 5’-GACGAGGTGAAGTGAATGGC-3'</td>
<td>322</td>
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<td>95/2</td>
<td>95/60</td>
<td>60/60</td>
</tr>
<tr>
<td>Ppia</td>
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<td>95/60</td>
<td>60/45</td>
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<td>174</td>
<td>NM_017101.1</td>
<td>95/2</td>
<td>95/60</td>
<td>60/45</td>
</tr>
</tbody>
</table>

bp, base pair.
for prazosin against 5-HT was much lower than theoretical unity (Fig. 3, A and C; Table 3), while the antagonism presented against norepinephrine was consistent with a competitive antagonism (Fig. 3, B and C; Table 3). Therefore, the effects of subtype-selective 5-HT receptor antagonists were evaluated in the presence of 100 nM prazosin to prevent activation of α1-ARs by 5-HT.

Increasing concentrations of the 5-HT2A/5-HT2C receptor antagonist ketanserin (1–30 nM) produced rightward shifts on the CRCs to 5-HT, although insurmountable antagonism was observed with concentrations from 3 to 30 nM, which reduced the E\text{\textsubscript{\text{max}}} value for 5-HT (Fig. 4A). To further investigate the putative serotonergic receptors involved in 5-HT-induced contraction in the CE duct, the contraction evoked by 5-HT was evaluated in the presence of increasing concentrations of WAY 100635 (5-HT1A antagonist) and buspirone (5-HT1A partial agonist). Both acted as noncompetitive antagonists (slope less than unity), providing estimated p\text{A\textsubscript{2}} values of 8.90 ± 0.04 and 7.34 ± 0.14, respectively (Fig. 4, B and C; Table 3). At concentrations in which fluoxetine displays affinity at 5-HT\textsubscript{2A} and 5-HT\textsubscript{2C} receptors (Bonhaus et al., 1997; Ni and Miledi, 1997; Owens et al., 1997; Rothman et al., 2000), 5-HT-induced contractions of the CE duct were right shifted in a surmountable manner with an estimated p\text{A\textsubscript{2}} value of 7.44 ± 0.16 (Fig. 4D; Table 3).

\textbf{Htr}1\textit{a}, \textbf{Htr}1\textit{b}, \textbf{Htr}2\textit{a}, and \textbf{Htr}2\textit{b}, but Not the \textbf{Htr}2\textit{c} Transcript, Are Expressed in the Rat Epididymis.

Based on the pharmacological data, we performed conventional RT-PCR assays to investigate the expression of \textit{Htr}1\textit{a}, \textit{Htr}1\textit{b}, \textit{Htr}2\textit{a}, \textit{Htr}2\textit{b}, and \textit{Htr}2\textit{c} transcripts along the rat epididymis (initial segment, caput, corpus, and cauda regions). The detection of these transcripts either in the brain or testis was used as positive controls (Fig. 5). Both the \textit{Htr}1\textit{a} and \textit{Htr}2\textit{b} transcripts were detected in the corpus and CE, while the \textit{Htr}2\textit{a} transcript appeared in the CE only (Fig. 5). Conversely, the \textit{Htr}1\textit{b} transcript was found to be ubiquitously expressed throughout the epididymis, with higher abundance in the CE (Fig. 5). The expression of the \textit{Htr}2\textit{c} transcript was not detected in any region of the epididymis (Fig. 5).

\textbf{Discussion}

We revealed that 5-HT is a contractile agent in the rat CE. The 5-HT\textsubscript{1A} and 5-HT\textsubscript{2A} receptors are involved in the 5-HT-mediated contractions of the rat CE smooth muscle, suggesting that this serotonergic transmission might contribute to the coordinated contraction of the CE duct during the transport of spermatozoa within the epididymis. This epididymal event is crucial for the production of a fertile ejaculate since its disruption may affect fertility (Ricker et al., 1997; Solomon et al., 1997; Kempinas et al., 1998). Indeed, the blockade of epididymal and vas deferens contractions during the emission phase of ejaculation has been proposed as a pharmacological strategy for male contraception (Sanbe et al., 2007; White et al., 2013). Our results underscore that the serotonergic system in the epididymis should also be considered when targeting epididymal/vas deferens smooth muscle as a potential target for male contraception.

When used at concentrations within their selectivity windows, some of the most selective SERT inhibitors including paroxetine (0.1–10 nM) and fluoxetine (1–10 nM) were unable to increase the potency of 5-HT in the CE, indicating that the SERT is not involved in the removal of 5-HT. However, paroxetine at concentrations higher than 100 nM caused a 5-fold increase in the potency of 5-HT, which likely results from paroxetine’s selectivity loss, and hence NET inhibition.

![Figure 1](https://example.com/fig1.png)

**Fig. 1.** Contractile effects of 5-HT and norepinephrine in the rat CE duct. (A) Mean concentration-response curves in the presence of cocktail of inhibitors for contractions induced by norepinephrine (○) and 5-HT (●), as measured by the peak of the phasic effect. Values represent mean ± S.E.M. of 11 and seven independent experiments to norepinephrine and 5-HT, respectively. (B) Representative recording of baseline before CRCs. (C and D) Representative recordings of contractions of the CE duct in vitro to norepinephrine and 5-HT, respectively.
In fact, at such high concentrations paroxetine also potentiated the effect of norepinephrine, although to a greater extent than for 5-HT; this observation is supported by the higher affinity of the NET for norepinephrine than for 5-HT (Andersen, 1989; Tatsumi et al., 1997; Rothman et al., 2001). The selective NET inhibitor desipramine also potentiated the effects of both norepinephrine and

Fig. 2. Differential effects of SERT and NET inhibitors on the 5-HT- and norepinephrine-induced contraction in the rat CE duct. Concentration-response curves to 5-HT are shown on the left and norepinephrine are shown on the right, in the presence of different concentrations of the selective SERT inhibitors paroxetine (A–D) and fluoxetine (E–H) and in the presence of the NET inhibitor desipramine (I and J). Each symbol represents the mean and the vertical bars, when larger than the symbols, represent the S.E.M. of independent experiments performed with CE ducts from four rats.
TABLE 2

pEC50 values for 5-HT and norepinephrine in the absence and presence of monoamine reuptake inhibitors with the respective concentration ratio

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>Concentration</th>
<th>5-HT pEC50</th>
<th>Norepinephrine pEC50</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>nM</td>
<td>CR</td>
<td></td>
</tr>
<tr>
<td>Paroxetine</td>
<td>0</td>
<td>6.31 ± 0.11</td>
<td>5.83 ± 0.10</td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>6.39 ± 0.16</td>
<td>5.94 ± 0.10</td>
</tr>
<tr>
<td></td>
<td>0.3</td>
<td>6.36 ± 0.17</td>
<td>5.97 ± 0.10</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>6.21 ± 0.16</td>
<td>6.05 ± 0.13</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>6.18 ± 0.14</td>
<td>6.05 ± 0.11</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>6.41 ± 0.06</td>
<td>6.10 ± 0.11</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>6.32 ± 0.13</td>
<td>6.19 ± 0.25</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>6.74 ± 0.08</td>
<td>6.61 ± 0.10</td>
</tr>
<tr>
<td></td>
<td>300</td>
<td>7.03 ± 0.11</td>
<td>7.05 ± 0.11</td>
</tr>
<tr>
<td></td>
<td>1000</td>
<td>7.03 ± 0.08</td>
<td>7.35 ± 0.15</td>
</tr>
<tr>
<td>Fluoxetine</td>
<td>0</td>
<td>6.62 ± 0.13</td>
<td>5.90 ± 0.10</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>6.48 ± 0.13</td>
<td>5.66 ± 0.08</td>
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<td></td>
<td>3</td>
<td>6.42 ± 0.18</td>
<td>5.80 ± 0.12</td>
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<td>5.71 ± 0.08</td>
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<td>30</td>
<td>6.33 ± 0.20</td>
<td>6.12 ± 0.14</td>
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<tr>
<td></td>
<td>100</td>
<td>6.12 ± 0.23</td>
<td>6.23 ± 0.07</td>
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<tr>
<td></td>
<td>300</td>
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<td>6.33 ± 0.10</td>
</tr>
<tr>
<td></td>
<td>1000</td>
<td>ND</td>
<td>6.21 ± 0.14</td>
</tr>
<tr>
<td>Desipramine</td>
<td>0</td>
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<td>5.70 ± 0.12</td>
</tr>
<tr>
<td></td>
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<td>6.72 ± 0.17</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>6.87 ± 0.19</td>
<td>4.1 ND</td>
</tr>
</tbody>
</table>
| ND, Not determined.

5-HT, further supporting the role of the NET in the removal of these two agonists in the CE. Although the presence of the SERT has been reported in epithelial, endothelial, and mast cells of the rat caput epididymis by immunohistochemistry (Jiménez-Trejo et al., 2007), we are not aware of studies describing its presence in epididymal smooth muscle. This may indicate the existence of region- and cell-specific mechanisms for 5-HT removal in the epididymis. It is worthwhile to establish the mechanisms of 5-HT removal in the epididymis in vivo considering both adverse and therapeutic effects of SSRIs on sperm parameters as well as on ejaculatory function (Gigliano and Clément, 2005; Kendirci et al., 2007; Rowland et al., 2010; Tanrikut et al., 2010; Abu El-Hamd and Abdelhamed, 2018).

It is known that 5-HT contracts vascular smooth muscles in vitro through α1-AR activation (Innes, 1962; Purdy et al., 1987; Shaw et al., 2000). Our results showed that part of the 5-HT-mediated contraction of the CE smooth muscle is due to activation of α2A-ARs. Indeed, the selective α1-AR antagonist prazosin, which exhibits low affinity for serotoninergic receptors (Norman et al., 1985; Cossery et al., 1987; Lyon et al., 1987; Pauwels et al., 1993), antagonized 5-HT-induced contraction, yielding an estimated potency of pA2 = ~8.9 (Table 3) that correlated with the value obtained for prazosin against norepinephrine as well with previous reports of pK5 values at α1-ARs (Pupo, 1998; Lima et al., 2005). Moreover, the Schild plot for prazosin against 5-HT yielded a regression line with a slope that was less than theoretical unity, indicating that 5-HT activates other receptor populations in addition to α2A-ARs.

Considering that the presence of 5-HT G protein-coupled receptors (at least 5-HT1A, 5-HT1B, and 5-HT2B) has already been demonstrated in the rat epididymis by histochemical and pharmacological approaches (Leung et al., 1999; Jiménez-Trejo et al., 2007), it is reasonable to hypothesize that the 5-HT metabotropic receptor(s) may be involved in the contractions of the CE smooth muscle to 5-HT. To explore this, the effects of selective 5-HT receptor antagonists on 5-HT-induced contractions were determined in the presence of desipramine to block the removal of 5-HT by the NET and prazosin to antagonize α1-ARs.

The potencies found for ketanserin (~9.4) and fluoxetine (7.4) in antagonizing the contractions of the CE to 5-HT are consistent with the participation of 5-HT2A/5-HT2C. This is due to the limited selectivity of ketanserin (Roth et al., 1992; Boess and Martin, 1994; Glennon et al., 2002; Knight et al., 2004) and fluoxetine (Sánchez and Hyttel, 1999; Rothman et al., 2000; Knight et al., 2004) for 5-HT2A over 5-HT2C receptors, and vice versa, thereby showing a weak ability to discriminate between 5-HT2A and 5-HT2C receptors. However, whereas mRNA encoding 5-HT2A receptors was readily detected in the CE, transcripts for 5-HT2C were not. Thus, it is likely that 5-HT2A receptors have an important role in the contractions induced by 5-HT in the rat CE. It is worth noting that ketanserin presented insurmountable behavior against 5-HT in the CE. This may result from hemi-equilibrium, where the slow dissociation of ketanserin from the receptor within the short time frame required for the contraction leads to reduction in the observed 5-HT maximal response (Kenakin et al., 2006). Interestingly, ketanserin also presented insurmountable antagonism in the contractions induced by 5-HT in the rat uterus, another tissue whose contractions are fast and transient (Ichida et al., 1983).

Fig. 3. Effect of prazosin on the 5-HT- and norepinephrine-induced contractions in the rat CE duct. Concentration-response curves for 5-HT (A) and norepinephrine (B). The Schild plot for these antagonisms is presented in (C). Each symbol represents the mean and the vertical line, when greater than the symbol, represents the S.E.M. of independent experiments performed with CE ducts from three to six rats.
In addition to the participation of 5-HT_{2A} receptors, we also found evidence for the involvement of 5-HT_{1A} in the contractions induced by 5-HT in the CE. The contractions induced by 5-HT were antagonized with high potency by the selective 5-HT_{1A} ligands WAY-100635 (p_{A2} \sim 8.9) and buspirone (p_{A2} \sim 7.3); however, the Schild slopes were much less than unity, further supporting the participation of multiple 5-HT receptors. Indeed, our RT-PCR experiments demonstrated that 5-HT_{1A} and 5-HT_{1B} transcripts are abundantly present in the rat CE. However, additional studies are warranted to investigate the role of 5-HT_{1B} in the contraction of rat CE by 5-HT.

A dense network of interstitial serotonergic fibers has been identified in close proximity to the smooth muscle layer of the distal segment of the CE (Leung et al., 1999). This pattern of innervation is similar to the sympathetic innervation, which is known to play a major role in the contraction of epididymal

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**TABLE 3**

<table>
<thead>
<tr>
<th>Antagonist</th>
<th>Agonist</th>
<th>n</th>
<th>p_{A2}</th>
<th>Schild Slope (95% Confidence Interval)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prazosin</td>
<td>Norepinephrine</td>
<td>4</td>
<td>8.92 ± 0.06</td>
<td>1.10 ± 0.10 (0.88–1.33)</td>
</tr>
<tr>
<td>Prazosin</td>
<td>5-HT</td>
<td>4</td>
<td>8.89 ± 0.20</td>
<td>0.58 ± 0.09 (0.37–0.79)</td>
</tr>
<tr>
<td>Ketanserin</td>
<td>5-HT</td>
<td>6</td>
<td>9.36 ± 0.17</td>
<td>—</td>
</tr>
<tr>
<td>Fluoxetine</td>
<td>5-HT</td>
<td>6</td>
<td>7.41 ± 0.16</td>
<td>0.44 ± 0.14 (0.13–0.75)</td>
</tr>
<tr>
<td>WAY 100635</td>
<td>5-HT</td>
<td>4</td>
<td>8.90 ± 0.04</td>
<td>0.62 ± 0.13 (0.26–0.97)</td>
</tr>
<tr>
<td>Buspirone</td>
<td>5-HT</td>
<td>4</td>
<td>7.34 ± 0.14</td>
<td>0.20 ± 0.13 (~0.07 to 0.47)</td>
</tr>
</tbody>
</table>

*This could not be examined because the antagonism was insurmountable.*
smooth muscle, transport of spermatozoa, and fertility (Kempinas et al., 1998; Ricker, 1998). In agreement with these observations, our results showed that the abundance of all 5-HT receptor transcripts analyzed was higher in the cauda epididymis when compared with proximal regions of this organ. It is recognized that proximal and distal epididymal regions display different morphologic and functional properties (Hinton and Robaire, 2015). Thus, further studies are warranted to understand the roles of 5-HT as a factor regulating the smooth muscle contractions also in the proximal regions of the epididymis.

It has been proposed that 5-HT regulates anion secretion from the CE epithelium via 5-HT1B and 5-HT2B receptors (Leung et al., 1999) and our data provide new insights into the potential roles of 5-HT as a signaling molecule regulating sperm transport and storage in the epididymis. Our study further contributes to the understanding of the peripheral effects of serotonergic drugs in the male reproductive tract.

Authorship Contributions

**Participated in research design:** Mueller, Kiguti, Silva, Pupo.

**Conducted experiments:** Mueller, Kiguti.

**Contributed new reagents or analytic tools:** Silva, Pupo.

**Performed data analysis:** Mueller, Kiguti, Silva, Pupo.

**Wrote or contributed to the writing of the manuscript:** Mueller, Kiguti, Silva, Pupo.

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