Urol Res (1989) 17:265–268

Urological Research

In-vitro contractility of human seminiferous tubules in response to testosterone, dihydrotestosterone and estradiol

M. Yamamoto, T. Nagai, H. Takaba, J. Hashimoto, and K. Miyake

Department of Urology, Nagoya University School of Medicine, Nagoya, Japan

Accepted: September 1, 1988

Summary. The effects of steroids (testosterone, dihydrotestosterone and estradiol) on human seminiferous tubules in vitro were ascertained by recording the intratubular pressure with a servonull micropressure measuring device. We describe here the first response of the human seminiferous tubule to steroids. Testosterone and dihydrotestosterone had a biphasic effect on tubular contractility. Higher doses of both testosterone and dihydrotestosterone induced contractions of the seminiferous tubules whereas lower doses of these compounds induced relaxation. Estradiol (10^{-9} M to 10^{-6} M) induced relaxation of the seminiferous tubules in a dose-dependent manner. The results from these experiments suggested that steroids may be involved in the control of contraction of the human seminiferous tubule and may regulate the movement of spermatozoa from the testes.

Key words: Contraction – Human seminiferous tubule – Steroids

Introduction

The mechanism of the transport of non-motile spermatozoa from the seminiferous tubules of the testis to the epididymis has long been a matter of question. Recently Miyake et al. reported that the seminiferous tubule of human testis was capable of responding to noradrenaline and acetylcholine [1]. It has been speculated that peritubular myoid cells were responsible for the contractility of the seminiferous tubules [2, 3]. Previous studies have suggested that the maturation of the peritubular myoid component of the seminiferous tubules may be under pituitary control [4]. It was also suggested that androgens could maintain and alter the nature of the rat tubule contractions [5]. However, a clear-cut physiological role for the androgens in the regulation of contractility of the human seminiferous tubule has not been previously reported. Therefore, this investigation was undertaken to evaluate the effects of testosterone, dihydrotestosterone and estradiol on human seminiferous tubule. The present communication describes for the first time that human seminiferous tubule contraction can be altered by steroids.

Materials and methods

All experiments were performed on human seminiferous tubules obtained by biopsy from seven healthy individuals (aged 23–24, individuals having undergone vasectomy). After tubules were dissected from the adhering interstitial tissue with microdissecting forceps using a dissecting microscope, a single tubule was transferred to Tyrode solution previously infused with 95% O_2 and 5% CO_2. A seminiferous tubule was tied to the cork platform (diameter 4 mm) by a fine thread and placed in the tissue bath (Fig. 1). The temperature of the bath was maintained at 33 °C.

Intratubular pressure was recorded with a servonull micropressure measuring device with the use of microelectrodes filled with 2 M NaCl colored with lissamine green. The electrodes were drawn on a horizontal pipette puller (Narishige Scientific Instrument,

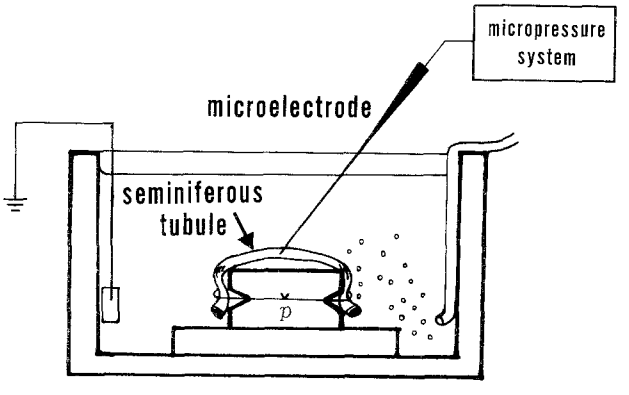


Fig. 1. Diagram of tissue bath (noth to scale). The seminiferous tubules were tied to the cork platform, p

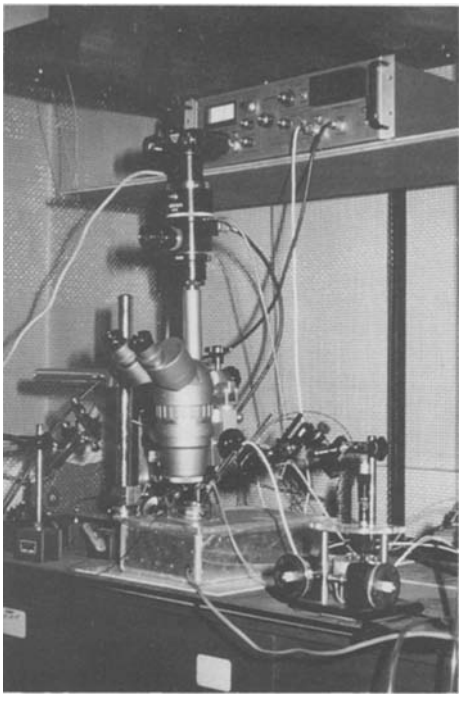


Fig. 2. Whole appearance of experimental apparatus

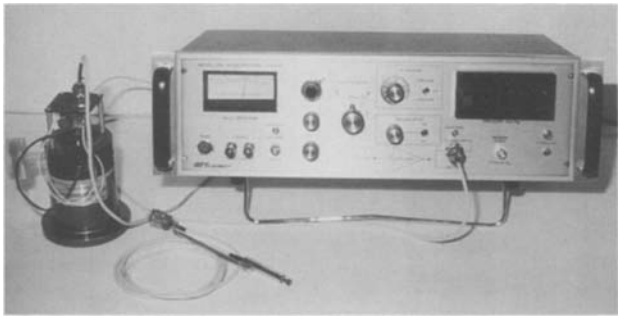


Fig. 3. The model 900 micropressure system includes microelectrode holders, preamplifier with active probe, power amplifier, hydraulic servo system, pressure transducer, and digital pressure readout

Tokyo, Japan) from constant-bore flint glass tubing with an outside diameter of one mm. The electrodes were immersed in sodium chloride (2 M) overnight. In order to facilitate penetration of the tubular wall, the pipette tips were sharpened on a rotating wet stone grinder (Narishige, type EG-1) and their outside diameters at the tips were two to five micron (tip impedances: 5 to 10 megohms). They were placed in a micromanipulator and inserted longitudinally from above into the tubules under visual control using an Olympus stereozoom microscope (Olympus optical, Co., Ltd., Tokyo, Japan) (Fig. 2). They were connected via a pipette holder with an Ag/AgCl to the input of a Model 900 Micropressure system that was designed to measure hydrostatic pressure in limited volume biological compartments (W-P Instruments, Inc., New Haven, CT) (Fig. 3). Intratubular pressure change was recorded with a pen recorder (Bioresearch center, Co., Ltd., Nagoya, Japan) with a paper speed of 2 cm/m. The Ag/AgCl reference electrode was connected via a sodium chloride bridge to the bath fluid (Fig. 1).

Successful insertion into the lumen of the seminiferous tubule was confirmed by rapid washout of dye injected through the micropipette. When stable pressure was obtained, the effects of various concentrations of testosterone, dihydrotestosterone and estradiol on the intratubular pressure were examined. Stock solutions of these steroids were made up using 50% ethanol as a diluent. Aliquots of the stock solution were diluted with Tyrode's solution. Each steroid was added to the tissue bath in an amount of 0.1 ml. Seminiferous tubules taken from the same individual were often used for testing several kinds of steroids, but only one steroid was tested on a given tubule. Effect of each steroid was tested four times. Replicates for each concentration level were performed on a given tubular preparation. After effect was determined at each concentration of steroid, and when pretreatment levels of intratubular pressure were reestablished, an additional assay was undertaken. Representative recordings for various treatment levels are included in this report.

Results

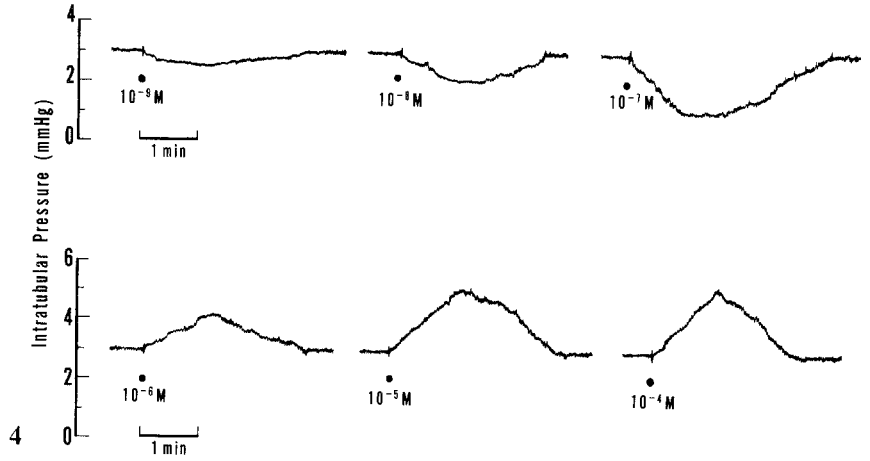
Pretreatment intratubular pressure of the human seminiferous tubule was approximately 3 mmHg (3 ± 0.28, mean $\pm$ standard error, $n = 14$). Spontaneous contractions of seminiferous tubules did not seem to affect the intratubular pressure.

Additions of testosterone and dihydrotestosterone in concentrations of 10^{-9}, 10^{-8} and 10^{-7} M produced a relaxation of the seminiferous tubule which decreased intratubular pressure to 1 mmHg at a final concentration of 10^{-7} M. Both testosterone and dihydrotestosterone in concentrations of 10^{-6} and 10^{-5} M produced a contraction of the seminiferous tubule which reached a maximum level within one min of its addition to the bath (Fig. 4, 5). Estradiol produced a relaxation of the seminiferous tubule which decreased intratubular pressure to 1 mmHg at a final concentration of 10^{-6} M. Minimum and maximum decreases in intratubular pressures induced by estradiol were found to occur at concentrations of 10^{-9} M and 10^{-6} M, respectively. Decrease in intratubular pressure reached a maximum level within one min of its addition (Fig. 6).

When ethanol was assayed for its effects on intratubular pressure in a range of 10^{-9} M to 10^{-5} M concentration no response of the seminiferous tubule was noted (Fig. 6).

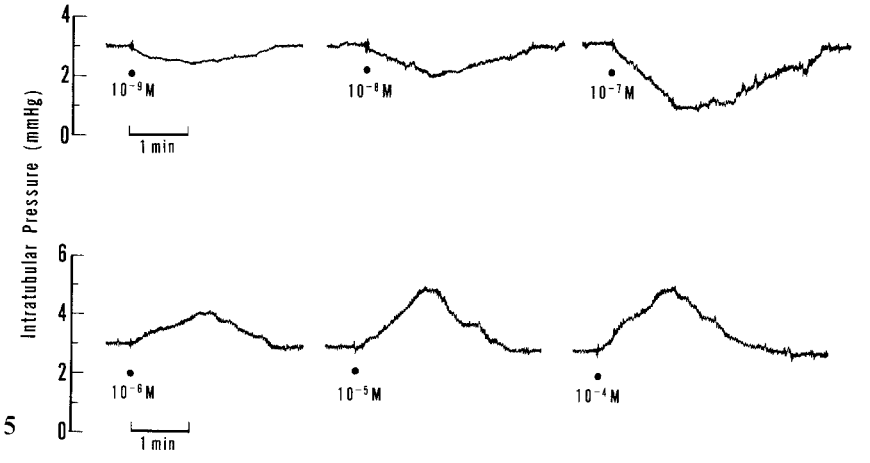
Discussion

The fact contractile cells of the seminiferous tubule mature during puberty of murine species leads to speculation that such development is androgen-dependent. Bressler and Ross [6] tested this hypothesis in the mouse by removing testes from newborn animals and implanting them in adult hosts. Development appeared to proceed in parallel with that of the littermates if



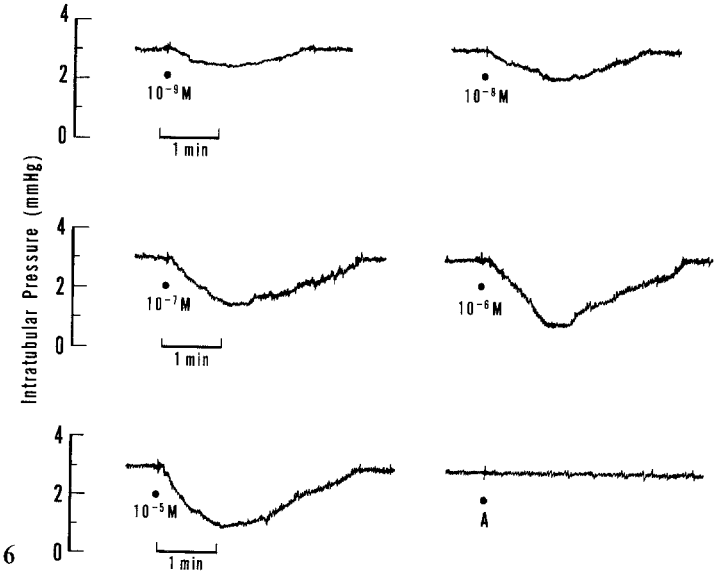
4

Fig. 4. Response to testosterone. Recordings show biphasic intratubular change. No response was recognized at concentration of 10^{-10} M. Testosterone at each concentration level was added at dots



5

Fig. 5. Response to dihydrotestosterone. Recordings show biphasic intratubular change. No response was recognized at concentration of 10^{-10} M. Dihydrotestosterone at each concentration level was added at dots



6

Fig. 6. Response to estradiol. Recordings show dose-dependency for reduction of intratubular presure. No response was recognized at concentration of 10^{-10} M. Estradiol at each concentration level was added at dots. A: lack of effect of diluent (ethanol) on tubular contraction. Ethanol concentration was 10^{-5} M

the testes were put into normal males, whereas those implanted into hypophysectomized hosts retained an immature appearance. The administration of supplemental doses of testosterone to the latter animals resulted in partial development [4]. Kormano et al. suggested that the contraction of the seminiferous tubule appeared at 15 days of age in the rat and reached adult values at about 40 days of age [7]. It was suggested that the development of the contractions could be due to testosterone as well as to the pituitary hormones [5]. Testosterone has been shown to increase the percentage of tubules contracting in tissue culture and to increase the number of filaments in the myoid cells that are thought to be responsible for causing the tubule contractions [5]. The antiandrogen, cyproterone acetate, has also been shown to inhibit both the functional and structural development of the myoid cells and to cause the disappearance of tubule contractility in tissue culture [5].

The data from our experiments indicated that the effects of testosterone and dihydrotestosterone on human tubule contractions were biphasic. High doses of testosterone and dihydrotestosterone induced contraction of the seminiferous tubules whereas low doses of these compounds induced relaxation. The biphasic effects of testosterone as seen in this investigation indicate two possible mechanisms of action. The higher concentration of steroid (10^{-6} M) is considered physiological and is required for optimal spermatogenesis. It is unlikely that human testicular androgen concentration would normally drop much below 10^{-6} M so that the inhibitory effect of low androgen concentration would probably not occur under physiological conditions. When the intratesticular levels of testosterone are high, conditions would be optimal for spermatogenesis and accessory sex organ function and the tubules would exhibit contractions. On the contrary, when the intratesticular levels of testosterone are low, conditions for spermatogenesis and accessory sex organ function are not optimal and the contractions would be inhibited.

The mechanism underlying the transport of nonmotile spermatozoa from the seminiferous tubules of the testis to the epididymis is not clarified. Many hypotheses have been proposed including fluid secretion and fluid flow in the seminiferous tubules [8], testicular capsular contractions [9] and contractions of the seminiferous tubules [10]. It is conceivable that steroids may be involved in the control of tubular contractions either directly or indirectly and may have, together with the aforementioned other factors, an important role in the transport of spermatozoa and tubular fluid to the rete testis.

References

1. Miyake K, Yamamoto M, Narita H, Hashimoto J, Mitsuya H (1986) Evidence for contractility of the human seminiferous tubule confirmed by its response to noradrenaline and acetylcholine. Fertil Steril 46:734–737
2. Roosen-Runge EC (1951) Motions of the seminiferous tubules of rat and dog. Anat Rec 109:413
3. Ross MH, Long IR (1966) Contractile cells in human seminiferous tubules. Science 153:1271–1273
4. Bressler RS, Ross MH (1972) Differentiation of peritubular myoid cells of the testis, effects of intratesticular implantation of newborn mouse testes into normal and hypophysectomized adults. Biol Reprod 6:148–159
5. Hovatta O (1972) Effect of androgens and antiandrogens on the development of the myoid cells of the rat seminiferous tubules (organ culture). Z Zellforsch Mikrosk Anat 131:299–308
6. Bressler RS, Ross MH (1969) Pituitary involvement in testicular peritubular cell maturation. Anat Rec 163:158–159
7. Kormano M, Hovatta O (1972) Contractility and histochemistry of the myoid cell layer of the rat seminiferous tubules during postnatal development. Z Anat Entwicklungsgesch 137:239–248
8. Smith G (1962) The effects of ligation on the vasa efferentia and vasectomy on testicular function in the adult rat. J Endocrinol 23:385–399
9. Davis JR, Langford GA (1969) Response of the testicular capsule to acetylcholine and noradrenaline. Nature 222:386–387
10. Niemi M, Kormano M (1965) Contractility of the seminiferous tubule of the postnatal rat testis and its response to oxytocin. Ann Med Wxp Biol Fenn 43:40–42

Masanori Yamamoto, MD
Department of Urology
Nagoya University School of Medicine
65 Tsurumai-cho, Showa-ku
Nagoya
466 Japan