Prostaglandin $F_{1\alpha}$ Induced Stimulation of Rabbit Testicular Contractions *in Vitro*[1] (35901)

JAY M. JOHNSON, JAMES L. HARGROVE, AND LEGRANDE C. ELLIS
Department of Zoology, Utah State University, Logan, Utah 84321

An autorhythmically contracting capsule exists around the rabbit testis [Langford and Davis (1), Davis *et al.* (2), and Hargrove *et al.* (3)]. These workers showed that acetylcholine, norepinephrine, and carbachol stimulated these contractions, while isoproterenol (1, 2) and PGE_1 (3) inhibited them.

The mechanism for prostaglandin synthesis is present in the testis and is age dependent (4). The mechanism appears to be distributed in the male gonad with the same distribution as the steroid biotransforming enzymes (4). Our data indicate that prostaglandins can modulate androgen synthesis and affect the oxidative mechanism that Van Dorp *et al.* (5) and Bergstrom *et al.* (6) have demonstrated to be involved in biotransforming dihomo-γ-linolenic and arachidonic acids into prostaglandins. In addition, Carpenter and Wiseman (7) have isolated prostaglandins from rat testicular tissue, and Hargrove *et al.* (3) have reported that physiological concentrations of PGE_1 do inhibit rabbit testicular contractions *in vitro*.

Prostaglandin $F_{1\alpha}$ is known to stimulate smooth muscle of the rat uterus (8), the pregnant human myometrium (9), and rabbit fallopian tubes (10). Moreover, Ramwell and Shaw (11) detected a release of $PGF_{1\alpha}$ after stimulating the nerve leading to the rat epididymal fat pad. The above information suggests that $PGF_{1\alpha}$ may stimulate testicular capsular contractions. The investigations reported here were undertaken to determine any such effects.

Materials and Methods. Six mature male rabbits of mixed breed were anesthetized while the testes were excised as described elsewhere (3). Contractions of *in vitro* testes were recorded using a sensitive myograph transducer (Statham Co.) connected to a polygraph (Gilson minipolygraph) as previously described (3). The testes were bathed in a continuously oxygenated Tyrode's solution that was maintained at 35° in a water-jacketed muscle warmer. Solutions of $PGF_{1\alpha}$ were made up using 95% ethanol as a diluent; aliquots of these solutions were assayed in the above system for their effects on smooth muscle contractions.

Replicates for each treatment level were performed on a given testicular preparation. After each determination, the medium was changed, and when pretreatment levels of contractions were reestablished, an additional assay was undertaken. Representative observations for various treatment levels are included in this report.

Results. Generally, 15 to 30 min elapsed before testicular capsular contractions began and became regular; prostaglandin $F_{1\alpha}$ assays were begun at this time. Some effects were noticeable at a concentration of 0.4 n*M* $PGF_{1\alpha}$ (Fig. 1A). The rate of contraction (12/5 min) was not affected, but the amplitude decreased by about one-half. A slight but immediate increase in tone was, however, observed with this treatment.

A gradation of response was observed from 3.6 to 60 n*M* concentration of $PGF_{1\alpha}$ (Fig. 1A to D). In this treatment range certain general characteristics were noted: the rate of contraction doubled over the pretreatment level, and there was an overall increase in tone. In some cases, amplitude increased.

The treatment range from 71 to 213 n*M* $PGF_{1\alpha}$ (Fig. 2A–C) produced an increase in

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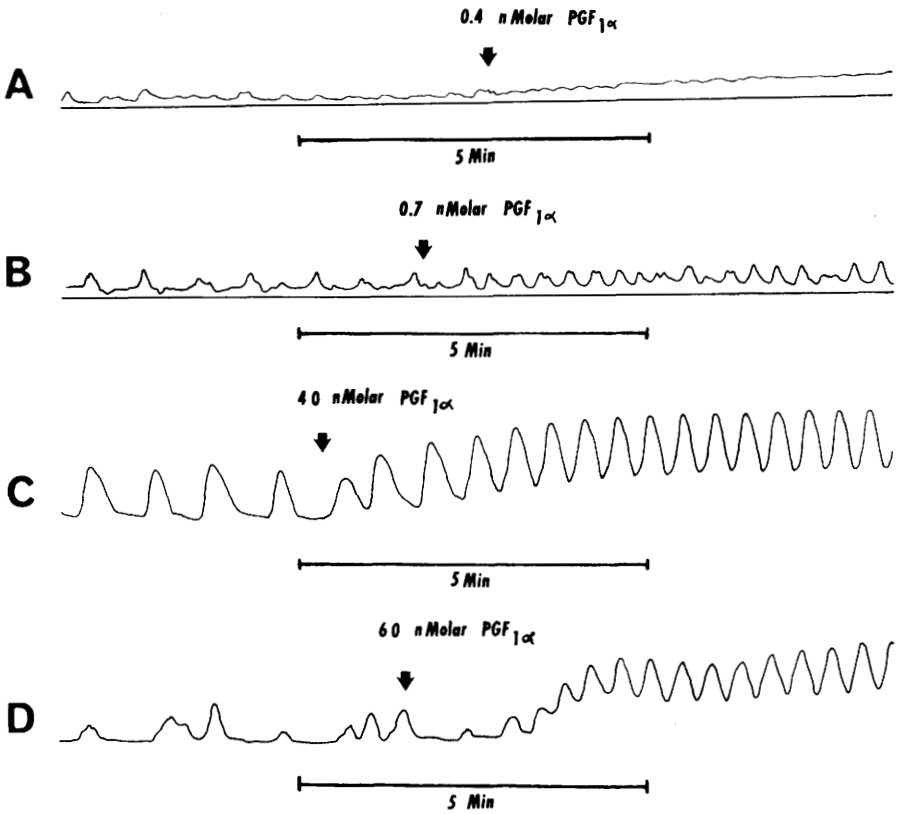

FIG. 1. Stimulation of rabbit testicular contractions *in vitro* with prostaglandin $F_{1\alpha}$: (A) $PGF_{1\alpha}$ concentration was 0.4 n*M* and the recorder sensitivity was 0.03 g/5 mm of pen deflection; (B) $PGF_{1\alpha}$ concentration was 0.7 n*M* and the recorder sensitivity was 0.2 g/5 mm of pen deflection; (C) $PGF_{1\alpha}$ concentration was 40 n*M* and the recorder sensitivity was 0.2 g/5 mm of pen deflection; (D) $PGF_{1\alpha}$ concentration was 60 n*M* and the recorder sensitivity was 0.2 g/5 mm of pen deflection.

rate, an increase in overall tone, and a decrease in amplitude. The responses were more pronounced at the highest level.

In one animal, testicular contractions could not be observed after the usual 15 to 30 min latent period. However, treatment with $PGF_{1\alpha}$ within the latent period resulted in changes (Fig. 3A and B). With 14 n*M* $PGF_{1\alpha}$, an increase in tone was seen, followed several minutes later by sustained contractions. The contractions were initially weak, but increased in rate (to 23/5 min) and amplitude with time. With 57 n*M* initial contractions were more rapid and became modulated in cycles (Fig. 3B) with a progressive increase in amplitude with each successive cycle.

Discussion. These data corroborate the observations of Langford and Davis (1), Davis *et al.* (2), and Hargrove *et al.* (3) that autorhythmic contractions do exist in the rabbit testicular capsule. Our results now indicate that there are two groups of prostaglandins: one that inhibits and one that stimulates testicular contractions *in vitro*. In addition to the possible *in vivo* control of testicular contraction through acetylcholine and norepinephrine postulated by other workers (1, 2), we now have the possibility of control by prostaglandins that may be synthesized in the testis.

Further support for the role of prostaglandins as possible mediators of physiological phenomena comes from observations by Ramwell and Shaw (11). They reported $PGF_{1\alpha}$ release following stimulation of nerves

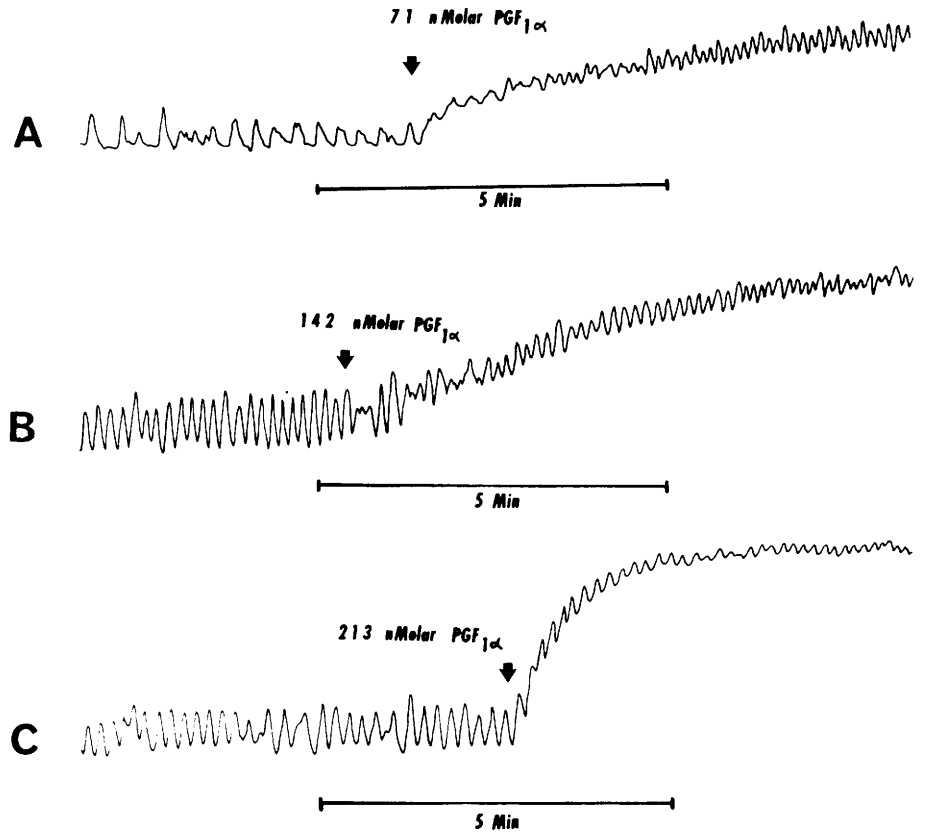

FIG. 2. Stimulation of rabbit testicular contractions *in vitro* with prostaglandin F$_{1\alpha}$: (A) PGF$_{1\alpha}$ concentration was 71 n*M* and the recorder sensitivity was 0.078 g/5 mm of pen deflection; (B) PGF$_{1\alpha}$ concentration was 142 n*M* and the recorder sensitivity was 0.078 g/5 mm of pen deflection; (C) PGF$_{1\alpha}$ concentration was 213 n*M* and the recorder sensitivity was 0.078 g/5 mm of pen deflection.

innervating the epididymal fat pads of young rats *in vitro*. PGE$_1$ was released spontaneously in their preparation.

The modulated response of testicular contractions (Fig. 3B) is often seen in other testicular preparations (3) that have not been pretreated with prostaglandins.

Summary. Excised rabbit testes were observed to contract autorhythmically *in vitro* at a frequency of 2–3 times/min. When PGF$_{1\alpha}$ was added to the bathing media, an increase in rate and overall tone with a

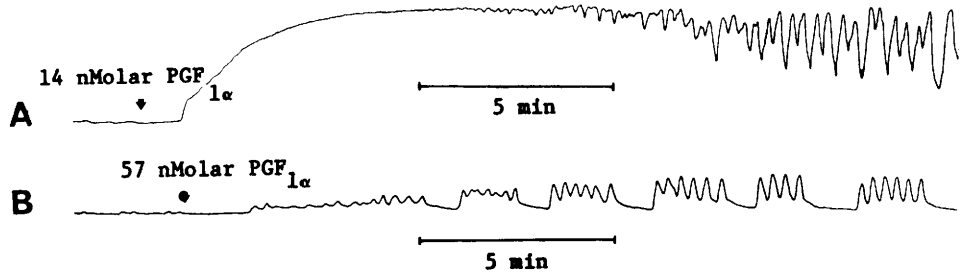

FIG. 3. Initiations of rabbit testicular contractions *in vitro* with prostaglandin F$_{1\alpha}$: (A) PGF$_{1\alpha}$ concentration was 14 n*M* and the recorder sensitivity was 0.143 g/5 mm of pen deflection; (B) PGF$_{1\alpha}$ concentration was 57 n*M* and the recorder sensitivity was 0.2 g/5 mm of pen deflection.

change in amplitude was observed. At lower concentrations of prostaglandin, the amplitude increased; but at higher levels (greater than 71 n*M*), amplitude was decreased. The response of the testicular capsule was dose dependent from 0.4 to 213 n*M* concentration of $PGF_{1\alpha}$. When $PGF_{1\alpha}$ was added to a testicular preparation during the latent period, before contractions were initiated spontaneously, an overall increase in tone was observed at 14 n*M* concentration, and the appearance of contractions approximately 7 min after treatment. At 57 n*M* concentration, $PGF_{1\alpha}$ initiated a series of modulated contractions within 1–3 min that increased in amplitude with time. The available data now suggest that prostaglandins may modulate testicular contractions *in vitro*: one group inhibiting and a second group stimulating this activity.

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