Prostaglandin E_1 Induced Inhibition of Rabbit Testicular Contractions *in Vitro*[1] (35405)

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Prostaglandins constitute a family of hormones derived from linoleic and arachidonic acids. They are unsaturated fatty acids, 20 carbons in length, that possess one cyclopentano ring. Kurzrock and Lieb (1) and Goldblatt (2) studied the influence of human seminal fluid on uterine strips and reported that it stimulated smooth muscle. Later, Asplund (3) and Eliasson (4) observed prostaglandin activity with respect to smooth muscle contractions in human semen from subjects at an infertility clinic. Finally, Samuelsson (5, 6) isolated prostaglandins E_1, E_2, E_3, $F_{1\alpha}$, and $F_{2\alpha}$ from human semen. Recent observations in this laboratory have shown that the basic mechanism for prostaglandin synthesis is present in the testis (7). Moreover, this phenomenon is age dependent and has the same distribution in the male gonad as the steroid biotransforming enzymes. Prostaglandins were also shown by these workers to modulate androgen synthesis and to affect the basic oxidative mechanism that has been shown by Van Dorp *et al.* (8) and Bergstrom *et al.* (9) to biotransform the unsaturated fatty acids (linoleic and arachidonic acids) into prostaglandins. Prostaglandins have recently (10) been isolated from rat testicular tissue.

Aside from the initial observations of Ellis and Baptista (7), no physiological role has been firmly established for prostaglandins with respect to testicular function. However, prostaglandins may inhibit or stimulate smooth muscle contractions depending upon the type of prostaglandin and the source of the muscle (11). In this respect, Langford and Davis (12) and Davis *et al.* (13) have shown that the testicular capsule of rabbits contracted *in vitro* and *in vivo* and the contractions were stimulated by acetylcholine, norepinephrine, and carbachol, while isoproterenol inhibited them. The above data implicate a possible action of prostaglandins on testicular function. Therefore, the present investigations were undertaken to ascertain if prostaglandin E_1 (PGE_1) would affect testicular contractions *in vitro*.

Materials and Methods. Nine mature male rabbits of mixed breed, anesthetized with sodium pentabarbitol and supplemented with ether, were used in this investigation. Contractions of excised testes were recorded *in vitro* using a sensitive myograph transucer (Statham Co.) and a polygraph (Gilson minipolygraph). Continuously oxygenated Tyrode's solution, held at 35°, was used as the bathing medium. A stock solution of PGE_1 was made up in 95% ethanol. Aliquots of the stock solution, and ethanol as a diluent, were both assayed for their effects on smooth muscle contractions.

Results. After the testes were removed and attached to the transducer, 15 to 30 min elapsed before contractions were initiated and became regular; assays were begun at this time. Four observations on three different animals were made with a concentration of 3.6 nanomolar (n*M*) PGE_1. A typical recording from these observations is shown in Fig. 1A. The frequency of contractions remained unchanged in these observations (13 beats/5 min), but there was an immediate increase (from 16 mm to 25.6 mm) in amplitude of contraction and a decrease of overall tone. Overall tension remained less than the pretreatment level during the observation peri-

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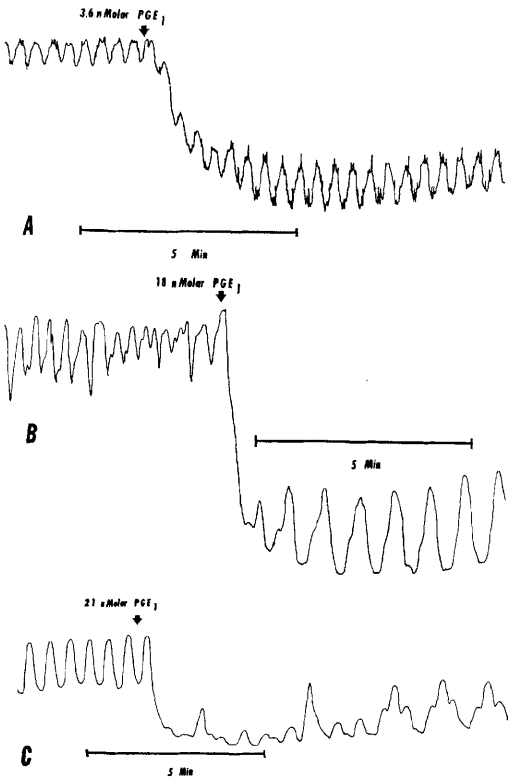

FIG. 1. Inhibition of rabbit testicular contractions *in vitro* with prostaglandin E_1 (A) PGE_1 concentration was 3.6 n*M* and the recorder sensitivity was 0.3 g/5 mm of pen deflection; (B) PGE_1 concentration was 18 n*M* and the recorder sensitivity was 0.4 g/5 mm of pen deflection; (C) PGE_1 concentration was 21 n*M* and the recorder sensitivity was 1.05g/5 mm of pen deflection.

od. Some recovery was evident 6 min after treatment.

When PGE_1 was increased to 18 n*M* concentration (Fig. 1B), overall tone was decreased. The rate of contraction decreased after treatment from 14 beats/5 min to 7 beats/5 min. Some recovery was evident after 5 to 6 min. With a concentration of 21 or 24 n*M* PGE_1, a characteristic decrease in overall tone and rate (from 9 beats/5 min to 3.5 beats/5 min) resulted as typified in Fig. 1C. There was, however, an unexpected change in mode of contraction with both preparations going from unimodal to trimodal contractions.

PGE_1 in a concentration of 36 n*M* (Fig. 2A) completely effaced testicular contractions for approximately 5 min. Some recovery was evident after this time period. When PGE_1 concentration was increased to 360 n*M* (Fig. 2B) overall tone was decreased and contractions were completely effaced. No recovery of contractility was evident during an observation period of 35 min. The data show a dose-dependency for rate of contraction, overall tone, and amplitude of contraction with the effects being minimal at lower concentrations and maximal at higher concentrations.

When ethanol was assayed for its effects on testicular contractions in a range of 11 to 32 μM concentration (Fig. 2C) no decrease in tone was noted. Frequency and rate decreased slightly, but these effects were transient and negligible when compared with the effects of PGE_1.

Discussion. Our data that whole rabbit testes do contract autorhythmically *in vitro*

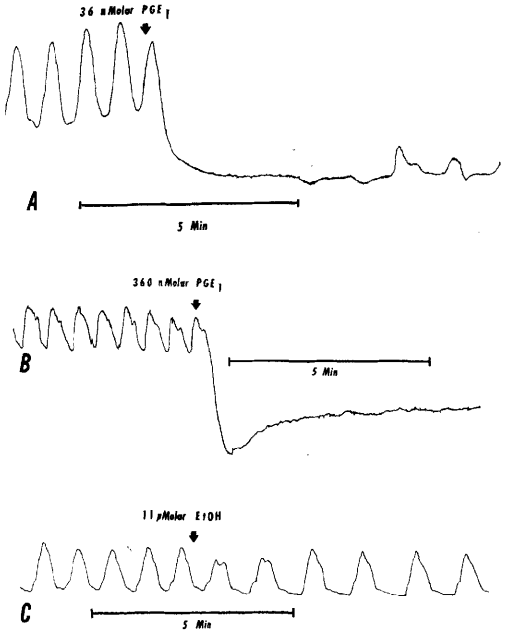

FIG. 2. Inhibition of rabbit testicular contractions *in vitro* with prostaglandin E_1 and the lack of effect of the diluent (ethanol) on this phenomenon: (A) PGE_1 concentration was 36 n*M* and the recorder sensitivity was 0.59 g/5 mm of pen deflection; (B) PGE_1 concentration was 360 n*M* and the recording sensitivity was 0.3 g/5 mm of pen deflection; (C) ethanol concentration was 11 μM and the recorder sensitivity was 0.4 g/5 mm of pen deflection.

corroborate the observations of Langford and Davis (12) and Davis *et al.* (13) that isolated testicular capsular preparations contract *in vitro*. Of importance are the observations by these workers that the capsule of the testis contracts autorhythmically while the parenchyma does not, and that the rabbit testis does contract *in vivo*. The above workers report a frequency of contraction of 4 beats/ min *in vitro* and *in vivo* that corresponds closely with the 2 to 3 beats/min that we found. This difference in rate can best be attributed to the higher temperature of incubation (37 vs 35°, respectively) and difference in type of preparation that was used in their studies as compared to ours.

The normal changes in tone of the capsule associated with the normal rhythmic contractions should correlate with changes in pressure inside of the testis. In this respect, Langford and Davis (13) postulated that the periodic contractions of the testicular capsule are important in propelling the nonmotile sperm out of the testis. If their conclusion is true, the contractions of the testis could be an important adjunct to the myoepithelial action of the seminiferous tubule (14) and the active movement of fluids produced by the tubular tissue (15) in propelling the nonmotile spermatozoa from the testis into the epididymus.

Davis and Langford (17) have shown that the rabbit testicular capsule contains two layers of smooth muscle that traverse the capsule in different directions. During the course of this investigation, various modes of contractions were noted before and after treatment with 21 and 24 n*M* PGE$_1$ (Fig. 1C). These data suggest that different layers of smooth muscle are present in the testicular capsule and may contract independently of each other. Since prostaglandins are found in the testis (10) and appear to be produced by the testis (7, 16) they may participate in regulating testicular function. In this respect, PGE$_1$ may act to modulate androgen synthesis as well as overall prostaglandin synthesis and testicular contractions (7). Conceivably, PGE$_1$ may serve to modulate testicular capsule contractions by serving as an inhibitory mechanism. This inhibition of testicular contractions could be of clinical importance if fertility were reduced as a consequence of the action of this hormone resulting from abnormal rates of synthesis or after exogenous administration. The action of PGE$_1$ on testicular contractions is consistent with the action of prostaglandins on other smooth muscle of the uterus, respiratory tract, and digestive tract (11).

Summary. Intact rabbit testes contracted autorhythmically *in vitro* with a frequency of 2–3 beats/min. When (PGE$_1$) was added to the bathing media, there were alterations in amplitude of contractions, rate, overall tone, and mode of contraction. At relatively high levels of PGE$_1$ (greater than 36 n*M*) the contractions were completely effaced; with lower concentrations of PGE$_1$, recovery was evident after 5 to 6 min. The effects of PGE$_1$ on amplitude, rate, and tonus were dose-dependent. The above observations, plus those of other workers that prostaglandins are present in mammalian testes, that a mechanism for their synthesis appears to be present in this organ, and that these compounds modulate androgen synthesis and inhibit testicular capsular contraction, suggest a possible physiological role for these unsaturated fatty acid derivatives in normal testicular function.

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