MASTER

Appendix VIII

Prostaglandins: Obligatory Role In
Regulating Contractions of Rabbit
Testicular Capsules In Vitro

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Abstract. Seasonally dependent, spontaneous rhythmical contractions were observed in the capsule of the isolated rabbit testis. Successive changes of the bathing medium abolished the contractions. Adding the original bathing fluid to the inactive testis reestablished the contractions. Prostaglandins E_1 , E_2 and $F_{1\alpha}$ but not acetylcholine, serotonin or epinephrine induced natural-appearing contractions. Extraction of the bath fluid for prostaglandins and related compounds yielded a smooth-muscle active substance.

Langford and Davis (1) have shown that rabbit testes have an autorhythmically contracting capsule that contains smooth muscle. These workers postulated that the contractions facilitate sperm transport. Inhibition of these contractions in vitro by prostaglandin E_1 (PGE_1) and the modulation of testicular contractions by $PGF_{1\alpha}$ have been reported by Hargrove et al. (2) and Johnson et al. (3). The present investigations now show: a) adult, scrotal and immature, abdominal testes both exhibited autorhythmia in vitro; b) all testes studied during the summer were active, but in winter many preparations slowly developed activity of low amplitude or remained inactive; c) contractions disappeared after repeatedly washing the testicular preparation with fresh media. Contractions were restored by adding the initial bathing medium obtained from a contracting preparation; d) prostaglandins E_1 , E_2 and $F_{1\alpha}$, but not serotonin, acetylcholine or epinephrine, restored natural-appearing contractions; and e) extracting the bathing fluid from an active, untreated testis for prostaglandins and related compounds yielded a substance that induced activity.

Fifty-three mature male rabbits were used in these investigations. The animals were sacrificed and their testes were excised. Capsular contractions were recorded in vitro using a sensitive myograph transducer (Statham Co.) and a Gilson minipolygraph. Continuously oxygenated Tyrode's solution, held at $35^\circ C$ in glass water-jacketed, muscle warmers, was used as the bathing medium. Prostaglandin stock solutions were made up in 95% ethanol. All treatments were repeated on at least five testes.

To recover smooth muscle-active substances, the bathing media were extracted twice with ethyl acetate after adjusting the pH to 3.0 with 3 N HCl. The organic solvent was then washed with 1 ml aliquots of distilled water until it was neutral. The remaining ethyl acetate was then evaporated with dry nitrogen gas at 40°C. The residue was taken up in Tyrode's solution and assayed for activity on the testicular capsule.

Rabbits from one month to two years of age were studied to determine if any changes occur in rhythmicity with respect to sexual development. In 5 rabbits from one to three months' age, the testes were abdominal and ranged in weight from less than 0.2 g to nearly 1 g. All of these testes showed natural rhythmicity corresponding to less than 0.5 g force, and responded in the same fashion as adult testes to prostaglandin stimulation (Fig. 1A). Testes from mature rabbits weighed from 2 to 4 g and exhibited contractions corresponding to 0.5 to 3.0 g. force. We concluded from these observations that the occurrence of natural spontaneous contractions is not dependent on age or previous sexual experience.

We have observed three states of capsular activity that appear to be seasonal. In this respect, all rabbits (N = 18) studied from May to August showed pronounced contractions within 1/2 hr of incubation. Adding PGE₁ in doses greater than 36 nM always decreased overall muscular tone, rate, and amplitude of contractions in a dose-dependent fashion. Concentrations of PGE₁ of 10⁻⁷ molar or greater completely effaced the contractions (Fig. 1B). From December to March, testicular contractions from 14 of 20 rabbits were either absent or less than 0.5 g force after one hour's incubation. Moreover,

addition of PGE_1 in concentrations from 10 to 70 nM always resulted in strong stimulation of tonus and contractility.

Successively changing the bathing media of contracting preparations resulted in effaced or markedly reduced motility irrespective of the initial magnitude of contractions. With each successive change, the contractions were serially diminished and recovery time was increased. Inactivity following the second rinse of a contracting testis is shown in figure 1C. Replacing the medium with the first bathing fluid restored the contractions. In non-contracting preparations, activity could always be induced with PGE_1 in concentrations of 10^{-9} to 10^{-8} molar while concentrations of PGE_1 greater than 10^{-7} molar were inhibitory. In these preparations containing inhibitory concentrations of PGE_1 , rinsing with Tyrode's solution initiated contractions (Fig. 2A). Further rinsing abolished the contractions.

In naturally contracting preparations, $\text{PGF}_{1\alpha}$ in concentrations of 10^{-8} molar increased overall tonus and rate of contraction (Fig. 2B). With higher concentrations of $\text{PGF}_{1\alpha}$, tetany resulted (Fig. 2C). When the testes were initially non-contracting, treatment with 10^{-8} molar concentrations of $\text{PGF}_{1\alpha}$ initiated contractions (Fig. 3A).

Adding the initial bathing medium from an active to an inactive rinsed preparation resulted in an immediate increase in tonus with eventual initiation in contractions (data not shown). Extraction of the medium at pH 3 with ethyl acetate, a system used for recovering prostaglandins, yielded a residue that stimulated contractions of the testes (Fig. 3B).

Addition of various concentrations of acetylcholine or serotonin (10^{-8} to 10^{-5} molar) failed to increase tonus or induce contractions in inactive preparations (Fig. 3C), although each compound increased tonus greatly in active preparations. Addition of epinephrine to either inactive or active preparations produced a large increase in tonus; however, extraction of the media at pH 3 with ethyl acetate did not yield a smooth-muscle active substance. These compounds were, therefore, excluded as being principal mediators of natural rhythmicity.

Disappearance of contractions due to changing the bathing medium, and resumption of activity after adding back the original bathing fluid, indicated that a factor was present without which spontaneous contractions were not observed. The fact that it partitioned into ethyl acetate at pH 3 indicated that it had prostaglandin-like solubility properties. Testicular homogenates do synthesize prostaglandins (4, 5). In the testis, prostaglandins also appear to be necessary for the smooth-muscle stimulating action of other agents such as serotonin and acetylcholine, since neither compound stimulated the rinsed, inactive preparation. In this respect, Coceani et al. (6) have shown that prostaglandin efflux from the rat stomach increased after infusion with serotonin or after the (cholinergic) vagus nerve was stimulated. Histamine and gastrin also increased prostaglandin efflux from the rat stomach (7). The release of prostaglandins from the rat stomach (6), the frog intestine (8) and their existence in the testes suggests that prostaglandins may be important modulators of the smooth-muscle motility.

In addition, the endogenous level of prostaglandins might severely affect bioassay procedures. This is pointed out by the observation that concentrations of PGE_1 that totally inhibited the maximally-contracting testis, stimulated the inactive preparation. This fact could partially explain the variability of response reported by other workers (9, 10) for other smooth muscle preparations.

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Figure 1. (a) Response of an immature, abdominal testis to 10^{-9} molar PGE_1 .
(b) Inhibition of testicular contractions by 10^{-7} molar PGE_1 .
(c) Disappearance of contractions in the capsule after the second change of medium. The vertical bar corresponds to 0.5 g of pen deflection.

Figure 2. (a) Absence of activity in the rabbit testis after treatment with 10^{-6} molar PGE_1 , followed by stimulation after the medium was changed.
(b) Increased capsular contractions following stimulation with 10^{-8} molar $\text{PGF}_{1\alpha}$. (c) Tetany following treatment of the capsule with 10^{-7} molar $\text{PGF}_{1\alpha}$.

Figure 3. (a) Initiation of contractions in an inactive testis with 10^{-8} molar $\text{PGF}_{1\alpha}$. (b) Addition of residue extracted from an active testis to the same rinsed, inactive preparation. These contractions are of the same magnitude as the original ones. (c) Absence of stimulation of the inactive testis in response to 10^{-6} molar serotonin.

A0.001 μM PGE_1 **B**0.360 μM PGE_1 Changed
Bath

A

0.04 μM $\text{PGF}_{1\alpha}$

B

5 Minutes

Added
Extract

C

0.65 μM Serotonin