Goat ureter—an alternative model for measuring ureteral peristalsis

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Received June 26, 2006; Accepted August 13, 2006

Abstract

An alternative model for the measurement of ureteral peristalsis is described using the goat ureter. Ureters from freshly slaughtered goats (Capra aegagous hircus) were collected from a local slaughter house. The peristaltic reflex of these preparations was recorded using a specially designed apparatus. The preparations were mounted so that contractile responses to drugs could be recorded isometrically. Histological studies were undertaken to enable a correlation to be made between the anatomical observations and the functional studies. The spontaneous peristaltic reflex of the goat ureter (7 ± 2 per 2 min) showed a 50% increase in the frequency of contraction (13.66 ± 1.6, P<0.001) after application of histamine at a concentration of between 6.512 µM and 13.024 µM, but was blocked completely by 10.4 µM of pheniramine (P>0.05). The reflex was not blocked by the H2 blocker ranitidine (P<0.001). The effects of acetylcholine were variable. Calcium chloride at 6.8 µM resulted in a tetanic response (P<0.001). Nicorandil showed partial inhibition of spontaneous peristaltic reflex at 189.4 µM and complete inhibition at 473.4 µM (P<0.001). Although acetylcholine did not show any appreciable effect on the isometric contractions at a maximum dose of 275.2 µM, adrenaline increased the frequency of contractions by 8.2 ± 6.5 (P<0.001), while salbutamol and isoprenaline had no effect. The histology revealed a striking resemblance to the human ureter, with a structure that explained the responses obtained. The anatomic, physiologic and histological similarities to the human ureter make it an effective alternative in tropical countries for research on ureteral peristalsis.

Key words: ureteral peristalsis, alternative tissue, goat ureter

Introduction

With the introduction of ethical controls on the use of laboratory animals for experimental purposes, there has been a restricted use of laboratory animals in scientific research. Newer methods and materials are being promoted by various scientific organizations as alternatives.
This is justified so as to balance scientific progress with the need to preserve the integrity of animal ethics. Many alternative methodologies like computer simulations, models, etc. may provide solutions that reduce the educational and academic use of animals, but for basic research it is mandatory that living tissue is used for investigating scientific questions. This issue has instigated our search for an alternative animal tissue that can be used in our research that does not involve killing of animals exclusively for experimental purposes. In spite of the presence of in vitro methods like tissue culture and cell culture, there are certain areas of research which still require a functional organ, such as an isolated heart, or segments of intestine, stomach, uterus or ureter, etc.

The study of the effects of drugs on ureteral peristalsis can help with the introduction of drugs into a wide range of therapeutic applications. Appropriate drug therapy could assist in pathological conditions related to the ureter, such as renal colic, and in preparation for procedures such as extra corporeal lithotripsy for avoiding retrograde propulsion of ureteral calculi, in providing relaxation of tissue preoperatively and also to ease the expulsion of ureteral calculus during flush therapy.

While many studies have been made of peristaltic mechanisms in the stomach and intestine, very little research has been conducted on peristalsis of the ureter. Many methods, both in vitro and in vivo are available to measure ureteral peristalsis. A number of laboratory animal species have been used for measuring the ureteral peristalsis in vitro (Table 1). Each of these species has their own advantages and disadvantages. Pig ureter is widely studied in various countries but it is not commonly consumed as meat in India and many Islamic countries as the killing of pigs is not permitted by local cultural beliefs. Sheep are easily available only in the areas near to wool based industries. Ethical clearance is becoming more and more difficult and laborious in the use of smaller animals. So we set out to find an animal which had the least ethical obstacles and which had maximum scientific advantages.

The domestic goat (Capra aegagous hircus) is widely distributed in tropical countries, and provided supplies of meat, leather and milk (Kraft, 1972). The gross anatomy of the goat ureter is very similar to that of humans. It receives an autonomic nerve supply from the pelvic nerve (Pasquini and Spurgeon, 1988) and a blood supply from the umbilical artery (Ghoshal and Habel, 1975).

Thus the easy availability of goats is an advantage for researchers working on ureteral peristalsis in tropical countries. In vitro experimentation using goat ureter does not require killing of the animal in the research laboratory as the tissue can be obtained from the slaughter house. Therefore it was of interest to study in detail the effect of drugs on the goat ureter. The present research was undertaken is an effort to introduce an alternative model for the measurement of ureteral peristalsis by using the goat ureter.

**Material and Method**

With ethical clearance from the ‘Animal Ethical Committee’, the present study was conducted in the Department of Pharmacology, Mahatma Gandhi Institute of Medical Science, Sewagram, India.
Collection of goat ureters

The ureters of freshly slaughtered goats weighing from 15 to 20 kg were immediately collected from a local slaughterhouse. They were then immersed in freshly prepared Mammalian Ringer (MR) with the composition given by Burn (1952), i.e. (in mM): sodium chloride 9.00 g/l (154), potassium chloride 0.42 g/l (5.6), anhydrous calcium chloride 0.24 g/l (2.2), sodium bicarbonate 0.50 g/l (0.595), dextrose 1.00 g/l (5.55), (distilled water up to 1,000 ml) maintained at 40°C and oxygenated with 100% oxygen. The pH of the MR was maintained at 7.4. Necessary precautions had been taken to avoid trauma and undue stretching of the tissue during its collection. The proximal 1/3rd of each ureter from the pelvic ureteral junction of about 4 to 11 cm in length was selected for experimentation.

Peristaltic reflex in the goat ureter

Peristaltic activity in the goat ureter was recorded using Trendelenburg’s, method (1917) with suitable modifications. The tissue was placed in an organ bath (MM 221, Inco Instruments, Ambala, India) containing warm MR solution. The distal end of the ureter was mounted over
the J tube connected to a modified reservoir (of length 15 cm, diameter 1 cm and capacity 10 ml, as it has been observed that the conventional reservoir suggested in the Trendelenberg method causes early fatigue of the tissue; Figs. 7 and 8). This modified reservoir also contained MR solution. The ureter was thoroughly washed with MR in order to expel air bubbles. The other end of the tissue was tied with thread to a lever. Before starting the experiment, the optimal intraluminal pressure required to elicit peristalsis of the ureter was determined by varying the height of the MR reservoir (between 0.5 and 1.0 cm) and then maintained throughout the experiment. Raising the MR reservoir to a critical height for 2 min induced peristaltic activity. This raise in intraluminal pressure triggered the peristaltic reflex and the fluid inside the ureter was driven to and fro. The changes in the volume were sensed by a volume transducer (T 303, Bio Devices, Ambala, India) attached to the reservoir. The recordings were made using a Students Physiograph (Bio device, Ambala) at a constant speed of 0.25 mm/sec and at 50 \( \mu V \) sensitivity. The peristaltic activity was recorded for 2 min, following which the MR reservoir was lowered down to stop the peristalsis. Care was taken to see that the temperature of the organ bath was maintained between 40 to 42 degree centigrade and the MR in the inner tube was constantly bubbled with 100% oxygen. Responses were only elicited when the spontaneous contractions remained stable in both amplitude and frequency. The drugs were used in logarithmic titers. Reversibility of the drug effect after washout was considered to be acceptable if the pre-drug and post drug values were within 10% of each other. The experimental apparatus is depicted in Fig. 1.

**Isometric contraction in goat ureter**

In studies of the isometric contraction for longitudinal muscle action, ureters of from 7 to 11 cm in length were suspended in the organ bath. The isometric forces were recorded using a force transducer (T 302, Bio Devices, and Ambala, India). The responses were recorded using a Students Physiograph at a speed of 0.25 mm/sec at 100 \( \mu V \) sensitivity. The tissue responses to the drugs were recorded for 2 min and prior to recording the next response, the ureter was
given a wash and rested for 5 min. Care was taken to see that the temperature of the organ bath was maintained between 40 to 42°C and MR in the inner tube was constantly bubbled with 100% oxygen. The experimental apparatus for measuring isometric contraction with a force transducer is depicted in Fig. 2.

The drugs were selected on the basis of previously published papers that used conventional laboratory species for the study of ureteral peristalsis. In this experiment the drugs were used from minimal concentration till an appreciable response was obtained and continued further until the maximal response was elicited. Logarithmic increments of the dose were made to elicit the range of responses. Different preparations were used to study the responses to each class of drugs. The tissue response to each drug and their specific antagonist was unique. At no point were two different classes of drug tried on the same tissue.

Drugs used for measuring the peristaltic activity in goat ureter are given in Table 2, while those used for measuring the isometric contraction in are shown in Table 3.

Chemicals used were as follows: histamine (Sigma Chemical Company, USA), pheniramine maleate (Aventis Pharma limited), ranitidine hydrochloride (Cadila Pharma chemicals), acetylcholine (Loba Chemicals, Mumbai), adrenaline (Arnoldo-otto Meyer, West Germany), calcium chloride (Sd. Fine Chem. Ltd, Mumbai), diltiazem (Torrent, India), glibenclamide, nicorandil (Nicoran, Torrent, India), isoprenaline (Unichem Pharma) and salbutamol (Cipla Pharmaceuticals). All chemicals were dissolved in distilled water for the *in vitro* experiments.

**Histology of goat ureter**

The segment of ureter from the upper 1/3rd was also used for this study. These ureters were also collected from the slaughter house immediately after the animal was slaughtered and its abdomen opened. After fixation in 10% formalin and routine processing and embedding, the transverse sections were cut and stained with Haematoxylin and Eosin and Masson’s Trichrome (Drury and Wallington, 1980). The above procedure was done on three ureteral preparations and the final histological reporting was done based on the finding from all tissues. The
histological structure was compared with that of the human ureter. The similarities and the differences are reported.

**Statistical analysis**

The following null hypotheses were assumed. Firstly that the goat ureter will not have normal peristalsis and secondly that if normal peristalsis is present then it will not respond to the drugs.

The significance values of P were taken as <0.05 significant, <0.01 very significant, <0.001 highly significant and >0.05 not significant and hence to accept the hypothesis. The mean of normal contractions with standard deviation was calculated and compared with a number of contractions after drug treatment expressed in terms of mean ± S.D. The significance was calculated by using one way ANOVA using SPSS version 11.0, USA.

**Results**

All preparations showed spontaneous activity within 5 to 15 min following mounting in the bath. The mean of the frequency of contraction observed was 7 ± 2 per 2 min. We also observed that tissue remained responsive for 8 to 10 h with constant environmental conditions. The contractions of individual preparations demonstrated a uniform pattern. Rhythmic motility was noted at times. Pre-drug activity was observed in all preparations and only those with optimum activity were used.

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**Table 2** Drugs used for measuring the peristaltic activity in the goat ureter

<table>
<thead>
<tr>
<th>Series Number</th>
<th>Drugs used in study</th>
<th>Dose Range/ml</th>
<th>Minimum</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Acetylcholine</td>
<td>1 µg</td>
<td>1 µg</td>
<td>1 mg</td>
</tr>
<tr>
<td>2</td>
<td>Histamine</td>
<td>1 µg</td>
<td>100 µg</td>
<td>10 µg</td>
</tr>
<tr>
<td>3</td>
<td>Pheniramine maleate</td>
<td>50 µg</td>
<td>–</td>
<td>50 µg</td>
</tr>
<tr>
<td>4</td>
<td>Ranitidine</td>
<td>100 µg</td>
<td>–</td>
<td>100 µg</td>
</tr>
<tr>
<td>5</td>
<td>Adrenaline</td>
<td>1 µg</td>
<td>200 µg</td>
<td>200 µg</td>
</tr>
<tr>
<td>6</td>
<td>Calcium chloride</td>
<td>1 µg</td>
<td>100 µg</td>
<td>100 µg</td>
</tr>
<tr>
<td>7</td>
<td>Diltiazem</td>
<td>1 µg</td>
<td>2 mg</td>
<td>2 mg</td>
</tr>
<tr>
<td>9</td>
<td>Nicorandil</td>
<td>1 µg</td>
<td>2 mg</td>
<td>2 mg</td>
</tr>
<tr>
<td></td>
<td>Need to add Glibenclamide</td>
<td>4 µg</td>
<td>4 µg</td>
<td>4 µg</td>
</tr>
</tbody>
</table>

**Table 3** Drugs used for measuring isometric contraction in the goat ureter

<table>
<thead>
<tr>
<th>Series Number</th>
<th>Drugs used in study</th>
<th>Dose Range/ml</th>
<th>Minimum</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Acetylcholine</td>
<td>1 µg</td>
<td>1 µg</td>
<td>1 mg</td>
</tr>
<tr>
<td>2</td>
<td>Adrenaline</td>
<td>1 µg</td>
<td>200 µg</td>
<td>200 µg</td>
</tr>
<tr>
<td>4</td>
<td>Isoprenaline</td>
<td>1 µg</td>
<td>1 mg</td>
<td>1 mg</td>
</tr>
<tr>
<td>5</td>
<td>Salbutamol</td>
<td>1 µg</td>
<td>1 mg</td>
<td>1 mg</td>
</tr>
</tbody>
</table>
Effect of histamine on peristaltic activity of goat ureter

Histamine was applied within the concentration range of (0.1628 \( \mu \)M to 16.28 \( \mu \)M (n=6). At concentrations of between 6.512 \( \mu \)M and 13.024 \( \mu \)M, the frequency of spontaneous ureteral contraction increased by a further 50% with a mean frequency of 13.66 ± 1.6 contractions (\( P < 0.001 \)). Pheniramine (1.4 \( \mu \)M) was able to completely block the effect of histamine (\( P > 0.05 \)) but did not affect either the frequency or amplitude of the spontaneous peristaltic contractions. Further increases in the concentration of pheniramine still had no effect on the spontaneous peristaltic contractions. However, ranitidine, an H\(_1\) antagonist, at a maximum dose of 1.59 \( \mu \)M did not block the histamine induced contractions (\( P < 0.001 \)). The effect of histamine persisted until it was washed out, after which baseline contractions were restored.

Effect of calcium chloride on the peristaltic activity of goat ureter

Calcium chloride was applied at a range of concentrations of from 0.34 \( \mu \)M to 34.0 \( \mu \)M. Calcium chloride (CaCl\(_2\)) produced a tetanic contractile response at concentrations of from 3.4 \( \mu \)M to 6.8 \( \mu \)M. At a concentration of 6.8 \( \mu \)M, calcium chloride increases the frequency of contractions by 64% (\( P < 0.001 \)) (Fig. 3). The amplitude of contractions was normal to slightly decreased at a concentration of 6.8 \( \mu \)M. Blockade by the calcium channel blocker diltiazem could not be achieved.

Effect of nicorandil on the peristaltic activity of goat ureter

Nicorandil was added to the bath at concentrations of between 0.237 \( \mu \)M and 473.4 \( \mu \)M. Nicorandil caused a 66% inhibition of peristaltic activity compared to control levels at a concentration of 189.4 \( \mu \)M (Fig. 4) and completely abolished peristalsis at a concentration of 473.4 \( \mu \)M (\( P < 0.001 \)) (Fig. 5). After a 15 min. wash-out, peristaltic activity had recovered by 64%. Glibenclamide at a concentration of 0.408 \( \mu \)M blocked this action.

Fig. 3. The effect of calcium chloride on peristalsis of the goat ureter.
Effect of acetylcholine on the peristaltic activity of goat ureter

Acetylcholine was applied at concentrations ranging from $275.2 \, \mu M$ to $275.2 \, \mu M$. It had no effect on the peristaltic activity of the goat ureter.

Effect of adrenaline on the peristaltic activity of goat ureter

Adrenaline at concentrations ranging from $0.2729 \, \mu M$ to $54.58 \, \mu M$ did not alter the peristaltic activity of the goat ureter ($P > 0.05$).

Effect of acetylcholine and adrenaline on the isometric contraction of the goat ureter

Acetylcholine at concentrations ranging from $0.2752 \, \mu M$ to $275.2 \, \mu M$ did not alter the isometric contractions of the ureter. Adrenaline caused an increase in the frequency of contractions at concentrations ranging from $0.2729 \, \mu M$ to $54.58 \, \mu M$ ($P < 0.001$) (Fig. 6). Salbutamol (a $\beta_2$ adrenoceptor agonist) within the concentration range of $0.208 \, \mu M$ to $208.9 \, \mu M$ and isoprenaline (a $\beta_1$, $\beta_2$ and $\beta_3$ adrenoceptor agonist) within the concentration range of $0.236 \, \mu M$ to $236.6 \, \mu M$ did not have any action on the isometric contraction of the goat ureter.

Histological examination of the goat ureter

Under a scanning objective lens the whole circumference of the ureter could be seen (Figs. 7 and 8). It was circular with a star shaped lumen and a thick wall. Under higher magnification,
Goat ureter for ureteral peristalsis

the following layers could be seen from the outside inwards. An outer thick tunica adventitia of loose connective tissue, which contained large muscular arteries. Internal to the tunica adventitia there was a thick muscular coat. The smooth muscle was arranged in three layers; both an outer and inner longitudinal layer with a much thicker circular layer in between. The innermost longitudinal muscle layer was the thinnest or in places quite scanty. Inside these muscle layers was the lamina propria containing thick collagen fibers, which was very thick and almost formed a membrane along the basal part of the epithelium. One peculiar observation was that many tubular alveolar glands could be seen in the lamina propria. They were at different layers; some were just at the depth of the epithelium, while others were nearer to the muscular layer.

The epithelium lining the ureter was a transitional epithelium with flattened surface cells having a regular margin facing the lumen. An irregular mass of surface coating were noted. The cellular layer was 4 to 7 cell thick. The cells were polyhedral with rounded nucleus and a prominent nucleolus. Typical pyriform cells of the second layer were not seen. Many cells seem to be vacuolated. Basal cells were cuboidal and form a row. The glandular epithelium was very thin with flattened or short cuboidal epithelial lining. There was no muscle around the glandular wall.

Discussion

The present study has provided the pharmacological and structural evidence that the goat ureter responds in the same way as that of both conventionally used ureteral preparations, as well as the human ureter, in studies that measure ureteral activity. The activity of the goat ureter was examined both in terms of the peristaltic reflex and its contractile properties. The long accepted method of measuring peristaltic activity as described by Trendelenburg (1917)
Fig. 7. Histology of goat ureter under 40 × H&E staining.

Fig. 8. Histology of goat ureter under 4 × Masson's trichom staining.
was used in this study. While the contractile response or the isometric contraction is used in most of the in vitro studies, some drugs have also been tested in our study. Histamine is known to have a modulatory effect on intestinal smooth muscle peristalsis. In agreement with previous studies done on the ureter (Borgstedt et al., 1962), histamine produces a stimulatory effect on goat ureteral peristalsis by increasing the frequency of contraction. This response was antagonized by pheniramine maleate (H₁ anti-histaminic), but not by ranitidine (H₂ anti-histaminic). Similar H₁ mediated responses were observed in a similar study done on the dog ureter (Doel et al., 1996).

Calcium is necessary for the development of the contractile response of the ureter. L-type voltage dependent calcium channels are responsible for the inward movement of Ca²⁺ into muscle cells, and for subsequently causing contraction. Previous studies have shown significant inhibition of ureteral contraction by calcium channel blockers (Maggi and Meli, 1984). Our study has shown the excitatory effect of calcium chloride in the form of a tetanic response.

The electrical activity of ureteral smooth muscle cells determines the peristaltic reflex. Potassium channels play an important role in the modulation of the electrical activity that also involves pacemaker activity as well as action potential generation and conduction (Weiss, 2002). Among the various types of potassium channels, K<sub>ATP</sub> channels have been known to bring about hyperpolarization and relaxation of ureteral smooth muscle in the rabbit, guinea pig and human (Klaus et al., 1989; Klaus et al., 1990). This property of potassium channels has been extended by use of a specific group of drugs called potassium channel openers—nicorandil, cromakalim etc. Nicorandil has been used extensively in many studies to bring about ureteral smooth muscle relaxation and to reduce the frequency of ureteral peristalsis (Weiss et al., 2002). In this study of the goat ureter, nicorandil at a concentration of 189.4 µM to 235.7 µM started to show the inhibitory effect which progressed to complete inhibition at 473.4 µM. The opening of K<sub>ATP</sub> channels by nicorandil induced a K⁺ efflux leading to a hyperpolarized state and thus smooth muscle relaxation. This suggests that K<sub>ATP</sub> channels are present in the goat ureter, but immunohistochemical confirmation of this observation is required.

Using a non-occlusive ureteral catheter, hyoscine butylbromide, an antimuscarinic agent was used symptomatically to decrease ureteral activity in vivo (Ross et al., 1972). The role of the parasympathetic nervous system in control of ureteral peristalsis has not been well defined (Weiss, 2002). In our study the effect of acetylcholine on goat ureter was variable. An in vivo study conducted on pig ureter has shown that acetylcholine does not alter peristaltic activity (Roshani et al., 2003). As the studies using Tendelenberg’s method have shown that the activity of the circular muscle is predominantly responsible for the peristaltic reflex, we studied the effect of acetylcholine on isometric contraction of the longitudinal muscle, which we measured using a force transducer. We found that acetylcholine had no effect on isometric contraction.

To further study the role of the autonomic nervous system, the effect of adrenaline on both the peristaltic reflex as well as on isometric contraction was determined. Adrenaline did not have any effect on peristalsis when applied in concentrations ranging from 1 µg to 200 µg/ml. However, the effect of adrenaline on isometric contraction, was to cause an increase in the frequency of contraction of the longitudinal muscle of the goat ureter. The ureter contains excitatory α adrenergic receptors and inhibitory β adrenergic receptors (Dean, 1988). The
inhibitory role mediated through adrenergic receptors is receptor specific and it has been also shown that the distribution of receptor types varies with the species (Tomiyama et al., 1998; Yamamoto and Koike, 2000).

As our results have shown a stimulatory action of adrenaline on the goat ureter, we studied the effect of β specific agonists on isometric contraction. Isoprenaline ($\beta_1$, $\beta_2$ and $\beta_3$ agonist) and salbutamol ($\beta_2$ agonist) did not have any effect on isometric contraction. This suggests that the stimulatory action of adrenaline on the goat ureter may be $\alpha$-adrenergic receptor mediated and that $\alpha$-receptors may be the predominant adrenoceptor in the goat ureter. Further confirmation of these results is needed.

For the application of results obtained from animal studies to humans, both in vitro and in vivo, it is mandatory that the preparation studied anatomically and functionally resembles that in human beings (Bennett and Brown, 2004). Histological examination of the goat ureter used in this study was performed in order to prove its anatomical resemblance. The histological report showed striking similarities when compared to that of humans (Bloom and Fawcett, 1978). Thus the resemblance of the goat ureter to that of the human ureter suggests that the responses obtained from the goat ureter can be scientifically correlated and the results can be suitably interpolated.

Goats are a common animal occurring abundantly in tropical countries, where they are slaughtered for meat. Utilizing the tissues of such animals killed provides a good source of different tissues that can be used for various experimental purposes. This does not pose ethical issues and allows for easier scientific investigation. In this study we have shown that the goat ureter as an alternative preparation that can allow studies to be conducted on ureteral peristalsis.

In conclusion our results suggest that goat ureter responds in the same way to standard drugs like acetylcholine, adrenaline, calcium and histamine as in the conventionally used preparations for measuring the ureteral peristalsis. The $P$ value of <0.05 observed during the course of the responses obtained allows us to reject the null hypothesis and confirm our assumption. The possibility of the presence of $K_{ATP}$ channels suggest that the mechanism involved in goat ureteral peristalsis is similar to that of humans. Further, the histological resemblance of the goat ureter with that of the human ureter gives a strong evidence for the similarity of these preparations. We therefore suggest that the goat ureter is the best alternative for this purpose, and in addition overcomes the considerations of animal ethics that are involved with standard laboratory animals. This in-vitro method will allow us to answer a number of scientific questions relevant to the activity of the ureter.

Acknowledgements

We are indebted to Professor Gosh, Professor and Head, Department of Anatomy, M.G.I.M.S. Sewagram, India for providing his valued opinion and assessment of the histological results. We are also thankful to Mr. P.R. Khapre, Dept of Pharmacology, M.G.I.M.S. Sewagram, India for his invaluable assistance.
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