Thai J. Physiol. Sci 3(1), 39-49 (1990)

CONTRACTILITY OF THE CAUDA EPIDIDYMIDIS OF RATS *IN VIVO* AFTER CASTRATION

Ganyapong Chaturapanich and Chumpol Pholpramool

Department of Physiology, Faculty of Science, Mahidol University, Rama VI Road, Bangkok 10400

ABSTRACT

Intraluminal pressures of the distal cauda epididymidis of rats with and without the presence of luminal spermatozoa have been measured by cannulation of the proximal vas deferens. Spontaneous contractions, having an amplitude and frequency of 7.2 ± 0.2 (n=3) cmH_2O and 1.3 ± 0.2 (n=3)/min, respectively, were observed in the sperm-intact epididymis of normal rats. Electrical stimulation (5V, lHz) caused an elevation of the basal pressure from 2.0±0.6 to 7.1±1.0 (n=4) cmH_2O and doubled the frequency without affecting the amplitude of contractions. By 7 days after gonadectomy, the basal pressure was not changed but spontaneous activities ceased completely. Nevertheless, contractions with high amplitude and low frequency were induced by electrical stimulation (5V, 1 Hz). After perfusion to remove luminal spermatozoa, spontaneous contractions of the epididymis were observed in both intact and castrated rats. In the control rats, the amplitude and frequency were 20.7±1.4 (n=27) cmH_2O and 2.4±0.2 (n=27)/min, respectively. These values were unchanged by 5 days after castration. However, by Day 7 the amplitude had significantly decreased to 11.4±1.9 (n=5) cmH_2O while the frequency was unaltered. The responsiveness of the sperm-freed epididymis to both electrical stimulation (5V, 1-10 Hz) and intra-arterial injection of clonidine (0.1-100 ug/kg) was virtually unaffected 7 days after androgen withdrawal. It was concluded that castration produced a time-dependent decrease in the amplitude of contractions of the rat cauda epididymidis but did not modify its responsiveness to contractile agonists.

Key words: epididymis, contractions, intraluminal pressure, castration

INTRODUCTION

It is well established that castration produces atrophy of the smooth muscles of vas deferens, but induces spontaneous contractions and enhances the responsiveness of this organ to contractile agonists (Martins & Valle, 1939; Markus et al.,1980; Calixto & Rae, 1981). On the other hand, the dependence of epididymal contractility on testosterone is still unsettle. This is probably due to differences in the methods used and the duration after hormonal manipulations. Thus, from visual observations of contractile activities of rat epididymis *in vivo*, Risley (1958) found that spontaneous contractions continued for at least fifteen days after bilateral castration. However, the activities were completely absent by 30 days but were restored by testosterone propionate (Risley, 1959). Direct application of testosterone to the guinea-pig cauda epididymidis caused an increase in tonic contractures without affecting the rate or amplitude of spontaneous phasic contractions (Da Silva E Souza et al., 1974). By measuring the intratubular pressure of the distal cauda epididymidis of rats, Hib and Ponzio (1977) reported that spontaneous contractions disappeared by 4 days after gonadectomy but the contractility was maintained by testosterone replacements. The results in these studies suggest that testosterone has a stimulatory effect on and is required for the maintenance of epididymal contractions.

In contrast, evidence from the epididymal sperm reserve in the rat (Dyson & Orgebin-Crist, 1973; Foldsey & Bedford, 1982; Pholpramool et at., 1982) and in the hamster (Lubicz-Nawroki, 1974), and from the rate of sperm transport through the epididymis in rats (Sujarit & Pholpramool, 1985) indicates that contractile activities of the epididymis are either maintained or even enhanced shortly (within 7 days) after androgen withdrawal. Furthermore, direct measurement of intraluminal pressures in different regions of the rat epididymis *in vivo* by using a survonulling pressure device showed that bilateral castration produced time-dependent increases in contractility of the mid-caput, the mid-corpus and the proximal cauda. These effects were also mimicked by cyproterone acetate (Din-Udom et al., 1985). The results, therefore, indicate that testosterone suppresses spontaneous activities of the epididymal tubule in intact rats.

In order to clarify this discrepancy, the present study has determined the spontaneous activities of the distal cauda epididymidis of rats *in vivo* by using catheterization method shortly after castration. In addition, the role of androgens on the responsiveness of the epididymis to contractile agents, which is presently unknown, was investigated.

Materials and Methods

Animal preparations

Adult, male Wistar rats weighing 300-430 g from the Mahidol University colony were housed in groups of 6-8 animals separated from females under a 12:12h lighting regime, and with free access to food and water. They were fasted overnight before the day of experiment and anaesthesia was induced by sodium-5-ethyl-(l-methyl -propyl)-2-thiobarbiturate (Inactin, Byk Gulden Konstanz), 100 mg/kg injected into the peritoneal cavity. Tracheostomy was then performed and a short polyethylene tubing (PE240, Clay-Adams) was inserted and fixed in place to ensure a clear airway passage. The right femoral artery was cannulated with a polyethylene tubing (PE50) and connected to a strain-gauge pressure transducer for continuous recording of systemic blood pressure on a Grass polygraph (Model 79D). The rectal temperature was monitored by using a telethermometer (Yellow Springs, 73A) and was maintained at 37 °C by direct illumination with a desk lamp.

In the experiments in which drugs were given, the left femoral artery was exposed and catheterized with a polyethylene tubing (PE50) containing heparinized saline (50 I.U./ml). The tip of the cannula was advanced to the branch of common iliac artery.

Measurements of epididymal contractions

Contractions of the distal cauda epididymidis were measured *in vivo* by catheterization of the vas deferens according to the method described previously by Hib and Ponzio (1977). In some experiments the luminal contents of the distal cauda was flushed to remove spermatozoa (Wong & Yeung, 1978).

Briefly, the left testis and epididymis were exposed through a skin incision on the scrotal sac. Vas deferens was identified and carefully isolated from the associated blood vessels. A small opening was made on the wall of vas deferens about 3 cm from the epididymal end. A fine polyethylene tubing (PE 60), the tip of which had been pulled over a microflame to an external diameter of approximately 700 um, filled with paraffin oil was inserted towards the rostal end until the tip reached the junction between the distal cauda epididymidis and vas deferens. The catheter was then ligated close to the epididymal-vas deferens junction with silk thread (No. 5-0, Ethicon). The other end of the catheter was connected to a venous pressure transducer (Statham, Model P23BB) by means of a three-way valve. Changes in pressure, which were recorded on a Grass polygraph (Model 79D), reflected that the contractions originated from the distal cauda epididymidis and, perhaps, from a short segment (not longer than 2 cm) of the proximal vas deferens. This method of measurement of epididymal contractions is similar to that described by Hib and Ponzio (1977) except that, in the present study, paraffin oil was used to fill the cannula instead of physiological saline so as to prevent initiation of sperm motility *in situ* and hence entry of epididymal spermatozoa into the cannula. In spite of this modification, we usually observed the influx of epididymal content into the tip of the cannula and frequent obstructions occurred. In order to alleviate this problem, the epididymal duct was flushed to remove its luminal content by cutting off the epididymal tubule at zone 6 (Reid & Cleland, 1957) and retrogradely perfused through the vas cannula at a rate of 6.5 ul/min using a Harvard (Model 901A) infusion pump until the isolated segment was cleared of spermatozoa (approximately 45 min). The open end of the tubule was doubly ligated with silk thread (No. 5-0). The mean ± SEM length of the perfused segment was 15.3 ± 3.0 cm (n = 37 rats).

The perfusing solution had the following composition (in mM) : NaCl, 20; KCl, 55; $MgCl_2.6H_2O$, l; $CaCl_2$, 0.3; Tris-HCl, 10; sucrose, 16.5. The calculated osmolality and pH of this solution were 335 mOsm/kg H_2O and 6.85, respectively. This solution has similar electrolyte concentrations, osmolality and pH to those reported in the luminal fluid of the rat cauda epididymidis (Levine & Marsh, 1971).

Before the intratubular pressure was recorded, it was opened to the atmosphere for a few minutes to remove residual pressure accumulated during perfusion. The initial intraluminal pressure was then adjusted to about 7 cmH_2O, which was the normal basal pressure of the rat cauda epididymidis (Pholpramool et al., 1984), by means of a three-way valve connected between the vas cannula and pressure transducer. After the pressure recording was allowed to stabilize, the following parameters of the contractile activity were analyzed :

a) ***Basal pressure*** (cmH_2O), an average of the minimum pressure between contractions, which represents tonic contractions of the tubular smooth muscles.

b) ***Amplitude of contraction*** (cmH_2O), a height between basal pressure and the peak level of each contraction wave. This pressure represents the magnitude of phasic contractile activity.

c) ***Frequency of contraction*** (/min), the number of peak of contraction per min.

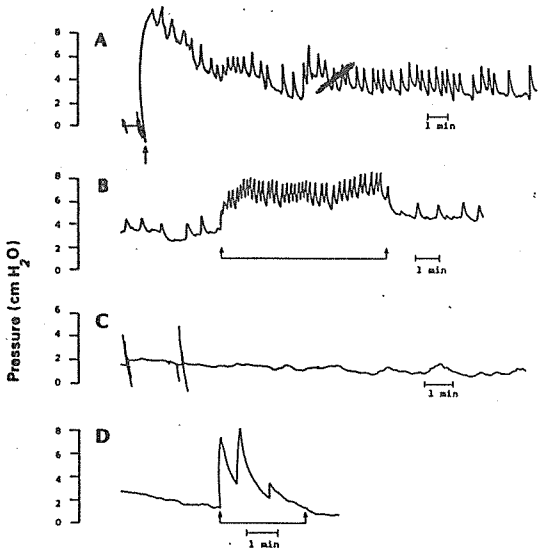

Figure 1. Typical tracings of the pressure waves recorded from the sperm intact distal cauda epididymidis of control and castrated rats. A) Spontaneous contractions of the control epididymis after opening the three-way valve connecting the vas cannula and the pressure transducer (at arrow). B) Responses of the control epididymis to electrical stimulation, 5V 1 Hz (between arrow heads). C) A tracing from the epididymis of a castrated rat showing no spontaneous activity. D) Responses of the epididymis from a castrated-rat to electrical stimulation, 5V 1 Hz (between arrow heads).

Electrical stimulation of the epididymis

A bipolar platinum electrode was placed on the epididymal end of vas deferens and the tubule was stimulated by trains of square pulses generated from a Grass stimulator (Model S48). The stimulus parameters were varied from 1-5 volts and 1-10 Hz with a constant train and pulse durations of 500 and 2 msec, respectively. Preliminary studies showed that this mode of stimulation induced reproducible contractile responses of the epididymis. Local applications of xylocaine onto the epididymal-vas deferens junction effectively and reversibly abolished the contractions induced by electrical stimulations indicating that activation of the epididymal tubule occurred through nerve conduction from the site of stimulation.

Bilateral castration

Bilateral gonadectomy was performed through an abdominal incision in the anaesthetized rats under aseptic conditions and the animals were allowed to recover. The details have previously been described (Din-Udom et al., 1985).

Statistics

Nonparametric methods, i.e. Wilcoxon's rank sum test, Spearman's rank order correlation and Kruskal-Wallis test, were used where appropriate. The critical probability for rejection of the null hypothesis was 0.05 throughout. All data are presented as mean ± S.E.M.

Table 1. Contractility of the distal cauda epididymidis in normal and 7-day castrated rats and their responsiveness to electrical stimulation +

Group	Basal pressure (cmH20)		Amplitude of contraction (cmH20)		Frequency of contraction (/min)	
	Before	After	Before	After	Before	After
Control	2 0±0.6 (4)	7.1±1.0[a] (4)	2.7±0.2 (3)	2.7±0.1 (4)	1.3±0.2 (3)	2.6±0.3[a] (4)
Castration	2.1±0.5 (4)	1.8±0.9 (4)	0 (4)	4.9±0.6[a,b] (4)	0 (4)	1.0±0.3[b] (4)

Values are means ± SEM; numbers in parentheses are numbers of animals.
[a] Singificantly different at $P<0.05$ between before and after stimulation of the control group.
[b] Significantly different at $P<0.05$ between control and 7 days after castration.
+ Parameters for electrical stimulations are : strength, 5V; pulse duration, 2 ms; frequency of stimulation, 1 Hz

RESULTS

Contractions of the non-perfused epididymis

Normal intact rats

Figure 1 illustrates a typical recording of contractions of the distal cauda epididymidis. As soon as the valve connecting the epididymal tubule and the pressure transducer was open, a sudden rise in pressure was observed (Fig. 1A). This pressure gradually decreased to reach a steady level within 15-20 min, after which the basal pressure was maintained at 2.0 ± 0.6 ($n=4$) cmH_2O. Superimposed on this pressure were spontaneous contractions having a mean amplitude of 2.70 ± 0.2 ($n=3$) cmH_2O and frequency of 1.3 ± 0.2 ($n=3$) per min. Electrical stimulation at 5V, 1 Hz elevated the basal pressure and frequency of contractions to 7.1 ± 1.0 ($n=4$) cmH_2O and 2.6 ± 0.3 ($n=4$) per min, respectively (Fig. 1B). The amplitude of contractions was not altered by this parameter of stimulation (Table 1).

Castrated rats

By 7 days after castration, none of the four epididymides showed spontaneous activities (Fig. 1C). However, the basal pressure was virtually unchanged at 2.1 ± 0.5 cmH_2O (Table 1). When the epididymal tubule was stimulated at 5V, 1Hz an abnormal pattern of contractions was elicited (Fig. 1D). The amplitude and frequency of contractions were 4.9 ± 0.6 cmH_2O and 1.0 ± 0.3 per min, respectively. The former was almost double while the latter was about half of those of normal rats. However, the basal pressure was not elevated (Table 1). It should be mentioned that in many preparations attenuation of the amplitude of contractions occurred during stimulation indicating decay of pressure waves and a blockage of the cannula (Fig. 1D). Direct

observations confirmed the presence of epididymal fluid content inside the tip of the cannula. Thus, frequent flushing of the cannula was required to prevent obstruction.

Contractions of the perfused epididymis

Normal intact rats

After the epididymal duct was perfused to remove spermatozoa regular spontaneous contractions were observed. The pressure recordings of phasic contractions were sharp and smooth (Fig. 2A) compared to the non-perfused tubule. The amplitude and frequency of contractions were 20.7 ± 1.4 (n = 27) cmH_2O and 2.4 ± 0.2 (n = 27) per min, respectively (Table 2). Although the basal pressure was initially adjusted at 7.0 cmH_2O, it was either decreased or increased to yield, at steady state, a value ranging from 4.5 to 14.6 cmH_2O with a mean value of 7.3 ± 0.6 (n = 27) cmH_2O. There were no correlations between the amplitude and frequency of contractions versus the basal pressure. Similarly, tests for the relationships between the basal pressure, the amplitude and the frequency of contractions with

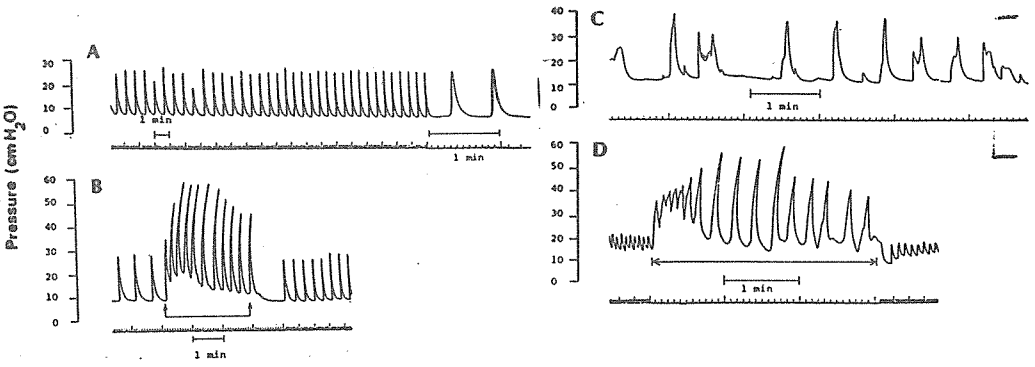

Figure 2. Typical tracings of the pressure waves recorded from the perfused distal cauda epididymidis of control and castrated rats. A) Spontaneous contractions of the control epididymis. B) Responses of the control epididymis to electrical stimulation, 5V 1 Hz (between arrow heads). C) Spontaneous activities of the androgen-deprived epididymis. D) Responses of the androgen-deprived epididymis to electrical stimulation, 5V 1 Hz (between arrow heads).

the length of the sperm-freed tubule ranging from 13.8 to 16.7 cm were also negative. Thus, perfusion of the epididymal duct in this study did not alter the contractility of tubular spontaneous activities.

Figure 2B shows the responses of the sperm-freed epididymis to electrical stimulation. At a strength of 5V, the basal pressure was elevated by an increase in the frequency of stimulation from 1 to 10 Hz (Fig. 3A). In contrast, the amplitude of contractions was a 2.5 fold increased at 1 Hz compared to those at 3 and 10 Hz (Fig. 3B). On the other hand, an optimal frequency of stimulation to produce maximal rhythmicity of contractions appeared to be 5 Hz (Fig. 3C).

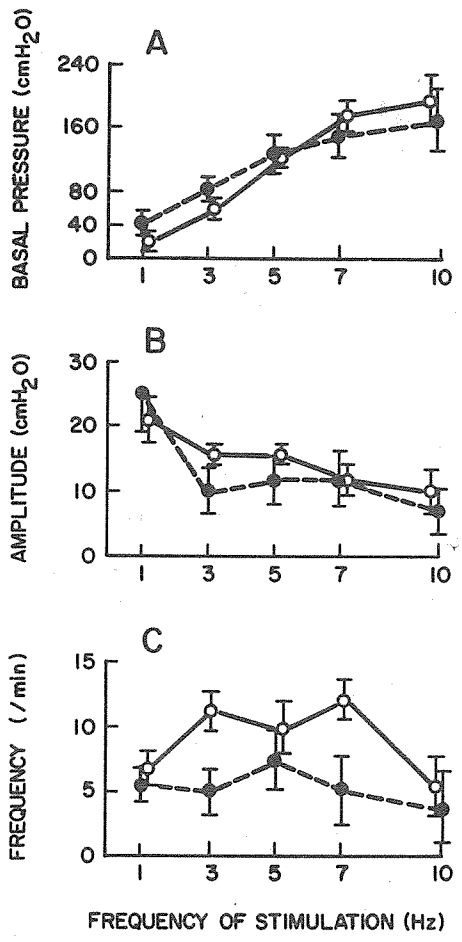

Figure 3. Effect of electrical stimulation at 5V and 1-10 Hz on the basal pressure (A), amplitude (B) and frequency (C) of contractions of the perfused cauda epididymidis of intact (●) and 7-day castrated (o) rats.

Castrated rats

Normal spontaneous contractions were still observed 5 days after castration (Table 2). However, the amplitude of contractions was significantly decreased by 7 days after androgen withdrawal while the basal pressure and frequency were not altered. It was noteworthy that after gonadectomy irregularity of the phasic contraction pressures showing doublet or multiple peaks occurred in most preparations (Fig. 3C).

Although the androgen-deprived epididymis revealed some abnormal pattern of spontaneous activities, its responsiveness to both electrical stimulation (Fig. 3) and to clonidine given intra-arterially at doses ranging from 0.1 to 100 ug/kg was virtually unaffected (Fig. 4).

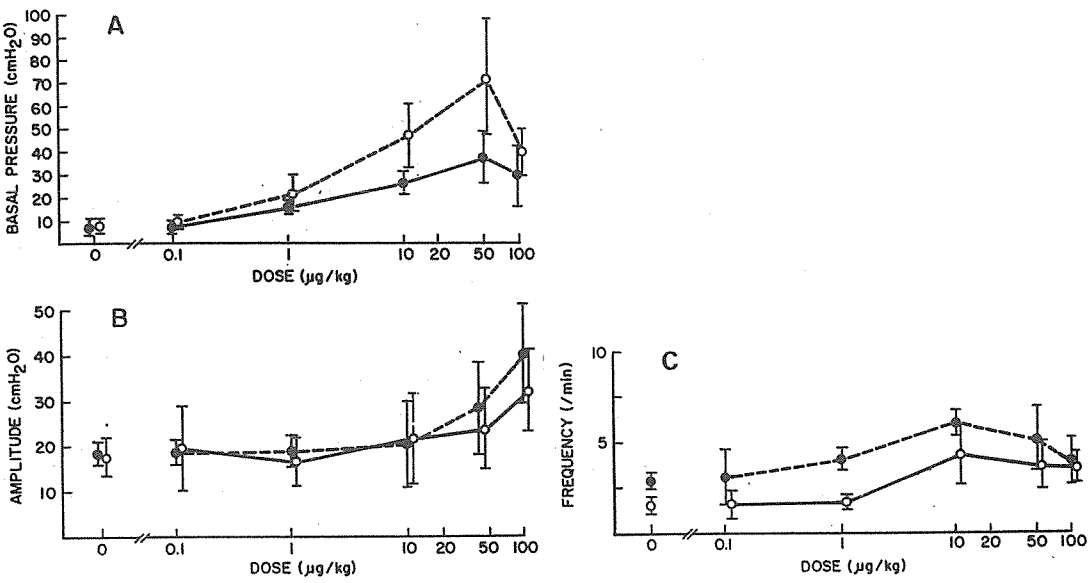

Figure 4. Effect of various intra-arterial doses of clonidine on the basal pressure (A), amplitude (B) and frequency (C) of contractions of the perfused cauda epididymidis of intact (●) and 7-day castrated (○) rats.

Table 2. Spontaneous contractions of the perfused segment of distal cauda epididymidis of normal and castrated rats

Group	Basal pressure (cmH_2O)	Amplitude of contraction (cmH_2O)	Frequency of contraction (/min)
Control	7.3 ± 0.6 (27)	20.7 ± 1.4 (27)	2.4 ± 0.2 (27)
5 days after castration	7.5 ± 0.7 (5)	16.2 ± 3.1 (5)	2.2 ± 0.5 (5)
7 days after castration	7.8 ± 0.8 (5)	11.4 ± 1.9[a] (5)	2.7 ± 0.5 (5)

Values are mean ± SEM; numbers in parentheses are numbers of animals.
[a]Significantly different at P 0.05 from control.

Discussion

In this study, contractility of the distal cauda epididymidis of rats was investigated by measuring intraluminal pressure after retrograde catheterization of the proximal vas deferens in the presence or in the absence of luminal spermatozoa. Spontaneous contractions were observed in 3 of 4 sperm-intact epididymides of

normal rats. The amplitude of contractions was considerably less than, but the frequency was comparable to, those reported by Hib and Ponzio (1977). By 7 days after bilateral castration, spontaneous activities were completely absent. This result confirms the earlier study by Hib and Ponzio (1977). Furthermore, we have shown that the androgen-deprived epididymis was still responsive to electrical stimulation. The amplitude of contractions was somewhat exaggerated while the frequency was suppressed when compared to the controls (Table 1). It would, therefore, appear that, by using a cannulation method to measure intraluminal pressures, spontaneous activities of the distal cauda epididymidis ceased completely shortly after castration, but its responsiveness to electrical stimulation was still partially intact. However, close inspection of the pressure recording and direct visual observations revealed frequent blockage by aggregates of spermatozoa in the tip of the catheter. Similar findings have previously been noted (Markkula-Viitanen et al., 1979). We, therefore, believe that this method of pressure recording does not register actual contractility of the epididymal smooth muscles.

When the epididymal tubule was perfused to remove its luminal fluid content and to replace it with a simple electrolyte solution, spontaneous contractions with regular and sharp pressure waves were recorded (Fig. 2A). However, the amplitude of the contractions was almost ten times that in the non-perfused tubule and the frequency was also increased. This may be due to the low viscosity of the perfusion fluid compared to the native luminal fluid and, perhaps, activation of the smooth muscle during perfusion. It should be emphasized that a precaution had been made to reduce the residual pressure by adjusting the initial basal pressure at 7.0 cmH_2O (see Materials and Methods). We have also demonstrated in this study that the amplitude and frequency of contractions were independent of the basal pressure over the range 4.5 to 14.6 cmH_2O. In fact, the amplitude of spontaneous contractions of the sperm-freed epididymis was less than that of the sperm-intact tubule (30.5 cmH_2O) in the more recent study by Hib et al. (1982). It is, therefore, less likely that the high contractility of the sperm-freed epididymis resulted from an over stretching of the tubule by perfusion.

Castration produced a time-dependent reduction in the spontaneous activities of the sperm-freed cauda. There were no changes in all parameters measured by 5 days after androgen withdrawal. However, a significant decrease in the amplitude, but not in the basal pressure nor the frequency, occurred by 7 days. Thus, the weakness of muscle contractions together with an increase in spermatocrit (Pholpramool & Sornpaisarn, 1980), and hence the luminal fluid viscosity, and a partial blockage of the catheter may explain the failure to detect spontaneous activities in the non-perfused tubule shortly after castration described in this and previous studies (Hib & Ponzio, 1977). It may be concluded that with an appropriate method of study the spontaneous contractility of the rat epididymis remains intact at least by 7 days after gonadectomy. This conclusion is in accordance with the findings from visual observations by Risley (1958) and from our earlier study (Din-Udom et al., 1985).

Although the amplitude of spontaneous activities was reduced shortly after androgen deprivation, the responsiveness of the cauda epididymidis to both electrical stimulation and clonidine, a predominantly alpha-2 agonist, was not changed. The

results suggest that the integrity of the neural elements innervating the epididymal tubule as well as the contractility of the smooth muscle cells were not affected after short-term androgen withdrawal. Since the phasic spontaneous contractions became irregular after castration and the pattern of contraction pressures indicates ectopic foci of pacemakers, the coupling between pacemaker cells or the conductive pathways of which may be deranged. Yet the rhythmicity of the pace-makers was unaffected since the frequency of major peaks of spontaneous contractions was not changed. It would appear that short-term effects of androgen withdrawal on the contractility of the epididymis are at variance with those on the vas deferens. Thus, spontaneous contractions of vas deferens, which are not normally observed, occurred by 7 days after castration and these activities increased to maximum by 30-60 days (Martin & Valle, 1939; Miranda et al., 1985). Furthermore, changes in the sensitivity to contractile agonists were demonstrated after gonadectomy (MacDonald & McGrath, 1980; Calixto & Rae, 1981; Miranda et al., 1985; Longhurst & Brotcke, 1989).

It is clear, therefore, that further quantitative studies are required to confirm the cessation of spontaneous activities and to determine whether the responsiveness of the epididymal smooth muscles to contractile agents is altered after long-term androgen deprivation.

ACKNOWLEDGEMENT

We thank Mrs. Suthada Sukuttakij for typing the manuscript.

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บทคัดย่อ

ได้ทำการวัดความดันภายในท่อพักเชื้ออสุจิส่วนปลายที่มีและปราศจากเชื้ออสุจิ โดยการสอดท่อพลาสติกเข้าทางท่อนำเชื้อของหนู ในท่อพักเชื้อของหนูปกติที่มีเชื้ออสุจิอยู่เต็ม พบว่ามีการบีบตัวได้เองตลอดเวลาด้วยความแรง 7.2±0.2 ซม.น้ำ และความถี่ 1.3±0.2 ครั้ง/นาที หลังการกระตุ้นด้วยไฟฟ้า (5 โวลท์ 1 เฮิรสท์) ความดันพื้นฐานในท่อเพิ่มขึ้นจาก 2.0±0.6 เป็น 7.1±1.0 ซม.น้ำ ความถี่ของการบีบตัวเพิ่มขึ้น 2 เท่า แต่ความแรงของการบีบตัวไม่เปลี่ยนแปลง ภายหลังการตัดลูกอัณฑะนาน 7 วันไม่พบการบีบตัวของท่อพักเชื้อเลย แต่ความดันพื้นฐานภายในท่อไม่เปลี่ยนแปลง อย่างไรก็ตามท่อพักเชื้อยังสามารถตอบสนองต่อการกระตุ้นด้วยไฟฟ้า เมื่อไล่เชื้ออสุจิที่อยู่ในท่อพักเชื้อส่วนปลายออกจนหมด พบว่าท่อพักเชื้อของหนูปกติและหนูที่ปราศจากลูกอัณฑะมีการบีบตัวได้เองเช่นเดียวกัน แต่ความแรงของการบีบตัวจะค่อย ๆ ลดลงในหนูที่ถูกตัดลูกอัณฑะ จนเหลือประมาณครึ่งหนึ่ง ภายในวันที่ 7 หลังการผ่าตัด แต่ความถี่ในการบีบตัวไม่เปลี่ยนแปลง ความไวในการตอบสนองของท่อพักเชื้อในหนูที่ปราศจากอัณฑะต่อการกระตุ้นด้วยไฟฟ้า และสารโคลนิดิน (ขนาด 0.1-100 ไมโครกรัม/กก.) ไม่ถูกกระทบกระเทือน จากการทดลองสรุปได้ว่าความแรงในการบีบตัวของท่อพักเชื้อส่วนปลายของหนูค่อย ๆ ลดลงตามเวลาภายหลังการตัดลูกอัณฑะแต่ความไวต่อการตอบสนองต่อสิ่งเร้าไม่เปลี่ยนแปลง