Prostaglandin-Like Substances: Initiation and Maintenance of Rabbit Testicular Contraction *in Vitro* (36989)

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Langford and Davis (1) have demonstrated that rabbit testes have a capsule containing smooth muscle that contracts autorhythmically *in vivo* and *in vitro*. These workers postulated that the contractions facilitate sperm transport and circulation in the testes. Davis and Langford (2) found that the parenchymal contribution to the contractile response *in vitro* was negligible. They observed that acetylcholine, epinephrine, histamine and carbachol stimulated the contractions, and that isoproterenol inhibited them. Hargrove *et al.* (3) and Johnson *et al.* (4) reported that prostaglandin E_1 (PGE_1) inhibited, while prostaglandin $F_{1\alpha}$ ($PGF_{1\alpha}$) stimulated, testicular motility.

Ellis and Baptista (5) observed that: the mechanism for prostaglandin synthesis was present in rat testes; it appeared to be age dependent; and it had the same distribution as the steroid biotransforming enzymes. Preliminary data suggested that prostaglandins E_1, E_2, $F_{1\alpha}$ and $F_{2\alpha}$ occur in rat testes (6). Furthermore, enzymes for prostaglandins inactivation have been observed in rat (7) and swine (8) testes.

In several smooth-muscle-containing systems [*e.g.*, gut, stomach, and uterus (9–11)], prostaglandins have been demonstrated to be released into the superfusate and account in a large measure for the stimulatory activity of such fluids. Prostaglandins also potentiate the response of smooth muscle to other agents (11, 12). The investigations reported here were undertaken to determine to what extent endogenous and exogenous prostaglandins in the bathing medium would contribute to testicular motility *in vitro*.

Materials and Methods. Fifty-three mature male rabbits of mixed breeds were sacrificed and their testes were excised. Capsular contractions were recorded *in vitro* using a sensitive myograph transducer (Statham Co.) and a Gilson minipolygraph as previously reported (4, 5). Continuously oxygenated Tyrode's solution, held at 35° in glass, water-jacketed, muscle warmers, was used as the bathing medium. Prostaglandin stock solutions were made up in 95% ethanol. Serotonin solutions (Sigma Chemical Co.) were made up fresh on the day of use in distilled water. All treatments were repeated on at least five testes. All pharmaceutical agents used in this investigation were also tested for activity on fresh preparations of castrate, estrogen-treated rat uteri.

To recover prostaglandin-like, 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 remaining ethyl acetate was then evaporated with dry nitrogen gas at 40°. The residue was taken up in Tyrode's solution and assayed for activity on the testicular capsule.

Results. Rabbit testes developed contractions within an hour after suspension *in vitro*. Typically, tonus increased gradually, prior to the onset of contractions. 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, tonus and frequency were serially diminished. Inactivity usually followed the second rinse of a naturally contracting testis (Fig. 1A).

The addition of the initial bathing medium from an active to an inactive, rinsed preparation resulted in an immediate increase in tonus with eventual initiation of contractions (Fig. 1B). Extraction of the medium at pH 3 with ethyl acetate (a preliminary system

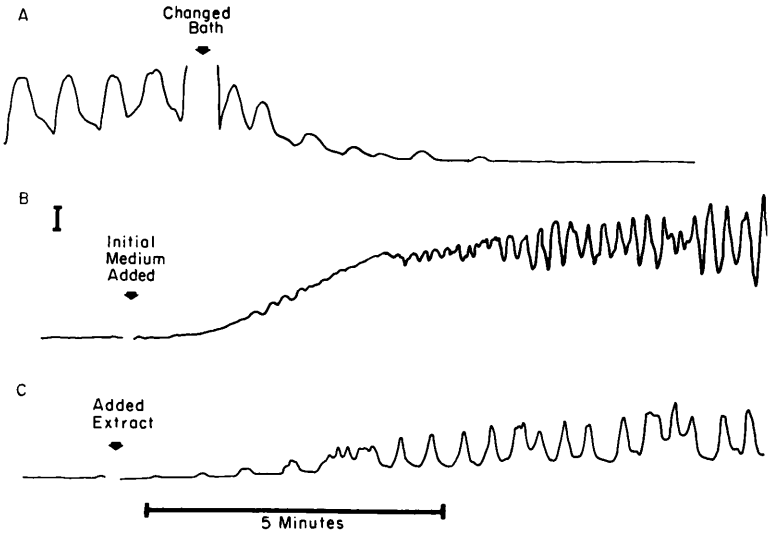

FIG. 1. (A) Inactivity following the second rinse of a naturally contracting rabbit testis. (B) Addition of the initial bathing medium of an actively contracting rabbit testicular preparation to an inactive rinsed preparation. (C) Addition of an extract of the initial bathing medium from an active rabbit testicular preparation for prostaglandin-like compounds to an inactive, rinsed preparation. The vertical scale represents 0.5 *g* force.

used for recovering prostaglandin-like compounds) yielded a residue that stimulated contractions of the testes (Fig. 1C).

PGE_1 in concentrations of 10^{-9} to 10^{-8} *M* was stimulatory to inactive preparations (Fig. 2A), although when added to active preparations it was inhibitory (Fig. 2B). Concentrations of PGE_1 greater than 10^{-7} *M* were inhibitory in both active and inactive testes. In preparations containing inhibitory concentrations of PGE_1, rinsing the testis with fresh prewarmed and oxygenated Tyrode's solution initiated contractions (Fig. 2C). Further rinsing with the above medium abolished the contractions (Fig. 2C). Treatment with PGE_2 gave results similar to those resulting from treatment with PGE_1 (data not shown).

Concentrations of acetylcholine or serotonin from 10^{-8} to 10^{-5} *M* failed to induce contractions and only slightly increased tonus in inactive preparations Fig. 3A and B). Oxytocin produced negligible stimulation of either active or inactive testes at concentrations as high as 0.1 IU per ml (Fig. 3C). Addition of epinephrine to inactive preparations produced a large increase in tonus (Fig. 3D). Contractions did not appear, however, and extraction of the medium at pH 3 with ethyl acetate did not yield a smooth-muscle active substance. When authentic prostaglandin was added to fresh media as a control and similarly extracted with ethyl acetate, a smooth-muscle active substance was recovered.

Discussion. The gradual onset of contractions, their rapid disappearance after a change of the bathing medium, and the resumption of activity when the original bathing fluid was re-introduced indicated that a soluble factor was present in the incubation medium without which spontaneous contractions would not occur. The fact that this material was extracted by ethyl acetate at pH 3 suggested that the material had prostaglandin-like solubility properties. This concept is strengthened by the fact that testicular homogenates have the capacity to synthesize prostaglandins (5, 6).

We have previously reported that PGE_1 inhibited normal spontaneous testicular contractions at 10^{-3} to 10^{-9} *M* concentration. We now report that PGE_1 induces contractions in the inactive testicular preparations. Our present data indicate that low concentra-

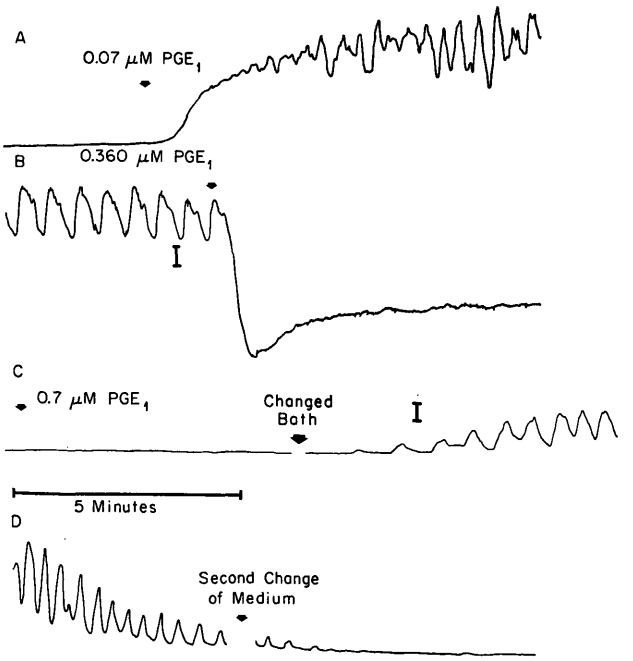

FIG. 2. (A) Initiation of rabbit testicular contractions *in vitro* with the addition of 0.07 μM PGE_1 to an inactive, rinsed preparation. (B) Inhibition of rabbit testicular preparations *in vitro* with the addition of 0.360 μM PGE_1 to an actively contracting preparation. (C) Addition of 0.7 μM PGE_1 to an inactive, rinsed rabbit testicular preparation resulted in no induced activity. Contractions were initiated after changing the bathing medium. (D) Inactivity following the second rinse of a preparation which was inhibited with 0.7 μM PGE_1 and then made active by changing the bathing medium once. The vertical scale represents 0.5 *g* force.

tions of PGE_1 are stimulatory while higher concentrations are inhibitory to the contractility of the male gonad. The inhibitory action of PGE_1 on the active preparation we now attribute to the presence of endogenous prostaglandins or prostaglandin-like material that increases the effective concentration in the bathing medium. Subsequent studies now show that this prostaglandin-like material does migrate with authentic PGE_1 upon thin-layer chromatography (unpublished data). Attempts are currently being made to positively identify this material using gas chromatography and mass spectroscopy. The above data are in agreement with recent observations of smooth muscle activity and the need for PG's in the rabbit jejunum preparation (9–13).

Acetylcholine and serotonin have been shown to stimulate the active testicular capsule (1, 2), however, our inactive preparations were not appreciably affected by either agent. The only difference between our active and inactive preparations appeared to be the fat soluble factor with prostaglandin-like characteristics found in the bathing medium of active capsules.

Oxytocin was incapable of eliciting significant activity in active or inactive preparations at any concentration. Epinephrine increased the tonus of both inactive and active preparations, but did not induce contractions, nor was a stimulatory factor with prostaglandin-like activity extracted by ethyl acetate at pH 3. Prostaglandins E_1 and E_2, on the other hand, initiated rhythmic activity and increased tonus in both inactive and active preparations. Of the compounds tested, only the prostaglandins as a group possessed all of the properties of the soluble factor that we isolated from the bathing medium of active preparations. In addition, various prostaglandins are known to potentiate the response of

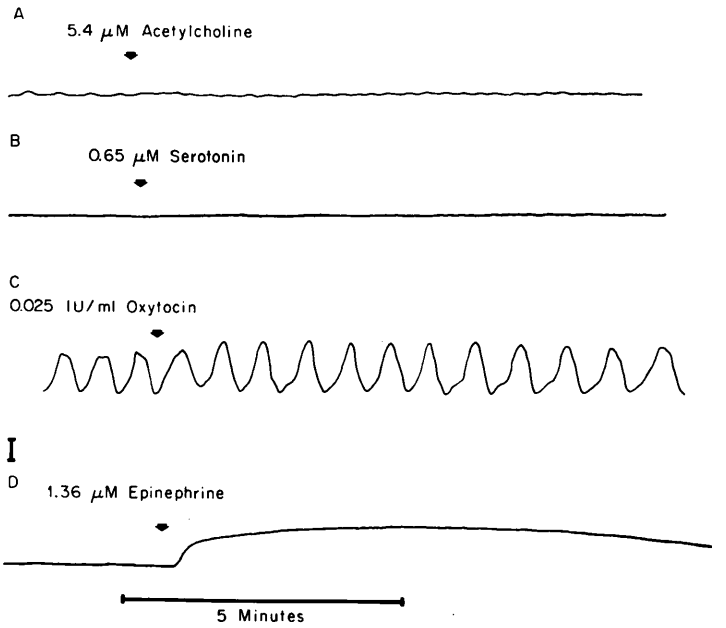

FIG. 3. (A) Addition of 5.4 μM Acetylcholine to an inactive, rinsed rabbit testicular preparation resulting in no increased tonus. (B) Addition of 0.65 μM Serotonin to an inactive, rinsed rabbit testicular preparation resulting in no increased tonus. (C) Addition of 0.025 IU/ml Oxytocin to a spontaneously contracting rabbit testicular preparation resulting in little change in contractility. (D) Additional 1.36 μM epinephrine to an inactive, rinsed rabbit testicular preparation resulting in an increase in tonus. The vertical scale represents 0.5 g force.

certain smooth-muscle-containing organs to various pharmacological compounds and calcium ions (11, 12). Therefore, we concluded that prostaglandin-like compounds appear to be responsible for modulation of testicular capsular activity *in vitro* and possibly *in vivo*.

Prostaglandins are released spontaneously and after stimulation into superfusates of several smooth-muscle-containing organs (10–12). Variability in response of smooth muscle to exogenous prostaglandins has been reported in the literature with either stimulatory or inhibitory action reported in the same organ with similar doses of prostaglandins (14, 15). We conclude that this variability in response may be due to endogenous levels of prostaglandin-like material present in the bathing medium.

Summary. The spontaneous contractions that occur in rabbit testes *in vitro* were abolished by changing the bathing medium. Adding the initial medium from an active to an inactive preparation induced activity in the inactive testis. Extraction of the initial medium at pH 3 into ethyl acetate, a system used for recovering prostagandins, yielded a residue that restored the activity. Acetylcholine and serotonin had little effect on the inactive preparation nor could a smooth-muscle active substance be extracted from these preparations. Oxytocin stimulated neither the active nor the inactive testis appreciably. Epinephrine increased tonus in both inactive or active preparations, but did not induce rhythmic contractions. Prostaglandins of the E-type were stimulatory at low, but inhibitory at higher concentrations. A myogenic agent with prostaglandin-like properties was present only in the bathing media from active preparations and appeared to determine the response that was obtained by treatment with exogenous prostaglandins or some pharmacological agents *in vitro*.

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1. Davis, J. R., and Langford, G. A., *in* "The Human Testis" (E. Rosemberg and C. A. Paulsen, eds.), p. 495. Plenum, New York, 1970.

2. Davis, J. R., and Langford, G. A., J. Reprod. Fert. **26**, 241 (1971).

3. Hargrove, J. L., Johnson, J. M., and Ellis, L. C., Proc. Soc. Exp. Biol. Med. **136**, 958 (1971).

4. Johnson, J. M., Hargrove, J. L., and Ellis, L. C., Proc. Soc. Exp. Biol. Med. **138**, 378 (1971).

5. Ellis, L. C., and Baptista, M. H., "Radiation Biology of Fetal and Juvenile Mammal," p. 963. U. S. Atomic Energy Comm. Div. Techn. Inform., Washington (1969).

6. Carpenter, M. P., and Wiseman, B., Fed. Proc., Fed. Amer. Soc. Exp. Biol. **29**, 248 (1970).

7. Anggard, E., Larsson, C., and Samuelsson, B., Acta Physiol. Scand. **81**, 396 (1971).

8. Nakano, J., Montague, B., and Darrow, B., Biochem. Pharmacol. **9**, 2512 (1971).

9. Coceani, F., Pace-Asciak, C., Volta, F., and Wolfe, L. S., Amer. J. Physiol. **213**, 1056 (1967).

10. Shaw, J. E., and Ramwell, P. W., *in* "Prostaglandin Symposium of the Worcester Foundation for Experimental Biology" (P. W. Ramwell and J. E. Shaw, eds.), p. 55. Interscience, New York (1968).

11. Vogt, W., Suzuki, T., and Babilli, S., Mem. Soc. Endocrinol. **14**, 137 (1966).

12. Eagling, E. M., Lovell, H., and Pickles, V. R., J. Physiol. **213**, 53 (1971).

13. Ferreira, S. H., Herman, A., and Vane, J. R., Brit. J. Pharmacol. **44**, 328 (1972).

14. Kurzrock, R., and Lieb, C. C., Proc. Soc. Exp. Biol. Med. **28**, 268 (1930).

15. Eliasson, R., Mem. Soc. Endocrinol. **14**, 77 (1966).

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