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Physiological and pharmacological impact of oxytocin on epididymal propulsion during the ejaculatory process in rodents and men

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Abstract

During the emission phase of ejaculation, the sperm is driven from the cauda epididymidis, where it is stored, through the vas deferens by strong contractions. These contractions are thought of as being mainly induced by the sympathetic nervous system and the neurotransmitter noradrenaline. In the present study, we investigated the effect of oxytocin (suggested to exert effects during ejaculation as well) on defined segments of the rat and human epididymis using live imaging. Our results indicate that it is the very last part of the epididymis, segment 19 (S19) in rat and likewise segment 9 in human, which responds in a uniquely strong and rapid manner to oxytocin (similar to noradrenaline). Because of the complex nature of this contractile response, we developed an imaging analysis method, which allowed us to quantify multidirectional contractions and to display them using heat maps. The reaction of S19 to oxytocin was concentration-dependent and could be inhibited by pretreatment with oxytocin antagonists (atosiban and cligosiban), but not with an arginine vasopressin 1_A antagonist (SR49059). In both rat and human tissue, pretreatment with the alpha-1 adrenoreceptor antagonist tamsulosin inhibited the response to noradrenaline, whereas the effect of oxytocin was unimpaired. Our data (from men and rodents) strongly suggest that the hormone oxytocin is involved in the ejaculatory process. Thus, oxytocin-based medications might be a promising non-adrenergic treatment option for ejaculatory disorders. Additionally, we propose that S19 could be an advantageous model (detecting very low concentrations of oxytocin) to test the bioactivity of new oxytocin agonists and oxytocin antagonists.

KEYWORDS

contraction, epididymis, human, noradrenaline, oxytocin, sperm transport

Abbreviations: BPH, Benign prostatic hyperplasia; DMSO, dimethyl sulfoxide; fc, final concentration; NA, noradrenaline; S, segment.

1 | INTRODUCTION

The physiological process of ejaculation is very complex and not fully understood to date. The sympathetic nervous system with the neurotransmitter noradrenaline (NA) is the main mechanism at play, but there are many more components involved such as dopamine, serotonin, acetylcholine, oxytocin, gamma aminobutyric acid, and nitric oxide.[1] The ejaculatory process can be divided into two phases: the emission phase and the ejaculatory phase.[2] During the emission phase strong contractions of the distal epididymis and the vas deferens drive the stored sperm from the distal cauda epididymidis into the prostatic urethra.[3] Although NA acting through alpha-1 adrenoreceptors seems to be essential for mediating these contractions, mice with all alpha-1 adrenoreceptor subtypes deleted still sired offspring in a few cases.[4] Therefore, other molecules such as oxytocin might play a role in the ejaculatory process as well.[3,5] Oxytocin is a hormone mainly known for its effects during parturition and lactation. It is synthesized in the hypothalamus and stored in the posterior pituitary gland until being released into the blood stream.

Ejaculatory disorders comprise premature ejaculation, delayed ejaculation, retrograde ejaculation, and anejaculation. There are a variety of possible etiologies ranging from psychological factors over serotonin level discrepancies to spinal cord injury and other nerve damages.[1] Often ejaculatory disorders also emerge after taking alpha-1-adrenergic receptor blockers such as after medical treatment with tamsulosin for benign prostatic hyperplasia (BPH). Interestingly in clinical trials to test the effectiveness of tamsulosin in treating BPH 4%-18% of the participants in short time[6] and 30% of the participants enrolled in longer clinical trials reported ejaculatory disorders as a side effect.[7] Therapeutic medical options for ejaculatory disorders are scarce and even more so if medication targeting the adrenergic pathway is found not appropriate. Therefore, a reliable non-adrenergic and non-neuronal option such as the hormone oxytocin is imperative.

Investigating the effect of oxytocin on the ejaculatory process started by the observation that plasma oxytocin levels rose after manual stimulation of the genital organs of male and female sheep[8] and sperm count increased after oxytocin application in experiments with rams and rabbits.9-11 This led to an increased interest in plasma oxytocin levels and the effect of oxytocin on the cauda epididymidis since this is where sperm is stored until ejaculation. Studies in ponies,[12] bucks,[13] bulls,[14] and men and women[15-20] all found a significant increase of oxytocin plasma levels related to sexual arousal and ejaculation/orgasm. Studies investigating the direct effect of oxytocin on the epididymis using either electrical activity, organ bath, or intraluminal catheter to record changes in contractility and tension found oxytocin to have a stimulatory effect in rat caput,[21] cow caput,[22] cow mid- and distal cauda,[22] rabbit cauda,[23] sheep cauda,[24] and mouse cauda[25,26] epididymidis. A relaxing effect was noted in cow corpus and proximal cauda epididymidis.[22] One study found no electrical or mechanical effects of oxytocin on rat caput or cauda epididymidis.[27] In rat and rabbit epididymis (without specifying which part exactly) another study reported oxytocin to have no effect[28] but found vasopressin (which differs to oxytocin only by two amino acids) to have an increasing effect, suggesting that the vasopressin 1_A receptor rather than the oxytocin receptor might be involved in signal transduction of oxytocin in the epididymis. Only one study used live imaging and found oxytocin to significantly increase contraction frequency in the initial segment of the rat caput epididymidis.[29] So far there have been no investigations regarding oxytocin effects on the cauda epididymidis using live imaging.

Although oxytocin levels rose in relation to arousal and ejaculation/orgasm in humans a few clinical studies on the effect of oxytocin on sperm parameters and time until ejaculation in men found oxytocin to have no effect.[30,31,32,33] However, to the best of our knowledge there have been no reported functional ex vivo studies on the contractility of the human epididymis, neither using oxytocin nor other agents.

The oxytocin receptor has been found to be present in the epididymis of all species investigated: human,[34] marmoset,[35] macaque[36] monkey, sheep,[37] cow,[22] rabbit[34,] and wallaby.[38]

In the functional studies mentioned above the epididymis was only divided into initial segment, caput, corpus, and cauda with only Mewe et al dividing further into proximal, mid-, and distal cauda in the cow epididymis. Newer research of the epididymis is suggesting that the differentiation of the epididymis should be extended further. Using so called "connective tissue septa" as segment limitations, 19 segments have been defined for the rat epididymis[39] and 10 for mouse epididymis.[39] For this study we followed the suggestion of Holstein[40] who divided the human epididymis into nine segments from aspect and, therefore, we named the last part of the human epididymis here segment (S) 9. More recently investigations into the segments of the rat epididymis found that each segment seems to express different genes, proteins, and segment-specific signal transduction pathways (for review see[41]) suggesting there might be segment-dependent differences in contractility.

In this study, we used live imaging in combination with an imaging analysis method to investigate the influence of oxytocin on the contractility of defined segments of the epididymal duct of the rat and human epididymis assuming that the most distal parts of the epididymis (where the sperm is stored until ejaculation) might respond greater to oxytocin. In addition, oxytocin receptor antagonists as well as a vasopressin receptor antagonist were tested for their ability to block the effect of oxytocin on the very last segment of the rat epididymis (S19). Furthermore, the effect of oxytocin was evaluated in both rat and human tissue that had been pretreated with tamsulosin and thereby made less or unresponsive to NA to test the potential of oxytocin-based medications in treating ejaculatory disorders.

2 | MATERIALS AND METHODS

2.1 | Study design

Rat (Wistar, Sprague Dawley) and human tissue were carefully dissected under a binocular microscope using fine surgical tools to isolate single loops of the epididymal duct. Tissue was kept in place for live imaging by embedding the dissected ductal segments in polymerized collagen gel. Minimal Essential Medium (Thermo Fisher Scientific, Waltham, MA, USA) was added to the dish to maintain tissue viability. Live imaging experiments at Justus-Liebig-University (JLU) were performed on a Leica DM5000 B microscope with an ORCA-Flash4.0 Hamamatsu digital camera (C11440) using LAS X software. The live imaging experiments at Monash University were recorded on a Nikon Eclipse Ti microscope with a Photometrics CoolSNAP MYO camera using Nikon photometrics software (Version 5.2). Dishes were kept at 33°C throughout the experiments. All experiments were recorded at 1 frame every 2 seconds. At the end of each experiment NA (norepinephrine or norepinephrine-bitartrate salt) (Sigma-Aldrich, Steinheim, Baden-Württemberg, Germany) (final concentration (fc) of 10 μM) was added to check for tissue viability. In rat tissue experiments the number of experiments was 6 (n = 6) except the experiments using S19 with 500 nM oxytocin addition or experiments with tamsulosin pretreatment (each n = 7) as well as experiments using dimethyl sulfoxide control (n = 3). In human tissue experiments the number of experiments with cauda epididymidis (S9) was 2 (n = 2) and for caput epididymidis was 2 (n = 2).

First experiments with S19 and 500 nM oxytocin were performed with both rat lines (Wistar or Sprague Dawley) on the respective imaging set ups to test for possible differences and were found to be interchangeable (data not shown).

First set of experiments investigated the effect of oxytocin (oxytocin acetate salt) (Bachem, Bubendorf, Basel-Landschaft, Switzerland) on ductal segments from mid-caput (S5), mid-corpus (S12), proximal cauda (S15), and distal cauda (S18 and S19) of the rat epididymis as well as the very last segment of the human epididymal duct (here called S9) and a piece from the human caput epididymidis. After a 10-minute period without treatment oxytocin was added into the dish (fc of 500 nM) and recorded for another 10 minutes.

Additionally, a set up using NA was conducted to evaluate if the response induced in S19 of the rat epididymis by 500 nM oxytocin was comparable to the one induced by a high concentration of NA. After a 10-minute period without treatment NA was added into the dish (fc of 10 μM) and recorded for another 10 minutes.

Second set of experiments investigated the concentration-dependent effect of oxytocin on S19 of the rat epididymis. A similar set up as in the first set of experiments was used but with oxytocin at the respective fc of 1, 10, or 100 nM.

Third set of experiments investigated three agents for their effectiveness to inhibit the previously observed oxytocin effect on S19 of the rat epididymis. A 10-minute period without treatment was recorded. Next either one of the oxytocin receptor antagonists Atosiban (Sigma-Aldrich, Steinheim, Baden-Württemberg, Germany) (fc of 5 μM) or Cligosiban (MedChemExpress, Monmouth Junction, NJ, USA) (fc of 40 μM) or the arginine vasopressin 1_A receptor antagonist SR49059 (Cayman Chemicals, Ann Arbor, MI, USA) (fc of 40 μM) was added for a 15-minute time period to record any direct effects and allow for maximal effectiveness in blocking oxytocin (fc of 500 nM) which was then added for another 10-minute period. To exclude possible effects of the soluble agent dimethyl sulfoxide (Roth, Karlsruhe, Baden-Württemberg, Germany) (DMSO), experiments were performed in the same set up but with DMSO (at fc 1,6%) alone without antagonizing agents.

In the fourth set of experiments, to evaluate the potential of oxytocin in treating ejaculatory disorders, oxytocin's ability to stimulate adrenergically blocked tissue was investigated. S19 of the rat epididymis or, respectively, S9 of the human epididymis was recorded for a 10-minute period without treatment followed by a 15-minute period of treatment with the alpha-1 blocker tamsulosin (tamsulosin hydrochloride, Sigma-Aldrich, Steinheim, Baden-Württemberg, Germany) (fc of 40 μM). Then, NA (fc of 10 μM) was added for another 10 minutes and oxytocin (fc of 500 nM) was added at the end of the experiment for 5 minutes.

All antagonist concentrations (atosiban, cligosiban, SR49059, and tamsulosin) were chosen through pre-experiments where they showed a successful and complete blocking effect. In case of SR49059, pre-experiments failed to find a concentration in which SR49059 could completely block the oxytocin effect. Due to the similarities between the arginine-vasopressin and the oxytocin receptor off target receptor effects (of SR49059 at the oxytocin receptor) should be taken into consideration especially with using high concentrations.

Image processing and analysis were performed using the Fiji distribution of ImageJ.[42]

Two types of analytical methods were used to best translate the different types of contractile responses. For the distinct contractions occurring throughout most of the rat epididymis, with the exception of the very last segment (S19), contractions per minute were counted using the previously established method[43] by reslicing the data.

Due to the complexity of the contractile response of the very last segment, S19 of the rat (Figure $2B_1$,B_2, 3, 5–7) and S9 of the human epididymis (Figure 4), another analysis method was needed. Here, we used an evolution of the Wiggle Index[44,45,46] to quantify the intensity of the

complex contractile activity in S19 https://doi.org/10.26180/13653614. The adaptation allowed us to compare how the sum of movements (displaying frequency in combination with amplitude of multidirectional contractions) of the entire tissue evolved throughout the different parts of the experiments. It also measured the fold change relative to baseline (and to each other addition) while expressing the data as a population distribution allowing subtle differences to be detected. Additionally, specific spatial information visualized in the heat maps allowed to pinpoint which specific areas of the tissue were moving more or less relative to the rest of the tissue. Thus, it was possible to show the area of interest in one image.

2.2 | Statistical analysis

Contractions per minute data were analyzed with Wilcoxon matched-pairs signed-rank test. Data of S19 and S9, respectively, generated with the adaptation of the Wiggle Index were analyzed with frequency distribution and nonlinear regression. GraphPad (GraphPad Prism 9, Version for Windows, GraphPad Software, La Jolla, California, USA, www.graphpad.com) was used to analyze all data. Differences were considered significant if $*P < .05$, $**P < .01$, and $***P < .001$ and non-significant (ns) if $P \geq .05$. Cumulative frequency distribution data are displayed ± standard error of means (SEM).

2.3 | Ethics

The adult rat tissue was obtained from Wistar rats (6-8 weeks old) housed in the animal facility of JLU, Giessen and Sprague Dawley rats (6-8 weeks old) housed in the animal facility of Monash Institute of Pharmaceutical Sciences (MIPS), Melbourne. Housing, animal care, and all procedures were conducted according to the guidelines for animal care and approved by the committee for laboratory animals of JLU (487_M) and Monash University (MIPS 2018-14149).

The adult human epididymal tissue was obtained from patients undergoing semicastration due to testicular cancer at the University's hospital (Clinic for Urology, Pediatric Urology and Andrology at JLU Giessen) and was approved by an ethics committee (AZ 152/16).

3 | RESULTS

3.1 | First set of experiments investigated whether there is a special reaction to oxytocin in the distal epididymis compared to other regions and to NA

Oxytocin only slightly but significantly increased the frequency of contractions in mid-caput (S5) ($P < .05$) and proximal cauda (S15) ($P < .05$), but not in mid-corpus (S12) ($P \geq .05$) of the rat epididymis (Figure 1). The rat

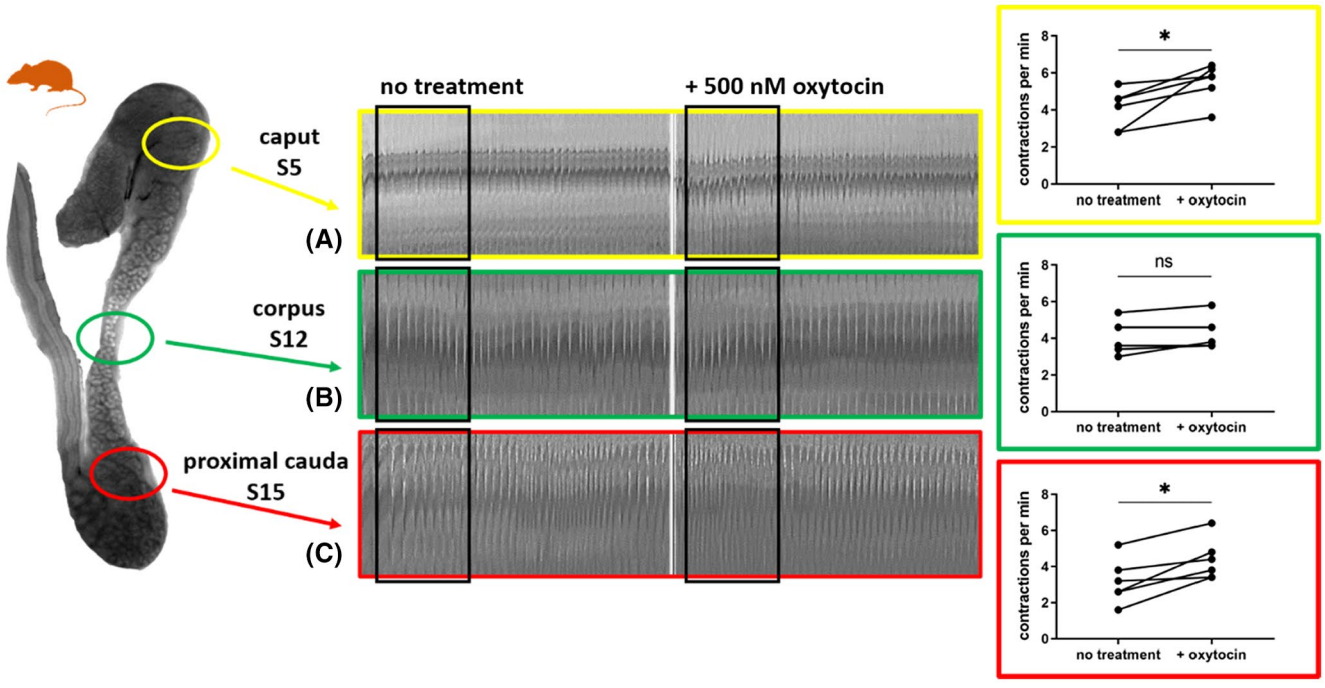

FIGURE 1 The effect of oxytocin on defined segments of the epididymal duct of the rat. Contractions are displayed by reslicing the data and countable as vertical "stripes." Contractions were counted for 3 minutes each (black frames) and summarized as contractions per minute. Oxytocin only slightly increased contractions per minute in caput, corpus, and proximal cauda of the rat epididymis (each n = 6). This effect was significant ($P < .05$) in segments of the caput (S5) (A) as well as proximal cauda (S15) (C) of the rat epididymis, but not significant ($P \geq .05$) in the corpus epididymidis (S12) (B)

epididymis in the most distal segments 18 and 19 showed little to no spontaneous activity. In S18, oxytocin had a significant effect ($P < .05$) by inducing distinct single contractions (Figure 2A). In S19, oxytocin induced a strong complex reaction comprised of multiple forceful contractions that displaced and compressed the duct, thereby expulsing the spermatozoa from inside ($P < .0001$) (video provided as Supporting Information SI1) (Figure 2B). These contractions had an instant onset, lasted for 3-5 minutes and then proceeded to subside eventually, leaving S19 to return to its relatively quiet state during the 10 minutes recorded. This reaction could be observed by reslicing the data (Figure 2B1) but could not be quantified with this method. Our method (Figure $2B_2$) considered the tissue in its entirety and did not restrain its complex multidirectional movement, thus showing the significant increase of movement ($P < .0001$) after oxytocin addition.

Regarding the potential role of oxytocin during the emission phase of ejaculation, rapid and short-term effects of oxytocin were compared to NA. The experiments with NA also using our method showed that 10 μM NA induced a series of strong and complex contractions very similar to the ones observed in the oxytocin experiments. The responses measured during the first 30 seconds after application of the respective agent (NA or oxytocin) were statistically not significantly different when compared ($P \geq .05$) and therefore the reactions to either agent deemed comparable (Figure 3) in relation to the ejaculatory process.

To validate if results found in rat experiments could be interpolated to human tissue, a limited number of experiments were conducted with the respective human tissue. In agreement with data from rat tissue, in two experiments the very last segment of the human epididymal duct (S9) was relatively quiet without treatment, while 500 nM oxytocin induced strong contractions (Figure 4) (video provided as SI2). The proximal part of the human epididymis (caput) was also only used in a further two experiments. The duct displayed regular spontaneous contractions which did not markedly increase after oxytocin addition (figure provided in SI3) (similar to our previous observations in the rat caput epididymidis (Figure 1)).

3.2 | Second set of experiments investigated the responsiveness of S19 to different concentrations of oxytocin

The intensity of the complex contractions induced by oxytocin in S19 of the rat epididymis changed in a concentration-dependent manner (Figure 5) (video provided as SI4). A concentration of 1 nM oxytocin induced very small but significant ($P < .05$) movements of the epididymal duct's wall (Figure 5A). A concentration of 10 nM oxytocin induced single strong contractions ($P < .0001$) with little to no expulsion of contents and only small displacement of the duct (Figure 5B). A concentration of 100 nM oxytocin induced the strong complex reaction comprised of multiple forceful contractions that displaced and compressed the duct including expulsion of content ($P < .0001$) (Figure 5C). In relation to each other 100 nM oxytocin elicited a significantly greater effect than 10 nM oxytocin ($P < .0001$), and 10 nM oxytocin greater than 1 nM oxytocin ($P < .0001$) (Figure 5D).

3.3 | Third set of experiments to determine which signaling pathway is responsible for the strong reaction to oxytocin

Three agents (and DMSO control) were evaluated for their effectiveness in inhibiting a post addition of 500 nM oxytocin (Figure 6). Blocking effects were only defined as "complete" in the case where no response was detected in the video for 8 minutes after addition of oxytocin. The otherwise seen intense response of S19 to oxytocin as seen in the DMSO control ($P < .0001$) (Figure 6A) was not recorded after pretreatment with either of the two oxytocin antagonists atosiban ($P \geq .05$) (Figure 6C) or cligosiban ($P \geq .05$) (Figure 6D). The arginine vasopressin 1_A antagonist (SR49059) failed to prevent a reaction to oxytocin ($P < .001$) (Figure 6B) over the 8 minutes analyzed. It did, however, delay the onset of oxytocin effects for a short time (roughly 2 minutes) and lessened the intensity of the response of S19 to oxytocin compared to DMSO control ($P < .0001$).

3.4 | Fourth set of experiments to determine if oxytocin could be an option in treating ejaculatory disorders in adrenergically impaired tissue

Pretreatment with 40 μM tamsulosin mostly prevented ($P \geq .05$) a response to the post addition of NA in S19 of the rat epididymis during the observation period (Figure 7A). In the last segment of the human epididymis (S9) the same concentration of tamsulosin was not able to prevent the entire effect of NA. However, instead of the strong complex series of contractions, NA only induced single strong contractions in the tamsulosin pretreated S9 (Figure 7B). In both tissues (S19 in rat and S9 in man) oxytocin then induced the forceful reactions as seen in previous experiments, and these were significantly stronger than the responses to NA (S19 $P < .0001$) (Figure 7A,B).

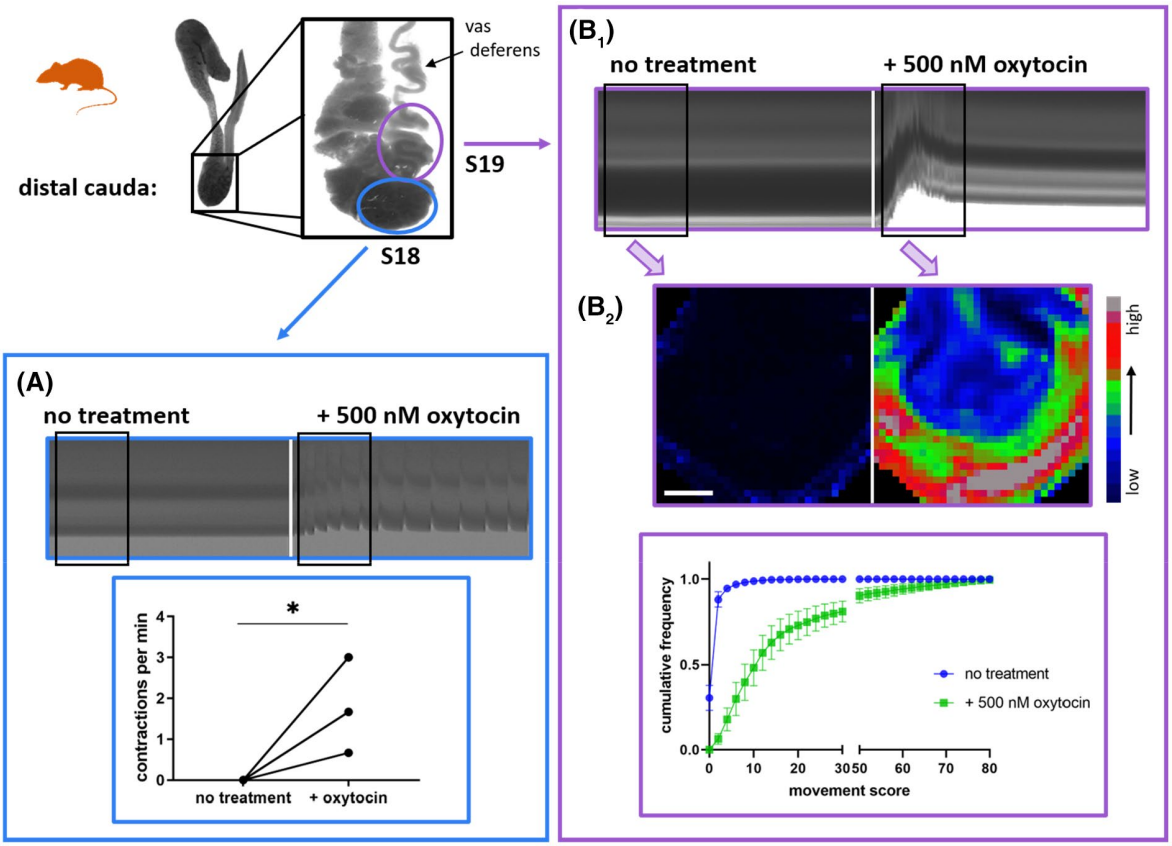

FIGURE 2 The effect of oxytocin on S18 and S19 of the epididymal duct of the rat. A, In S18 contractions are displayed by reslicing the data and countable as vertical "stripes." Contractions were counted for 3 minutes each (black frames) and summarized as contractions per minute. Oxytocin had a significant effect in S18 of the rat epididymis (n = 6) ($P < .05$) by inducing distinct single contractions. B, In S19 oxytocin induced a strong series of contractions quickly following each other thereby creating a complex movement with displacement of the duct (including expulsion of content). Reslicing this data (B_1) displayed the difference in quality of the reaction compared to the other segments investigated (Figure 1A-C and Figure 2A) and is indicative of a very forceful reaction. However, it was not possible to quantify the reaction this way, therefore a new analyzing method (B_2) was introduced to display and quantify the intensity of the movement. In the heat map representation of the results of this new analyzing method, the intensity of the sum of movements over the 3 minutes analyzed is displayed in a color-coded manner with blue representing low intensity and red and gray the high intensity movements (white scale bar: 300 μm). The data collected through this new method showed that the reaction to oxytocin in S19 (n = 7) was significant ($P < .0001$) (± SEM)

4 | DISCUSSION

Live imaging was used to investigate the effect of oxytocin on multiple well-defined segments of the epididymal duct, showing differences in spontaneous contractility, as well as responsiveness to oxytocin. Live imaging has been shown to be an excellent tool to investigate the contractility of the epididymal duct[43,29] with as little disturbances to the tissue as possible ex vivo and allowed us in this study in combination with our analysis method to identify and display even small contractions.

From our results reported above, it can be deduced that only the most distal part of the epididymis is essential in driving sperm through the vas deferens during the emission phase of the ejaculatory process since only S19, the very last segment of the rat epididymis showed a uniquely strong effect to oxytocin or NA compared to the other segments investigated (S5, S12, S15, and S18). In agreement, the respective most distal part of the human epididymis reacted in a similar strong manner. This strong reaction to oxytocin, which was comparable to the one induced by NA, supports the hypothesis that oxytocin plays a role in the physiology of the ejaculatory process.[47,48,2,5] Thus, targeting oxytocin signaling might be an alternative to targeting the adrenergic pathway for the treatment of ejaculatory disorders.

S5, S12, and S15 of the rat epididymis and the caput of the human epididymis presented with regular contractions during the "no treatment" period (and a slight increase in frequency after stimulus) as can be assumed to be the case for nearly the entire length of the epididymis. This contractile pattern suggests the occurrence of a single-unit smooth muscle type including the presence of pacemaker cells similar to the gastro-intestinal tract.[49]

Interestingly, both the unstimulated S18 as well as S19 of the rat epididymis were found to be relatively quiet, but

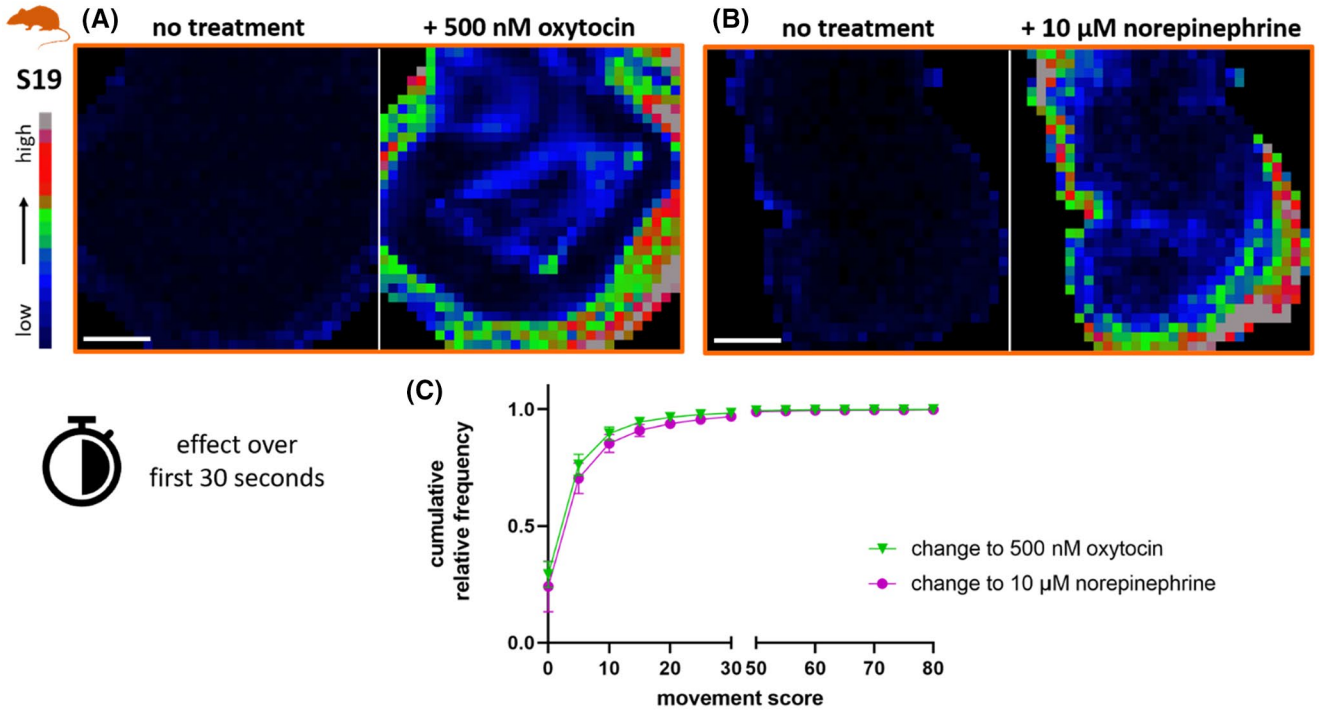

FIGURE 3 The effect of 500 nM oxytocin in comparison to 10µM norepinephrine on S19 during the first 30 seconds after administration of the agents. The sum of movements analyzed is displayed using a color-coded heat map representation with blue representing low intensity and red and gray the high intensity movements (white scale bar: 300 µm). A, The effect of 500 nM oxytocin on S19 (n = 7) during the first 30 seconds after application. B, The effect of 10 µM norepinephrine on S19 (n = 6) during the first 30 seconds after application. C, Graph of the changes of "no treatment" to either oxytocin or norepinephrine during the first 30 seconds after application, respectively (± SEM). The difference between the two changes was not significant ($P \geq .05$)

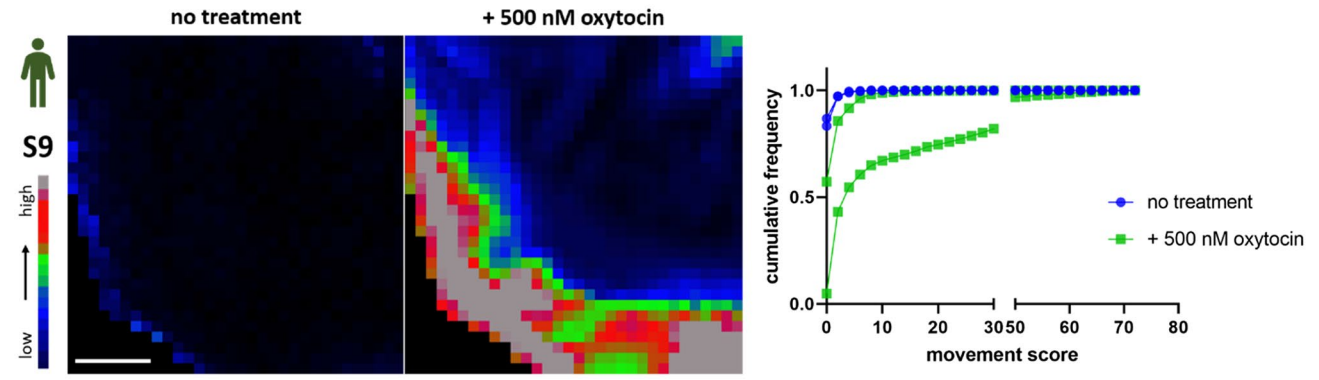

FIGURE 4 The effect of oxytocin on distal cauda of the epididymal duct in men. The sum of movements analyzed (during the 3 minutes) is displayed using a color-coded heat map representation with blue representing low intensity and red and gray the high intensity movements (white scale bar: 300 µm). Oxytocin induced strong contractions in the most distal part (S9) of the human epididymal duct (n = 2)

with only the S19 (not S18) reacting in a uniquely strong manner to either oxytocin or NA. The last segment of the epididymis (S19 in rat, S9 in man) responded with a complex series of contractions which is indicative of a multiunit smooth muscle type without the presence of pacemaker cells. The multiunit smooth muscle type has been described for the adjacent vas deferens.[49] In addition, the cauda epididymidis and the vas deferens have been found to be heavily innervated[50,51] which would also be necessary in a multiunit smooth muscle type since the signal cannot be transmitted from cell to cell as it would be in a single-unit type. All this suggests that the very last segment of the epididymis functionally resembles more the vas deferens than the rest of the epididymis. As for S18 showing no contractions during the "no treatment" period and then responding with coordinated single contractions, we would suggest the occurrence of a single-unit smooth muscle type but without the presence of pacemaker cells.

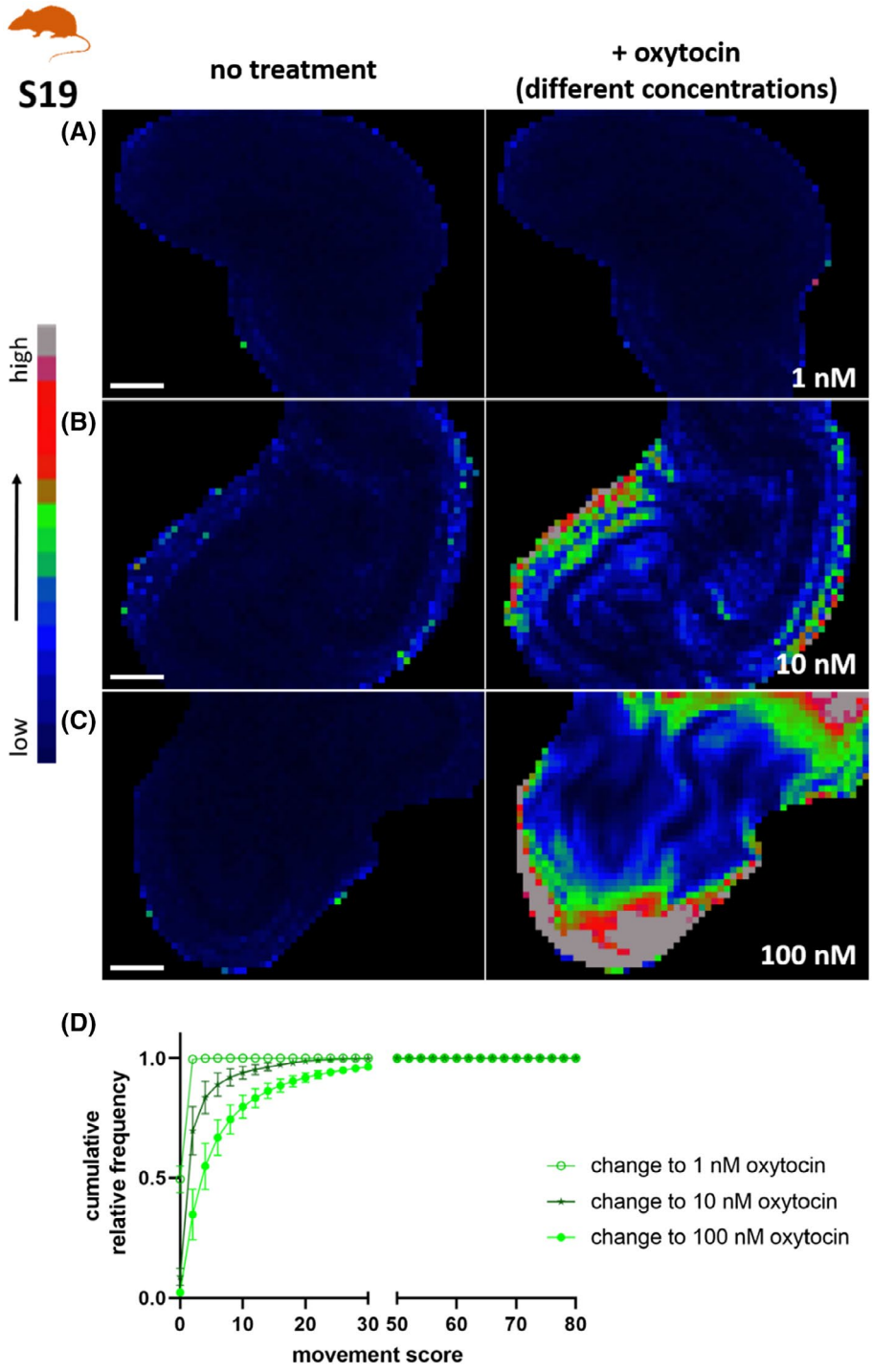

FIGURE 5 Concentration-dependent oxytocin effects on S19 of the epididymal duct of the rat. The sum of movements analyzed (during the 3 minutes) is displayed using a color-coded heat map representation with blue representing low intensity and red and gray the high intensity movements (white scale bar: 300 µm). A, 1 nM oxytocin induced very small contractions in S19 (n = 6), which were still significant ($P < .05$). B, 10 nM oxytocin induced single strong contractions in S19 (n = 6) ($P < .0001$). C, 100 nM oxytocin induced a strong series of contractions quickly following each other thereby creating a complex movement with displacement of the duct (including expulsion of content) (n = 6) ($P < .0001$). D, Graph of the three changes of "no treatment" to one of the three doses of oxytocin (1, 10, or 100 nM), respectively (± SEM). In relation to each other, the 100 nM oxytocin dose elicited a significantly greater effect than 10 nM oxytocin ($P < .0001$), and 10 nM oxytocin greater than 1 nM oxytocin ($P < .0001$)

Therefore, we postulate that the underlying cause for the different effect of the hormone oxytocin observed in the distal epididymis is not due to receptor distribution but due to a change in smooth muscle cells toward a multiunit smooth muscle type.

This quiescence in S19 also allowed the sensitivity to low concentrations of oxytocin (1 nM) to be effectively measured, visualized by live imaging, in combination with our image analysis method. Thus, S19 might also be an attractive

tool to test the bioactivity of oxytocin agonists and oxytocin antagonists.

In accordance with previous anatomical and histological findings,[52,40] we also found that the total and luminal diameter of the most distal part of the human cauda epididymidis is small in relation to S19 of the rat epididymis. In conducting contractility experiments in the human epididymal duct, we found that the last segment of either the rat or the human epididymal duct both reacted similar to NA (big strong complex contractions), whereas the caput of either species showed no such big response. Compared to the rat, the responsiveness of the human tissue to both 500 nM oxytocin as well as 40 μM

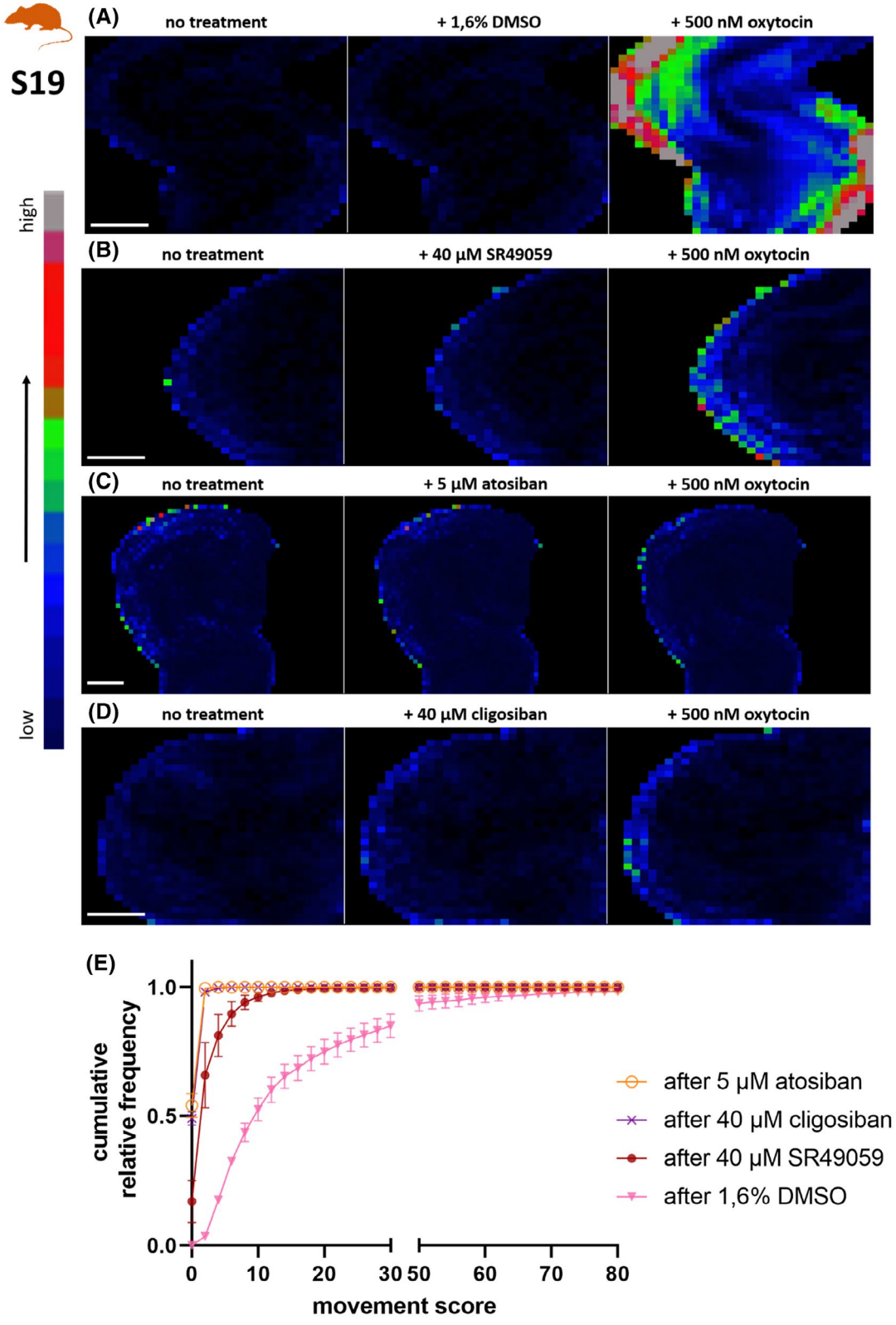

FIGURE 6 Oxytocin effects after pretreatment with atosiban, cligosiban, or SR49059 in S19 of the rat epididymal duct. The sum of movements analyzed (during the 8 minutes) is displayed using a color-coded heat map representation with blue representing low intensity and red and gray the high intensity movements (white scale bar: 300 µm). A, Pretreatment with 1,6% DMSO did not prevent the oxytocin effect and is used as a control in this set of experiments (n = 3) ($P < .0001$). B, Pretreatment with 40 µM SR49059 did not completely prevent the oxytocin effect (see above, A) resulting in a significant difference between SR49059 and SR49059 + oxytocin (n = 6) ($P < .001$). C, Pretreatment with 5 µM atosiban prevented the oxytocin effect (see above, A) resulting in no significant difference between atosiban and atosiban +oxytocin (n = 6) ($P \geq .05$). D, Pretreatment with 40 µM cligosiban prevented the oxytocin effect (see above, A) resulting in no significant difference between cligosiban and cligosiban +oxytocin (n = 6) ($P \geq .05$). E, Graph of the oxytocin effects after pretreatment with either of the three agents (atosiban, cligosiban, and SR49059) and the soluable agent DMSO used as a control in comparison to each other (± SEM). The oxytocin effect after pretreatment with SR49059 was significantly decreased compared to DMSO control ($P < .0001$), but only atosiban and cligosiban completely blocked a reaction to oxytocin

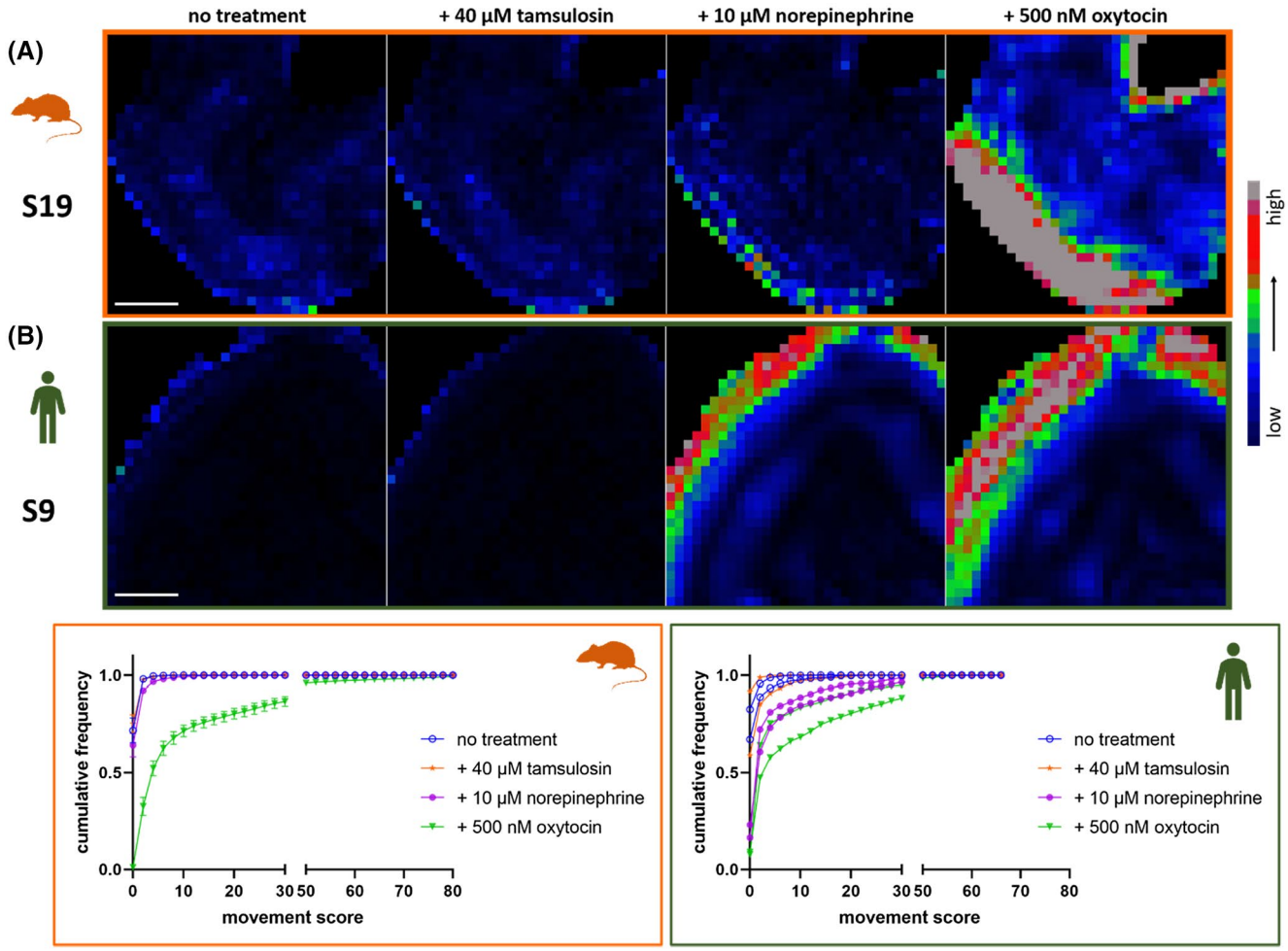

FIGURE 7 Oxytocin effect after pretreatment with adrenergic-blocker tamsulosin. The sum of movements analyzed (during 3 minutes) is displayed using a color-coded heat map representation with blue representing low intensity and red and gray the high intensity movements (white scale bar: 300 µm). A, In the rat S19 (n = 7) pretreatment with 40 µM tamsulosin prevented a significant reaction to an afterward addition of NA ($P \geq .05$). Additional treatment with oxytocin induced a strong series of contractions quickly following each other thereby creating a complex movement with displacement of the duct (including expulsion of content) ($P < .0001$) (± SEM). B, In the human S9 (n = 2) pretreatment with 40 µM tamsulosin did not prevent a reaction to an afterward addition of NA, but lessened NA's effect. Oxytocin then induced forceful contractions that were stronger than the ones induced by NA in the tamsulosin pretreated tissue

tamsulosin (although similar) was markedly weaker. Since the response of the human S9 to 500 nM oxytocin closely resembled the response of the rat S19 to 10 nM oxytocin, we attribute the weaker responsiveness of the human tissue to the need for a higher oxytocin concentration. Furthermore, the reaction of the human tissue to oxytocin in our ex vivo experiments leads us to speculate that the results from previous studies (finding no effect of oxytocin on the sperm parameters or time until ejaculation in humans) might be either due to the need for higher concentrations of oxytocin in humans

or that psychological factors (especially concerning intimate topics such as those associated with sexual functions) have a much bigger impact in men than anticipated. Further ex vivo experiments with human S9 should be conducted to help clarify these aspects.

Our experiments with the antagonists indicate that the strong reaction observed in S19 of the rat epididymis is mainly mediated through the oxytocin receptor rather than the arginine vasopressin receptor 1_A since the highly selective oxytocin receptor antagonist cligosiban[53] was able to completely block any reaction to oxytocin, whereas the highly specific arginine vasopressin 1_A receptor antagonist SR49059 (known to show also weak affinity to the oxytocin receptor[54]) was only able to partially block a reaction of S19 to 500 nM oxytocin. Surprisingly, in clinical trials for premature ejaculation one study from 2019 found that cligosiban prolonged intravaginal ejaculation latency time compared to placebo,[55] whereas another study from the same year found no difference.[56]

Our data also demonstrate that tissue showing no or diminished responsiveness to NA (artificially induced through pretreatment with tamsulosin) was still excitable by oxytocin. Likewise, in our antagonist experiments, treatment with NA (to lastly check for viability of the tissue) showed that the adrenergic pathway was unimpaired. Thus, the adrenergic and oxytocin pathways seem to be completely independent in the distal cauda epididymidis (S19) while mediating similar responses in this tissue important during ejaculation. Therefore, oxytocin-based agonistic therapeutic agents which do not act via the nervous system could show great potential in promoting ejaculation.

Especially in cases of spinal cord injury in young men desiring to father children, oxytocin might be a pain free alternative to penile vibratory stimulation or electroejaculation or a supporting agent to yield more motile and fertile sperm. Results from our ex vivo studies compel to test in future clinical trials if oxytocin application in patients with ejaculatory disorders facilitates an antegrade ejaculation. Furthermore, in treating premature ejaculation a pharmacological option targeting multiple signaling pathways involved in the ejaculatory process such as the adrenergic and the oxytocin pathway simultaneously might yield better results than monotherapy.

We conclude that oxytocin is involved in the ejaculatory process and that S19 of the rat epididymis is an advantageous model for the respective human epididymal tissue and an excellent animal model to test bioactivity of oxytocin agonists and antagonists. We suggest that oxytocin-based medication could be a potent new option in treating ejaculatory disorders, especially in case of spinal cord injury.

CONFLICT OF INTEREST

The authors declare that they do not have any competing interest.

AUTHOR CONTRIBUTIONS

B. Stadler and R. Middendorff designed research; B. Stadler performed research; B. Stadler and C. J. Nowell analyzed data; C. J. Nowell developed software necessary to analyze experiments; B. Stadler, C. J. Nowell, M. R. Whittaker, S. Arnhold, A. Pilatz, F. M. Wagenlehner, B. Exintaris, and R. Middendorff wrote the manuscript.

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the Supporting Information section.

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