Porcine vas deferens luminal pH is acutely increased by systemic xylazine administration

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Title: Porcine vas deferens luminal pH is acutely increased by systemic xylazine administration

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Summary sentence:

Vas deferens luminal pH is acutely increased by xylazine, an alpha-2 adrenergic receptor agonist

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Abstract

Data are accumulating to demonstrate that pH regulation in the male reproductive tract has a vital role in modulating sperm cell fertilizing capacity and, therefore, male fertility. Bicarbonate uptake by sperm cells is required for the achievement of motility levels required for fertilization. Vas deferens epithelial cells can carry out measurable bicarbonate secretion, but the available literature to date reports that the vas deferens luminal content is typically acidic. This study aimed to determine pH in the boar vas deferens lumen and whether modulatory mechanisms exist for regulation of pH in this compartment of the male reproductive tract. A fiber-optic pH probe was used to assess pH in the vas deferens of anesthetized adult boars. The mean pH, derived from multiple measurements at variable positions along the vas deferens lumen, was 7.39 ± 0.09. Furthermore, administration of xylazine, an alpha-2 adrenergic receptor agonist, rapidly (< 10 min) alkalinized the vas deferens lumen in most cases. Since the duct was transected proximal to the site of measurements, the observations rule out the possibility that alkalinization resulted from secretion in more proximal portions of the duct. These results indicate that the boar vas deferens lumen can be alkaline, and suggest that porcine vas deferens epithelia increase net bicarbonate secretion in vivo, following systemic alpha-2 adrenergic stimulation. This secretory response greatly changes the luminal environment to which sperm cells are exposed, which will initiate or enhance motility, and is expected to modulate male fertility.

Introduction

Defined pH levels in specific compartments of the mammalian male reproductive tract are required for male fertility [1, 2]. A phenotype that included increased epididymal pH, reduced sperm motility, abundant sperm tail defects and male infertility was described in a knockout mouse model in which expression of the V-ATPase proton pump and bicarbonate exchanger SLC26A4 were disrupted [1]. Additionally, data derived from mice heterozygous for the cystic fibrosis conductance transmembrane regulator (CFTR) revealed that bicarbonate uptake through CFTR expressed in sperm cells is required for the achievement of sperm motility levels suitable for fertilization [2]. At the clinical level, men carrying dysfunctional mutations for the bicarbonate exchanger SLC26A3 are reportedly subfertile or infertile [3]. Taken together these observations suggest that distinct cellular components that are expressed in the excurrent duct and modulate luminal pH are required for fertility.

Luminal pH along the male excurrent duct is currently thought to be acidic throughout. Granted, the epididymal lumen exhibits the lowest pH levels described for the male excurrent duct [1, 4, 5]. Acidification is brought about by epididymal clear cells, where V-ATPase expression at the apical membrane contributes to proton accumulation in the luminal space [6]. However, V-ATPase expression is much reduced in the subsequent component of the male rat excurrent duct, the vas deferens [7]. The vas deferens epithelium is composed largely by columnar cells generally referred to as principal cells [8]. Data derived from in vitro systems support both absorptive and secretory ion transport by porcine and human vas deferens epithelia [9, 10]. Anion secretion was shown to be chloride and bicarbonate dependent in vitro [11] and bicarbonate co-transporters and exchangers, in addition to CFTR, are expressed in vas deferens epithelial cells [12]. Despite the presence of these transporters, the vas deferens luminal content is described as acidic. The available literature to date, reports that pH at this compartment is 6.85 in the rat vas deferens [4] and 6.46 in the proximal vas deferens of the boar [5].

Although in vitro data are available revealing that epithelia lining the male excurrent duct carry out water and solute secretion and/or absorption, it remains unknown whether epithelial transport can generate dynamic changes in pH of the luminal content in vivo, or if pH changes occur upon distinct physiological stimuli. Similarly, it is widely known that the vas deferens is innervated by sympathetic noradrenergic neurons and that the release of neurotransmitters by such noradrenergic nerve terminals causes robust contraction of vas deferens smooth muscle in

in vitro preparations [13]. However, it is not known whether adrenergic stimulation can modulate vas deferens epithelial transport in vivo or change pH in the vas deferens lumen.

This study aimed at determining pH in the vas deferens luminal content of the boar in vivo, and investigate whether modulatory mechanisms exist that can regulate vas deferens luminal pH. Data reported here reveals that the boar vas deferens luminal content is alkaline and that systemic administration of xylazine, an alpha-2 adrenergic receptor agonist, can induce rapid and further alkalinization in this compartment of the boar reproductive tract.

Material and Methods

Animals and Surgical Procedure. A total of 12 boars were employed in this protocol, which was reviewed and approved by the Kansas State University Institutional Animal Care and Use Committee. Boars were mixed breed, sexually mature and active, purchased from local swine facilities and transported to the Large Animal Section of the Veterinary Medical Teaching Hospital at Kansas State University on the day prior to assay. Pre-anesthesia was conducted with acepromazine (0.15 mg/kg, IM) and anesthesia was induced by ketamine (20 mg/kg, IM) and maintained by an inhaled 3-5 % isoflurane in oxygen mixture. A parasagittal scrotal incision was made to fully expose the testis. The serpentine vas deferens was identified and a transection made on the proximal vas deferens immediately distal to the serpentine duct. A plastic tubing (MicroLumen Inc., Tampa, FL) either 30.5 or 40.6 cm in length, internal and external diameters 1.00 and 1.10 mm, respectively, was introduced along the lumen of the vas deferens to cannulate the duct from the transection point to the extent of 10, 15 and 25 cm, towards the distal or prostatic end. The exposed glass fiber tip of a micro pH-sensor (World Precision Instruments, Sarasota, FL) was introduced into the plastic tubing and moved towards the end of the tubing. The pH-sensing tip was pushed 1-2 mm beyond the end of the cannula, therefore exposed to the vas deferens luminal content and considered ready for pH measurement and recording. Throughout the surgery, vital signs such as pulse rate, cardiac function assessed by auscultation, depth and quality of chest respiratory movements were monitored.

pH Measurement System and Data Acquisition. Measurements of vas deferens luminal pH began immediately after the placement of the micro pH-sensor and were carried out by a pHOptica micro (World Precision Instruments) commanded by a portable computer (Dell, Round Rock, TX) and a software application (pHOpt_v1.exe for pHOptica micro, World Precision Instruments) at 1 hertz. This system allowed for real time visualization of the acquired data in an auto-scrolling chart. Two to three minutes after the onset of each measurement, visual inspection of these tracings and the observation of a steady pH tracing was interpreted as the achievement of a basal state and/or stable baseline. Subsequently, systemic pharmacological interventions were conducted while pH measurements and recordings were maintained for several minutes during and after drug administrations.

Internal calibration settings, specific for each micro-pH sensor, were pre-determined by the instrument's manufacturer and applied in each experiment. In addition, measurements of pH

standards 6.5, 7.0, 7.5 and 8.0 (Thermo Fisher Scientific Inc., Waltham, MA) were conducted prior to and after each in vivo measurement, in order to verify stability of the probes and to generate standard pH curves for subsequent use in data analysis. Measurements of pH standards were conducted while each pH standard solution was kept at either room temperature or 37^{o}C. These two conditions did not produce detectable differences in pH measured from standard solutions.

Drug Administrations. To test if vas deferens luminal pH can be acutely modulated by alpha-2 agonists, xylazine (Bayer Corp., Pittsburg, PA) was administered to each of the 12 boars. Xylazine administrations were conducted with ongoing pH data acquisition and took place after pH recordings had reached a stable value. Two administrations were performed, each at 1 mg/kg, IV and spaced by 4-10 minutes. Xylazine injections were conducted in a bolus format, typically in a total volume of 2.5-4.0 ml, and lasted no longer than 1 minute.

Data Analysis and Statistics. The standard pH-curves generated before and after each measurement in vivo, were used to generate a linear regression equation that was then employed to correct the pH measurement conducted in vivo. Baseline and drug response pH values were determined by averaging data points acquired at each of these experimental phases, within each observation. Summaries of observations are expressed as mean ± standard error of the mean.

Results

Porcine vas deferens luminal pH is alkaline and dynamically regulated

To determine pH of the vas deferens luminal content in vivo, a surgical procedure was devised that included general anesthesia and transection of the proximal vas deferens to allow for the placement of a micro-pH sensor in the duct lumen. Among the 12 boars employed in this study, 5 had measurements conducted in 1 vas deferens only. In the remaining boars, measurements were conducted in both ducts simultaneously. Measurements began immediately after the placement of the pH-sensor at a pre-determined position, from the transection point. A minimum of 2 minutes of measurement and recording was conducted before a baseline was considered to have been achieved. Measurements of luminal pH conducted at 10, 15 and 25 cm distal to the serpentine vas deferens, indicated that baseline pH at these locations were 7.28 ± 0.08 (n = 11), 7.47 ± 0.11 (n = 5) and 7.69 ± 0.46 (n = 3), respectively; and no significant differences in pH were detected among such recordings performed at these locations ($P < 0.05$).

After pH recordings achieved baseline, administration of xylazine was performed intravenously at 1 mg/kg. Two administrations per animal, spaced by 4-10 minutes, were conducted. Xylazine induced an increase in vas deferens luminal pH (Fig. 1A), in 8 of 12 boars. Among these 8 boars, 2 had 1 vas deferens which did not present an increase in pH after xylazine administration. The remaining 4 boars exhibited minimal or no changes in luminal vas deferens pH (Fig. 1B). Increases in pH (Fig. 1A) were detected from a stable baseline and began approximately 2 minutes after systemic administration (arrows). Following their onset, pH increases lasted for approximately 4 minutes, exhibited an apparent linear rate of increase and pH values remained at the increased level over time, as to form a plateau. In some cases, such as the depicted in Fig. 1A, a second xylazine injection generated an additional phase of pH increment, also at a rate that was very similar to that of the initial response (Fig. 1A). Xylazine administrations did not cause detectable changes to the vital signs monitored.

Analysis undertaken with all paired observations available reveals that baseline pH was 7.38 ± 0.10, and that pH increased to 7.56 ± 0.09 as an effect of xylazine administration ($P < 0.05$, Fig. 2). It is obvious however, from the results presented in Fig. 1 that not all boars responded to xylazine administration. Thus, the data were examined in an attempt to identify a predictive parameter. Outcomes were categorized by the magnitude of responses to xylazine and observations that revealed a xylazine-induced pH increase smaller than 0.1 were grouped

separately from counterparts where pH increases were greater than 0.1 pH unit. Such segregation suggests that a lower starting pH was associated typically with a greater magnitude or likelihood of response. In the 8 responsive boars, 8 of 10 ducts showed starting pH values less than 7.3 whereas in the 4 non-responsive boars, 7 of 8 ducts showed starting pH values greater than 7.3.
145 Thus, one might generalize that xylazine typically induced a pH increase greater than 0.1 when baseline pH levels were below 7.3 (Fig. 2).

Discussion

Data are presented that demonstrate that the vas deferens luminal content of the boar is alkaline and that mechanisms exist for acute regulation of pH in this compartment of the male reproductive tract. The mean baseline pH in the vas deferens of boars was alkaline (pH = 7.39 ± 0.09). Importantly, ducts exhibiting lower pH typically showed the greatest responsiveness to adrenergic stimulation. Indeed, luminal pH was rapidly and significantly increased following xylazine administration. It is believed that these observations are the first evidence that epithelial cells lining the vas deferens can rapidly modulate luminal pH that ultimately would affect sperm function.

Vas deferens luminal content at pH 7.39, as reported here for the boar, contrasts with previous reports [4, 5]. An earlier study that reports pH in the luminal content of various compartments of the boar reproductive tract, determined that pH was 6.84 in the proximal segment of the vas deferens [5]. Distinct anatomical and technical differences might form the basis for the basal pH values reported here. In that study [5], the authors reported that measurements took place at the proximal segment of the vas deferens, a region of the duct that is not defined by distinct anatomical structures. Luminal pH in the proximal vas deferens is most likely influenced by acidic epididymal contents flowing distally towards the vas deferens. Measurements reported here were conducted at sites no less than 10 cm distal from the serpentine transition from epididymis, which is still within the proximal half of the vas deferens. In addition, the duct was severed distal to the serpentine region such that no luminal content from more proximal portions of the duct flowed on to the measurement sites during the assessments. The basal vas deferens luminal pH reported here also contrasts with what has been reported for the rat, where luminal pH was detected at 6.85 [4]. Here a combination of species-specific anatomy and epithelial tissue characteristics might be forming the basis for an acidic vas deferens luminal environment in the rat versus an alkaline one in the boar. Clearly, in absolute terms, the duct's length in rodent species is much less when compared to that of large domestic animal species. This spatial difference could exempt greater portions of the boar vas deferens from acidity that is maximal at the cauda epididymis. Factors such as slow flow, along with passive and active water and solute transport could favor such a scenario. Alternatively, there is substantial evidence to support that epithelia lining the vas deferens of species other than rodents might function differently. The rat vas deferens epithelium houses a number of proton secreting

cells and this cellular population is large enough to generate potential differences detectable to a proton selective vibrating probe [6]. Such acid secretion present in the rat vas deferens is bafilomycin-sensitive [6]. On the other side, porcine vas deferens epithelial cells cultured on permeable supports and voltage-clamped in Ussing chambers, do not exhibit short-circuit current that is sensitive to bafilomycin (Schultz, unpublished data). In addition, data are available to demonstrate how the absence of functional CFTR has very different impacts on reproductive performance and anatomy of the male excurrent ducts of men and mice [14-16]. It remains to be determined whether porcine vas deferens more closely approximates murine or human vas deferens.

Xylazine induced an increase in pH that was of greater magnitude when baseline levels were at the lower values recorded. In addition, responses were minimal when the luminal content was substantially alkaline at the onset of recordings. It seems reasonable to propose that in observations where baseline pH were relatively high at the time of baseline recording, that a modulatory mechanism could have already played out a pH increase, prior to the onset of recordings or that it can not move higher. It remains to be determined whether or not there are parallel mechanisms for active pH reduction of the vas deferens luminal environment following acute alkalinization.

The demonstration that pH in the lumen of the boar vas deferens can increase as much as six tenths of a pH unit, within approximately 8 minutes and at a nearly linear rate, is, most likely, a remarkable indication of the underlying epithelia's capacity to secrete bicarbonate. The possibility that proton reabsorption was the primary event accounting for the pH increases cannot be discarded. However, evidences for bicarbonate secretion as a significant component of the secretory activity of porcine vas deferens epithelial cells have been reported [11, 12]. Moreover, it was shown recently that sperm develops greater levels of motility by uptaking bicarbonate while yet in the male reproductive tract [2]. Data reported here support that the vas deferens luminal content may be the first source of bicarbonate to sperm leaving the epididymis. Data are also available to reveal that mammalian sperm is not only stored in the cauda epididymis, as it is primarily thought. In the mouse, 23 % of sperm cells are found in the vas deferens and close to half of these are found at the middle and distal thirds of the duct [17]. In men who undergo vasectomy, sperm cells located and possibly stored in the vas deferens lumen constitute a clinical concern that induce clinicians to recommend patients to observe a period of 4 weeks or several

ejaculations, to ensure complete clearance of sperm located at the vas deferens [18, 19]. However, the literature is currently conflicting to whether or not sperm is stored in the vas deferens or the seminal vesicles or both [18-20]. Therefore, it seems reasonable that, as sperm cells enter or remain in the vas deferens lumen for a given period of time, they are exposed to greater bicarbonate concentrations and may undergo initiation or enhancement of the motility acquisition process.

In conclusion, the pH of the boar vas deferens luminal content is alkaline and can be further and rapidly alkalinized. This should make bicarbonate available to sperm cells, might contribute to the process of acquiring fertilization capacity and, thus, modulate male fertility.

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Figure Legends

Figure 1. Porcine vas deferens luminal content is alkalinized by alpha-2 adrenergic agonist in vivo. Increases in pH, in response to intravenous xylazine administrations (arrows), occurred within 2-3 minutes. Responses had greater magnitude when baselines were at lower pH levels (A) than otherwise (B).

Figure 2. Luminal pH increases in the boar vas deferens are an effect of xylazine intravenous administrations. Statistical analysis (t-test) comparing baseline-pH to response-pH of all observations acquired (●, n = 18), indicates that xylazine induces a pH increase in the vas deferens luminal content ($P < 0.05$). Increases in pH induced by xylazine were statistically significant ($P < 0.05$) when baselines were at lower levels (■, n = 10) but not when the vas deferens luminal content exhibited greater levels of alkalinization by the onset of recordings (▲, n = 8).