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MODULATION OF RABBIT TESTICULAR CONTRACTIONS BY PROSTAGLANDINS,
 STEROIDS AND SOME PHARMACOLOGICAL COMPOUNDS¹

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ABSTRACT

Progesterone, testosterone, and pregnenolone inhibited spontaneous testicular capsular contractions in vitro with equal effectiveness and in a dose-dependent fashion. The three steroids assayed also inhibited contractions induced by prostaglandin⁴. Progesterone, testosterone, and pregnenolone were ineffective at a concentration of 10^{-5} M in inhibiting the tonus increase of the testicular capsule induced by epinephrine. However, the three steroids did inhibit the increased contractility of the testicular capsule induced by either acetylcholine or serotonin. Both acetylcholine and serotonin had little effect on testicular contractility unless the testes were spontaneously contracting or the testes were stimulated with the prostaglandins.

We concluded that steroids and prostaglandins may have a role in modulating testicular capsular motility in vivo.

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An autorhythmically contracting capsule exists around rabbit testes (1). The spontaneous contractions of this capsule that occur in vitro (1-3) respond to pharmacological agents by either increasing or decreasing their rate, amplitude, and overall tonus. We have shown that these spontaneous, in vitro contractions may be diminished in rate and amplitude or completely effaced by changing the bathing medium (2). Extraction of the initial media for compounds with prostaglandin-like (PG-like) properties produced an active principle capable of reinstating these spontaneous testicular capsular contractions (2). Recent investigations (1, 3, 4) showed that carbachol, epinephrine and $\text{PGE}_{1\alpha}$ stimulate, while isoproterenol inhibits the contractions. On the other hand, PGE_1 at low concentrations stimulates, but at higher concentrations inhibits, the contractions (2). Acetylcholine and serotonin increased motility in either spontaneously-active preparations or in preparations in which contractions were induced with prostaglandins, but inactive capsules were not affected by these two compounds (2).

The basic mechanism for prostaglandin biosynthesis exists in rat testes (5, 6) and appears to be distributed in the male gonad as are the steroid biotransforming enzymes. Preliminary data indicate that PGE_1 , $\text{PGF}_{1\alpha}$ and $\text{PGF}_{2\alpha}$ are synthesized by rat testes (7). Other work has indicated that prostaglandins can modulate steroid synthesis in vitro (5, 8, 9). Lastly, prostaglandins may mediate the effect of gonadotropins on adenyl cyclase in initiating steroidogenesis (8-11). That androgen synthesis occurs in the testis is well established, but the relationship (if any) between androgen synthesis and testicular capsular motility is unknown at this time. Progesterone and estradiol are known to inhibit uterine motility (12-15), and the inhibition of uterine motility by progesterone is believed to be important in the maintenance of pregnancy. Moreover, in previous studies (1) we have recorded seasonal changes in contractility of rabbit testes.

On the basis of the above observations, we undertook the following investigations in order to ascertain if naturally occurring steroids (progesterone, pregnenolone and testosterone) would affect the spontaneous contractions of rabbit testes in vitro. We also wanted to evaluate the effects of the above three steroids on testicular contractions induced in inactive preparations by PGE_1 or epinephrine, and on contractions potentiated by serotonin and acetylcholine.

MATERIALS AND METHODS

Fourteen mature male rabbits of mixed breed were sacrificed by a sharp blow on the head. Contractions of the immediately excised testes were recorded in vitro in a water-jacketed all-glass, muscle warmer using a Gilson minipolygraph and a sensitive myograph transducer (Statham Co.) as previously reported (2, 4). Continuously oxygenated Tyrode's solution, held at 35°C , was used as the bathing

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medium. Solutions of PGE_1 , progesterone, pregnenolone and testosterone were dissolved in 95% ethanol. Acetylcholine, epinephrine and serotonin were dissolved in Tyrode's solution. Aliquots of the stock solutions were assayed for their effect on smooth muscle contractions.

RESULTS

All of the rabbit testes developed spontaneous contractions within one hour after being suspended in Tyrode's solution. Progesterone inhibited both spontaneous (data not shown) and PGE_1 -induced contractions (Fig. 1) with equal effectiveness at a threshold

Figure 1. Dose-dependent progesterone inhibition of rabbit testicular capsular contractions induced by 70.0 nM PGE_1 .

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of 4×10^{-6} M. Amplitude and over-all tonus were the parameters primarily affected by progesterone. The response was dose-dependent with maximum inhibition observed at 20×10^{-6} M concentration (Fig. 1). Pregnenolone and testosterone inhibited testicular contractions and exhibited the same threshold dose and dose-dependency phenomena with either spontaneous or PGE_1 -induced contractions (data not illustrated).

Addition of progesterone, testosterone, or pregnenolone to the bathing medium of a non-contracting testis inhibited the induction of rhythmic contractions and tonus that were normally observed after PGE_1 treatment (cf. Fig. 2). Moreover, this phenomenon was also dose-dependent.

Figure 2. Effect of 70.0 nM PGE_1 on testicular capsular contractions which have been pretreated with 3.9 μM , 7.9 μM , 19.7 μM , and 27.8 μM progesterone, respectively.

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Addition of epinephrine to the bathing medium increased the tonus of non-contracting testes without inducing rhythmic contractions. The increase in tonus was dose-dependent and slowly decreased within five minutes after epinephrine was added. Addition of progesterone, pregnenolone, or testosterone to the bathing medium of a preparation stimulated with epinephrine did not appreciably reduce tonus at any concentration of the three steroids tested (i.e., up to 10^{-5} M-- cf. Fig. 3).

Figure 3. The lack of effect of progesterone on testicular capsular tonus induced by epinephrine.

Increased testicular motility induced by serotonin or acetylcholine was also inhibited by the addition of testosterone, pregnenolone, and progesterone (cf. Fig. 4).

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Figure 4. Progesterone inhibition of testicular capsular contractility induced by acetylcholine on a spontaneously active preparation, and the inhibition of testicular capsular tonus by progesterone on a preparation that was stimulated with prostaglandin and then with serotonin.

DISCUSSION

Certain steroids are known to inhibit uterine contractions (12-15); the postulated physiological function of these steroids is to inhibit uterine contractions during pregnancy. The concentration of steroids in the smooth-muscle capsule of the rabbit testis is unknown, but testicular contractions are maximally inhibited with concentrations of steroids that approach threshold for inhibition of the estrogen-dominated rat uterus (unpublished data). Therefore, it is likely that steroids are important in modulating testicular capsular contractions. Steroids as well as prostaglandins are synthesized in the testis (5-7). Both classes of compounds appear to be capable of modulating motility *in vitro*. Although PGE₁ and PGE₂ are inhibitory in relatively high concentrations, all prostaglandins that have been tested (PGF₁, PGF₂, PGA₂, PGE₁, PGE₂--unpublished data) are stimulatory, while the three steroids used are inhibitory at physiological concentrations.

The seasonal changes reported to occur in contractility of rabbit testes (2) could well be due to changes in metabolism of steroids and prostaglandins within the testis. Further studies

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are being undertaken to evaluate this possibility. That seasonal variations in the weight of rabbit testes occurs has been reported by other workers (16).

The fact that spontaneous contractions are inhibited by the three steroids studied, suggests that epinephrine may not be responsible for spontaneous activity in vitro, since the tonus increases due to epinephrine are unresponsive to steroids.

Acetylcholine or serotonin markedly increased the amplitude and tonus of capsular contractions in spontaneously active or prostaglandin-treated capsules, but had little effect on inactive preparations in vitro (1). Prostaglandins are present naturally (1, 5, 7) and may be necessary for the modulation of testicular motility by acetylcholine or serotonin. The acetylcholine- or serotonin-induced contractions were inhibited by the steroids tested. The interaction between prostaglandins and steroids is, therefore, likely to be important for in vivo modulation of testicular contractility.

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