Response of Rabbit Testicular Capsular Contractions to Testosterone, Prostaglandin E_1, and Isoproterenol *in Vitro* and *in Vivo*[1] (37584)

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The tunica albuginea of the human and rabbit testicular capsule has smooth muscle cells as a major component (1, 2). These cellular elements are responsible for the autorhythmic testicular capsular contractions that are postulated to facilitate sperm transport and circulation (2, 3). Rabbit testicular capsules respond to acetylcholine, carbachol, epinephrine, norepinephrine, and histamine by contracting while isoproterenol inhibits them (2, 3). Prostaglandin $F_{1\alpha}$ ($PGF_{1\alpha}$), in all concentrations tested, stimulated contractions in isolated testicular capsules. By contrast, PGE_1 and PGE_2 stimulated contractions in low concentrations, but inhibited contractility at higher concentrations (4–6).

Endogenous prostaglandin-like compounds may be important modulators of rabbit capsular contractions *in vitro*, and epinephrine, serotonin, oxytocin, and acetylcholine do not appear to be responsible for the occurrence of spontaneous contractions *in vitro* (4, 7). Furthermore, prostaglandin biosynthesis occurs in rat (8–11) and rabbit testes (12).

Prostaglandin involvement in testicular physiology has been demonstrated by a modulation of androgen synthesis (8–10, 13) similar to what has been observed for ovarian steroidogenesis through the adenyl cyclase system (14, 15). Moreover, progesterone, pregnenolone, and testosterone inhibit spontaneous and prostaglandin-induced contractions of the rabbit testicular capsule *in vitro* (7). It is, therefore, reasonable to assume that steroids may regulate testicular capsular motility *in vivo*. Furthermore, peripheral plasma testosterone and androstenedione concentrations increased in male rabbits after HCG treatment or copulation (16).

The present investigation was undertaken to compare the relative effects of testosterone, PGE_1, and isoproterenol on testicular capsular motility *in vivo* and *in vitro*. We also determined the relative effectiveness of other steroids (*e.g.*, androstenedione, 17α-hydroxyprogesterone, and 5α-androstan-17β-3-one) as inhibitors of rabbit testicular capsular contractions *in vitro*.

Materials and Methods. All animals used in this investigation (approx 25) were a mixed breed of domestic rabbit and were obtained from local sources. For *in vitro* studies, mature male rabbits were sacrificed and their testes were excised and suspended in oxygenated Tyrode's solution held at 35° in all-glass, water-jacketed, muscle warmers. Contractions were measured *in vitro* using sensitive myograph transducers (Statham Co.) and a Gilson minipolygraph (4–7).

Contractions *in vivo* were recorded by a modification of the methods described by Davis and Langford (2). Sexually mature rabbits were anesthetized by iv injections of sodium pentobarbital supplemented with ether anesthesia. The animal was restrained ventral side up, and an incision was made through the scrotal sac to expose the testis. After a piece of thread was anchored to each end of the testis, the organ was drawn into a water-jacketed muscle warmer and the thread (attached to the superior end of the testis) was connected to the myograph transducer. The inferior pole of the testis was secured to the base of the muscle warmer with the other

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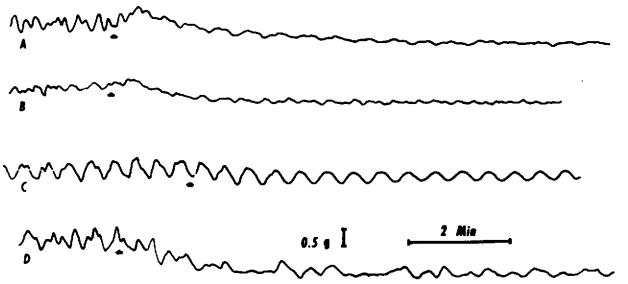

FIG. 1. Inhibition of testicular capsular contractions *in vitro* by the addition of 5×10^{-3} mg/ml of: (A) 5α-androstan-17β-ol-3-one; (B) androstenedione; (C) 17α-hydroxyprogesterone; and (D) testosterone in the bathing medium. The arrow indicates the addition of steroids to the bathing medium.

thread. The scrotal sac was sealed around the bottom of the muscle warmer with a circular wire tie. By carefully arranging the position of the muscle warmer with respect to the body of the rabbit, an effective seal between the testis and the body cavity was made without initiating ischemia in the testis.

Prostaglandins and steroids in 95% ethanol were added to the bathing medium. Isoproterenol in Tyrode's solution was either added to the bathing medium or injected iv. The inhibitory effects of steroids *in vitro* were tested on PGE_2-induced contractions.

Results. Rabbit testicular capsular contractions that were initiated with 1×10^{-5} mg/ml PGE_2 *in vitro,* were inhibited by all steroids tested (Fig. 1). Overall tonus, as well as rate and amplitude of contractions were reduced in response to both 5α-androstan-17β-3-one (Fig. 1A) and androstenedione (Fig. 1B). A lesser response was obtained with 17α-hydroxyprogesterone (Fig. 1C).

In vivo rabbit testes contracted autorhythmically within 15 min after suspension in the water-jacketed muscle warmer. Tonus was decreased during changes of the bathing medium, but recovery was relatively rapid (Fig. 2A). Capsular contractions were not eliminated by repeatedly changing the bathing medium of *in vivo* preparations. Adding testosterone at concentrations as high as 0.120 mg/ml to the bathing medium of *in vivo* preparations initiated some arrhythmia and a transient decrease in tonus. Recovery occurred within 2 to 3 min (Fig. 2B). Testosterone (18 mg/kg) had no effect on capsular motility when injected iv. However, capsular contractions *in vivo* that were potentiated with PGE_1 (5×10^{-7} mg/ml) could be

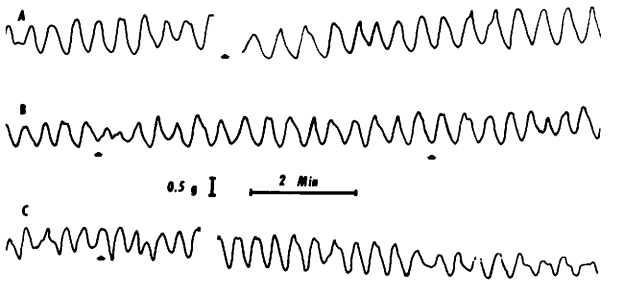

FIG. 2A. Lack of effect of changing the bathing medium of capsular contractions *in vivo*. (B) Lack of effect of testosterone on capsular contractions *in vivo*. First arrow indicates addition of 2.5×10^{-2} mg/ml testosterone in the bathing medium. The second arrow indicates the addition of 9.5×10^{-2} mg/ml testosterone in the bathing medium. (C) Inhibitory effect of 2.5×10^{-2} mg/ml testosterone on capsular contraction *in vivo* that were potentiated with 5×10^{-7} mg/ml PGE_1. The interruption of the tracing represents 2 min.

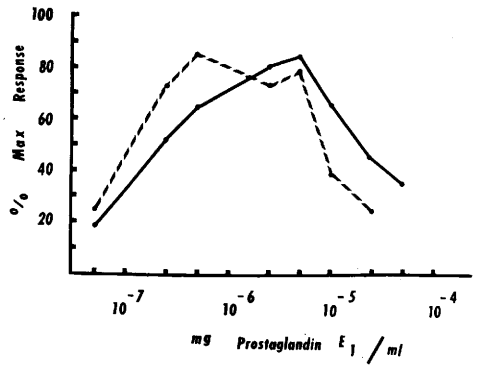

FIG. 3. Similarity of the dose–response curve for PGE_1 on capsular contractions *in vivo* and *in vitro*. (- -) *In vivo* contractions and (—) *in vitro* contractions. The concentration of PGE_1 in the bathing medium represented by points on the graph are 5×10^{-8}, 2.5×10^{-7}, 2.5×10^{-6}, 5×10^{-6}, 1×10^{-5}, 2.5×10^{-5}, and 5×10^{-5} mg/ml PGE_1. Each point is the mean of either 9 observations *in vitro* or 5 observations *in vivo*.

partially inhibited by testosterone (2.5×10^{-2} mg/ml) in the bathing medium (Fig. 2C).

PGE_1 stimulated capsular contractions *in vivo* and *in vitro* with approximately equal potency; the thresholds for inhibition of capsular motility by PGE_1 were also approximately equal in the two preparations (Fig. 3). However, the response of the testicular capsule *in vivo* was more variable, and the absolute magnitude of contractions was less than *in vitro* preparations. Isoproterenol inhibited capsular motility *in vivo* (5×10^{-3} mg/ml) when added to the bathing medium or when injected iv (1.7 mg/kg). A rapid reduction of overall tonus, and the rate and amplitude of rhythmic contractions was noted after treatment with isoproterenol. Recovery during the next 15 min was gradual and incomplete (Fig. 4).

Discussion. Pregnenolone, progesterone, and testosterone inhibited testicular capsular contractions *in vitro* (7). 5α-Androstan-17β-3-one, 17α-hydroxyprogesterone, and androstenedione also inhibited capsular contractions *in vitro*. The steroids tested were about equally effective in inhibiting capsular motility, however 17α-hydroxyprogesterone was less potent. Although HCG and exposure of male rabbits to females increased plasma levels of testosterone (16), the present data indicate that increases in testicular levels of androgens under physiological conditions would not be effective in diminishing contractility of the capsule since testosterone was ineffective in inhibiting capsular contractions *in vivo*. Since all steroids tested appear to be equally potent in inhibiting capsular contractions *in vitro*, it is doubtful that other steroids may appreciably affect testicular contractions *in vivo*.

Our observations indicate: (a) that the log dose–response of the testis to PGE_1 was essentially the same for either *in vivo* or *in vitro* preparations (Fig. 3), and (b) that isoproterenol inhibited the testicular preparations both *in vivo* (Fig. 4) and *in vitro* (17). In addition, isoproterenol, injected iv, also inhibited testicular contractions. Thus, failure of testosterone to inhibit the testicular contractions *in vivo* seems not to be due to an inability of this compound to reach the tunica albuginea in effective concentrations.

In vivo and *in vitro* capsular preparations also differ in that the *in vivo* motility was more stable. We have previously reported that contractions *in vitro* can be reduced or eliminated by successively changing the bathing medium and that a prostaglandin-like substance was extracted from the bathing medium that was capable of reinstating capsular motility (4). *In vivo* contractions, on the

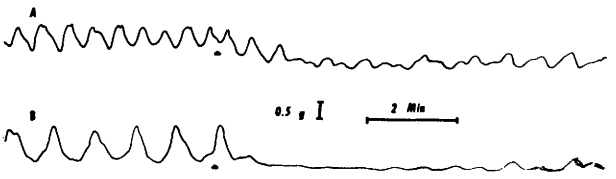

FIG. 4A. Response of capsular contractions *in vivo* to isoproterenol (5×10^{-3} mg/ml) added to the bathing medium. (B) Response of *in vivo* capsular contractions to isoproterenol (1.7 mg/kg injected iv.

other hand, although reduced by changing the bathing medium, recovered rapidly, and motility could not be markedly reduced by successively changing the bathing medium. Studies are presently underway to determine if prostaglandin biosynthesis is necessary to maintain *in vivo* rabbit testicular capsular contractions.

Testosterone inhibited motility potentiated by prostaglandins *in vivo* (Fig. 2C) and *in vitro* (7), but it did not inhibit tonus increases initiated by epinephrine *in vitro* (7). Epinephrine initiated tonus increases by stimulating α-adrenergic receptors in the rabbit testicular capsule (17, 18). Moreover, electrical stimulation of the perivascular nerve to the dog testes elicited contractions of the testicular capsule (19). It appears that nervous mechanisms as well as prostaglandins are important regulators of rabbit testicular capsular contractions.

Summary. Rabbit testicular capsular contractions *in vitro* were inhibited by 5α-androstan-17β-ol-3-one, androstenedione, 17α-hydroxyprogesterone, and testosterone with approximately the same potency. Testosterone did not inhibit spontaneous contractions *in vivo,* but it did partially inhibit PGE_1 potentiated contractions *in vivo.* Capsular preparations responded similarly to PGE_1 and isoproterenol both *in vitro* and *in vivo* indicating that it was not the route of administration that determined the response of the capsule to testosterone. By contrast, spontaneous contractions *in vivo* could not be abolished by repeated changes of the bathing media as previously reported for *in vitro* preparations. These data negate the possible regulation of testicular contractions by endogenous steroids.

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