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Contractile Behaviour of the Human Pyelo-Ureteral Musculature

I. Contraction Frequency/Force Relationship

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Summary. 405 normal and pathological ureteral and pyelic strips were electrically stimulated according to different schemes. Reflux strips produced stronger contractions than normal ones; transversally excised strips contracted stronger than longitudinal ones. The amplitude of a test pulse after a series of stimuli increased with increasing delay until a constant value (at 30 s). The first contraction after a rest period had a lower latency and higher amplitude ("rest contraction"). The time to peak force of contractions was constant. Elongation of the strips increased the amplitude but not the latency of the contractions. In the discussion the similarities between the contractile behaviour of the pyeloureteral muscle and of the heart are discussed.

Key words: Electrical stimulation, Normal and pathological ureter strips, Similarities heart/pyeloureteral muscle.

It is known that urine transport from pyelum to bladder at low and moderate diuresis depends on peristaltic activity [4]. At each ureteral contraction a bolus is pushed downwards; its configuration and the pressures within the bolus depend on the resistance at physiological narrowings or at pathological strictures [5]. The frequency of the contractions is related to the diuresis [12]. Furthermore the long refractory period of the ureteral muscle has been demonstrated in animals and man [7]. Since the contractile behaviour of the pyelo-ureteral musculature is important, we studied normal and pathological human pyelo-ureteral strips under different conditions caused by repetitive electrical stimulation.

The aim of the study was 1) to evaluate the contraction frequency/force relationship, 2) to determine if pathological conditions change the contractile force of the muscle and 3) to observe whether contraction force is different in a longitudinal versus transverse axis.

Since species dependent variations have been described [12], only human tissues are included in order to permit deductions for clinical practice.

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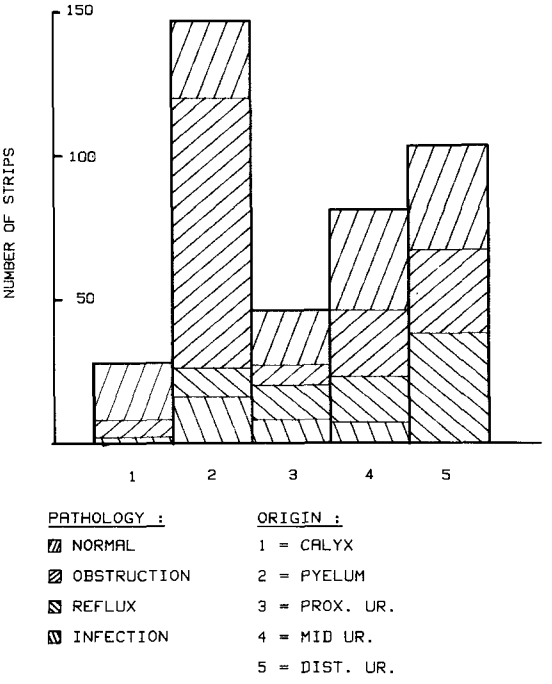

Fig. 1. Distribution of different strips according to their origin and pathology

Methods and Materials

This Study is carried out on 405 muscle strips of human upper urinary tract. The strips were obtained from operative specimens (nephrectomy or cystectomy for tumour, obstruction of the urinary tract, reflux, infection). The distribution of the different strips related to their origin and pathology is outlined in Fig. 1.

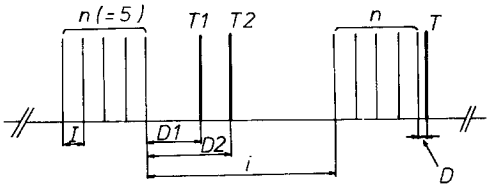

Fig. 2. Schme of electrical stimulation

4 Strips of 10 to 20 mm length and 2 to 3 mm width were simultaneously tested. The strips were carefully freed from connective adventitional tissue and cut in a distinct direction: longitudinally, transversally, helically or cut as a transverse closed ring. During this preparation the tissues were continuously moistened with Hepes or modified Krebs-Tyrode solution at 37 °C. The segments were mounted in a thermostable warmed organ bath, filled with the same solution at 37 °C, which was continuously aerated with 99.6% of O_2 and 0.005% of CO_2. The Hepes solution contains: (mM) NaCl 131,5, $CaCl_2$ 2.5, $MgCl_2$ 1.2, KCl 5.9, Hepes 11.5 and glucose 11.5. A pH of 7.35 was obtained by adding molar NaOH. The Krebs-Tyrode solution contained: (mM) NaCl 136.9, $CaCl_2$ 2.5, $MgCl_2$ 0.5, KCl 5.9, $NaHCO_3$ 11.9, NaH_2PO_4 0.3 and glucose 11.5; a pH of 7.35 being obtained by adding molar NaOH or molar HCl. One end of the muscle segment was fixed, the other end was connected to a home-made isometric force transducer with a sensitivity of 2 V/g, a range of 0–10 g, minimal force of 3 mg, hysteresis < 0.1 mg, revovery time 12 ms at 10 g force and drift of 5 mg/h. A slight initial tension of 1 g was allowed to equilibrate for at least 45 min; in some experiments the length of the strip at this value was increased by 1/10 or 1/5.

Electrical activation of the tissue was obtained by field stimulation through 2 platinum wires, attached 8 mm apart at opposite sites of the bath. The surface of the electrodes was 25 mm^2. Rectangular pulses of 10 to 80 V and 0.5 to 200 ms duration were provided by a Grass SD5 stimulator unit, triggered at variable intervals by a Devices digitimer. Several stimulation schemes were applied:

1. 10 to 200 ms, 10 to 80 V stimuli at a frequency of 1 per 40 s.
2. pulse trains of a fixed number (n), followed by a test pulse (T) at various delays (D) after the train (Fig. 2).

The activity was recorded on a Philips PM8252 dual pen recorder and on a Kipp and sons 2 channel BD9 recorder.

At the end of most experiments the muscle strips were dried between absorbing paper and weighted on a precision balance. By dividing maximal contraction strength, recorded during the experiment in normal conditions by the weight of the strip, a factor "force per weight unit" was obtained; this value was described as "the normalized force".

All specifications of the tissues (origin, pathology, direction of the strip, specifications of the bath solution, a. o.) and the results of the experiments were entered into a Hewlett-Packard computer, model 9826 and stored on a flexible mini disc. All data were processed by the computer, mathematical and statistical parameters were computed and graphs plotted on a H–P 7470A plotter. Comparisons between groups of strips were performed with the Student's t-test by rejecting the "null-hypothesis" below a level of 5% ($p < 0.05$ = sifnificant difference).

Results

1. Strength of Contractions

The normalized force as a function of weight of the tissue showed no mathematical correlation between force of contraction and weight of the strip. A statistically significant difference in forces was found between normal versus reflux cases and between longitudinal versus transverse strips (Table 1). Reflux strips produce stronger contractions than normal strips, and transversally excised strips contracted more strongly than longitudinal strips. Proximal segments did not differ from distal ureteral fragments, nor did chronically obstructed segments differ from normal ureters.

2. Miminal Excitation Voltage

In this test 81 strips were used. Stimuli of decreasing voltage were applied every 40 s at a stimulus duration of 200 ms until the disappearance of a measurable contractile response was observed. This series of pulses was repeated at a shorter stimulus duration. In this way the minimal excitation voltage necessary to elicit a measurable contraction of at least 5% of the maximal possible contraction amplitude was determined for the different pulse durations. As expected the

Table 1. Normalised force as a function of different origin and pathology of the ureter segment

n	X ± SE	*n*	X ± SE	*t*	*p*
Proximal ureter		Distal ureter			
4	3.2 ± 1.3	30	13.9 ± 3.2	1.207	$0.1 < p < 0.5$
Normal		Obstructed			
28	9.2 ± 2.5	24	14.1 ± 2.8	1.314	$0.1 < p < 0.5$
Normal		Reflux			
28	9.2 ± 2.5	9	28.8 ± 3.8	3.959	$p < 0.001$[a]
Longitudinal		Transverse			
37	10.9 ± 1.9	15	21.2 ± 5.1	2.299	$0.02 < p < 0.05$[a]

[a] statistically significant

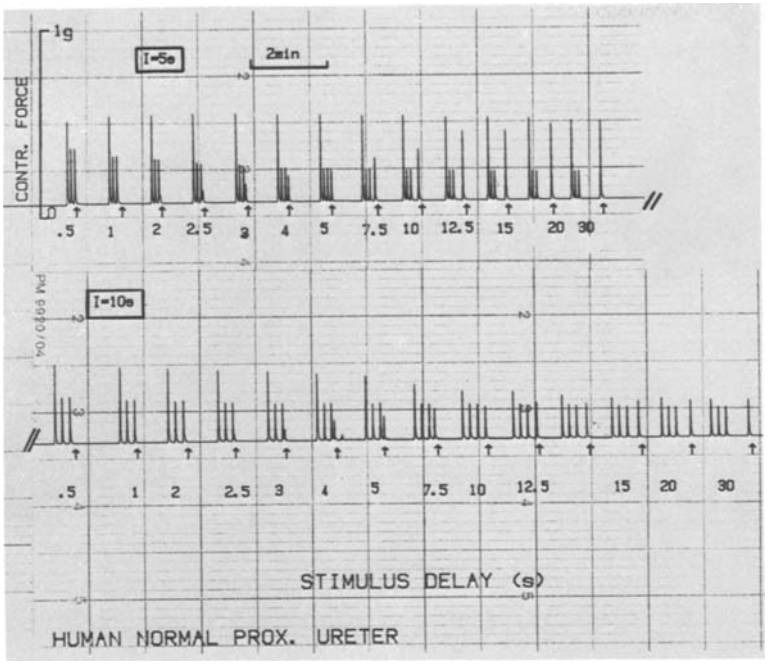

Fig. 3. Test pulse T (arrow) at increasing delays after 5 train stimuli (I = 5 s, *upper curve* and I = 10 s, *lower curve*) in a normal proximal ureteric strip. Note the large response to the first stimulus after each rest period (Woodworth staircase phenomenon)

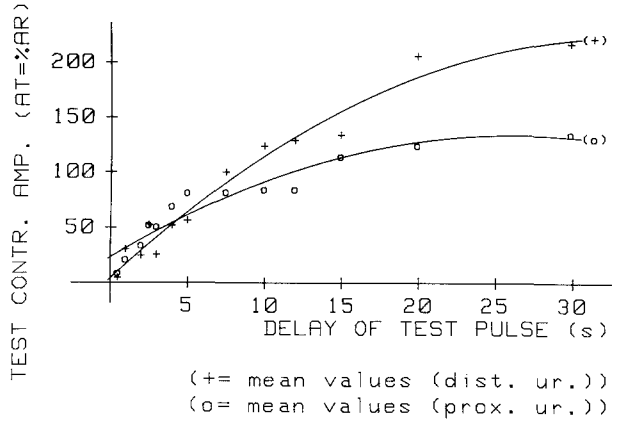

Fig. 4. Test contraction amplidutes in proximal and distal ureteric segments at increasing delays

minimal exitation voltage decreased with increasing stimulus duration until a constant value equal to the rheobase was reached. The rheobase was 39.7 ± 1.5 V (X ± SEM, n = 93) and the chronaxie was 28.0 ± 1.7 ms (X ± SEM, n = 90). No statistically significant differences existed for these values according to the pathology, origin or strip direction respectively.

3. Influence of Delay of Test Pulse

The test involved 47 strips. Since it was found by Cuthbert in the guinea pig that the true rested state can only be obtained after a period of 20 to 30 min, we preferred to apply train stimuli (Fig. 2) to produce steady state conditions instead of twin pulses separated by long periods. In all tested strips the amplitude (AT) of the contractile response to the test stimulus (T) as described above, increased with increasing delays (D) until a constant value was reached. An example of such an experiment is shown in Fig. 3. We tested the 5 and 10 s intervals which lie within usual contraction rates of the ureter.

If a pulse train is started after a resting period, the amplitude of the first – and sometimes also the second – contraction is markedly higher than the following ones, which then show stable steady state amplitudes serving as a reference value of 100%. In the same way the latency period of this first contraction was significantly lower (0.212 s ± 0.0047, X ± SEM; n = 11) than of the following ones (0.241 ± 0.0054; n = 10).

When the amplitude of the test contraction (AT) is expressed as a percentage of the mean amplitude of the last steady state contractions of the preceding train (reference value of 100% = AR), AT can increase to more than 200% of AR. The test contraction amplitude reached a constant maximal value at a mean delay of 30 s.

Comparison of test contraction amplitudes of proximal and distal ureter segments showed weak but significant differences at delays above 12 s, the distal segments were the strongest (Fig. 4).

The contraction amplitudes evoked by test pulses after reference pulse trains of 5 s were significantly higher than for 10 s intervals: the amplitudes for I = 5 s were higher than for I = 10 s. At smaller delays the differences were not statistically significant (Table 2).

With D and I held constant, no influence of the number of reference pulses (n) (3 to 20) on AT was found.

In most of the tested strips the time to peak of the contraction (rise time) was measured by recording pulse induced contractions at higher paper speed. Time to peak of contrac-

Table 2. Differences of test contraction amplitudes after reference trains of 5 resp. 10s intervals

Delay(s)	n °	AT (5 s) $\bar{X} \pm SE$	AT (10 s) $\bar{X} \pm SE$	t	P
0.5	21	21.2 ± 11.9	14.2 ± 10.1	0.45	> 0.5
1	24	53.3 ± 11.9	13.8 ± 6.3	2.93	< 0.02*
2	23	35.5 ± 10.5	46.8 ± 7.8	0.78	> 0.1
2.5	14	66.9 ± 9.1	53.3 ± 15.6	0.78	> 0.1
3	19	58.8 ± 12.7	40.4 ± 7.7	1.30	> 0.1
4	28	79.9 ± 6.5	51.8 ± 8.6	2.65	< 0.02*
5	29	91.1 ± 8.5	66.6 ± 6.1	2.38	< 0.05*
7.5	26	126.9 ± 13.5	77.5 ± 7.0	3.38	< 0.01*
10	24	134.5 ± 13.8	96.7 ± 4.9	2.41	< 0.05*
12.5	15	128.3 ± 9.7	114.7 ± 2.9	1.60	> 0.1
15	30	207.4 ± 23.8	123.9 ± 6.9	3.76	< 0.001*
20	23	210.8 ± 28.1	132.3 ± 9.5	2.55	< 0.02*
30	25	223.0 ± 33.4	145.8 ± 10.3	2.44	< 0.05*

Table 3. Influence of length on contractions at stimulus intervals of 0.1 to 30 s

Parameter of contraction	L = 100%			L = 120%			t	p
	nr	$\bar{X}$	SEM	nr	$\bar{X}$	SEM		
amplitude (mg)	8	67	18	11	960	266	2.8	< 0.02
rise time (s)	8	0.27	0.03	11	0.58	0.03	6.7	< 0.01
latency (s)	8	0.18	0	11	0.18	0	0	> 0.50

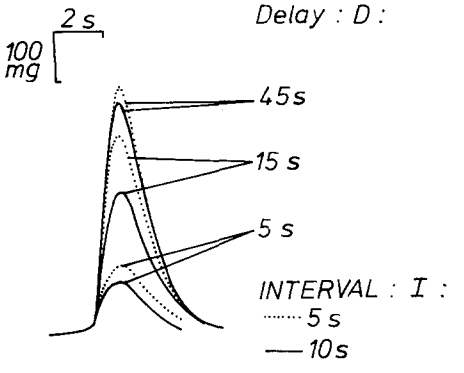

Fig. 5. Amplitude of test contraction af different delays after reference train contractions of 5 or 10 s intervals. Note that the time to peak remains unchanged

tion was not changed whatever the values are of D, n and I and averages 1.32 ± 0.7 s (SD). Since the AT varied with D, the rise rate increased with AT (Fig. 5).

4. Influence of Increase of Initial Length on the Contraction Parameters

If the strip is elongated to 110% or 120% of its initial value (L = 100%) amplitude and rise time of contractions at stimulus intervals of 0.1 to 30 s were increased, while the latency period showed no significant decrease (Table 3). However the number of contractions necessary to obtain a steady state amplitude were increased to 4 or 5 contractions.

Discussion

The contraction force exerted by a muscle strip depends not only on the weight of the strip; indeed large variability was observed for the "normalized" strength i.e. the maximal contraction strength diveded by the strip weight. The force of contractions was similar in all pyelo-ureteral segments; we therefore think that the higher intra-ureteral pressure waves observed in the proximal ureter by Ulmsten [10] reflect lower compliance of the wall. It is of interest to note that even calyx and pyelum considered as pacemaker areas, have no lower excitation levels.

Statistically significant differences were found between longitudinally cut and transversally cut or annular strips, the latter producing stronger contractions (Table 1). This may be related to the orientations of the muscle fibers. It is generally accepted that the ureter is composed of helical strucutres; if the slope of such helix is low [11] or if the helical fibers end in more circular windings, then the transversely directed forces are larger than along the longitudinal

axis. In this respect our findings disagree with observations of Malin et al. [9] in canine preparations, who reported tension amplitudes too small to evaluate in circular strips. All reflux ureter strips produce stronger contractions than corresponding normal strips (Table 1). This may be due, in the absence of infection, to muscular hypertrophy induced by increased load (transport of the sum of normally excreted urine plus refluxed urine).

Our chronaxie values were comparable with values observed by others [14]. Despite stronger contractions in transverse and reflux ureters in respect to normal ones, no statistical differences are found in their minimal excitation voltages (rheobase-chronaxie curves). This is in contrast with the findings of Weiss et al. [14] who needed higher currents and pulse durations for excitation of dilated human ureteral segments. Probably their segments were taken from very severely obstructed or refluxing cases with infection: indeed we excluded strips of dilated ureters classified as "rotten", because of atonicity and very low excitability.

We paid much attention to the normal force-interval relationship because alterations when tissue is exposed to an inotropic agent such as a sympathomimethic or to ionic changes such as high calcium concentration, then this relationship may provide a criterion for classifying the activity of drugs.

The stimulus schemes used were analogous to those applied in studies of the force-interval relationships in cardiac muscle [8]. The necessity of creating a steady state by a stimulus train was dictated by the long refractory period (fatigue) of the pyelo-ureteral tissue [12]. Cuthbert [3] mentioned that in the guinea pig ureter a rest period of 20 to 40 min was required to obtain a true steady state condition. The amplitude of the train contractions at an interval of 5 s or 10 s as in Fig. 3 were submaximal due to the negative inotropic effect which in the guinea pig ureter lasts for 40 s [3]. Note that a stimulus interval of 5 to 10 s is within physiological limits: ureteral contractions at a frequency of 6 to 12 per min at moderate diuresis are not exceptional [2] and the basic electrical rhythm of the calyx is 6 to 7 in the guinea pig [6] and 10 to 12 in the pig [4]. The contraction force at these frequencies however may be one half of the maximun force the ureter can generate after a resting period. At this frequency propulsion of the urine may therefore be impeded, especially if still higher frequencies are evoked as a first reaction to subobstruction [12].

A similar reaction was observed if the delay of the test contraction was longer than the interval between the pulses of a train: its amplitude was then larger than that of the contractions of the train while for shorter delays the reverse was true (Fig. 3, 4). We observed a high "rest contraction" and a so-called Woodworth staircase phenomenon, which is an enhanced contractility following a long pause. These terms were first used in cardiac physiology to describe the first stimulus after prolonged inactivity which is enlarged: this so-called "rest contraction" is an example of the reverse staircase phenomenon since the interval of rest can be regarded as a temporary shift to a lower frequency which causes an increase of twitch tension. This can be explained by the plateau type of action potential in both ureters [12] and cardiac tissue. A possible explanation is that at high frequency stimulation during the train pulses most contractions result from Ca^{++} entry derived from extracellular fluid, while at rest or low frequency stimulation cellular Ca^{++} sources are added [7]. Other similarities between ureter and cardiac muscle have been described by Weiss [13] who described Wenckebach periods in canine ureters. The existence of a "rest contraction" implies that the first and even the second response to a pulse train stimulus should be disregarded.

The larger sensitivity of the distal ureter to the repeated stimulation, as expressed by the higher test contraction in comparison to proximal segments may be partially caused by the formation of a cystoid in the proximal ureter, since more powerful contraction may impede passage of urine.

Analysis of the waveform of each contraction (force versus time) (Fig. 5) shows that changes in the rate of force development were related to the test stimulus delay while the time to peak force remained largely unchanged. The peak force as the traditional measure of contractile force is therefore an appropriate and complete measure of changes in waveform and is easier to measure than the rise time. We did not find variations in time to peak force as has been observed in the papillary muscle [8]. Since time to peak is unchanged at different forces, the velocity of the ureteric muscle shortening increases with increased amplitude of contraction. However the velocity of shortening is independent of the initial lengths of the strip (isometric tension). The maximum rate of rise of tension in a contraction, F_{max}, should be independent of length if it serves as a measure of contractility [1]. When the strips were elongated all parameters, except the latency periods were increased. It is known that elongation provokes membrane depolarisation and therefore increased excitability. In dilated strips (by obstruction or reflux) this may be the case although Weiss [14] found a lower excitability (see discussion higher).

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