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RABBIT TESTICULAR CAPSULAR CONTRACTIONS—PROSTAGLANDINS, CYCLIC ADENOSINE 3′,5′-MONOPHOSPHATE AND EPINEPHRINE*

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Abstract—Log dose–response curves for the effect of prostaglandin E_1, E_2, $F_{1\alpha}$, and $F_{2\alpha}$ on rabbit testicular capsules *in vitro* showed that F-type prostaglandins were stimulatory throughout the concentration range tested. On the other hand, E-type prostaglandins were stimulatory in low concentrations, but became less effective at higher concentrations, and exogenous cyclic AMP potentiated the testicular capsule's inhibitory response to PGE_1. Dibutyryl cyclic AMP inhibited tonus, whereas theophylline and isoproterenol inhibited both tonus and contractility *in vivo* and *in vitro*. Epinephrine stimulated contractions equally *in vivo* and *in vitro*; however, pretreatment of the preparations with ergotamine tartrate eliminated the stimulatory response to epinephrine. Pretreatment with propranolol eliminated the inhibitory response to isoproterenol. Ergotamine tartrate inhibited spontaneous contractions *in vivo*, but propranolol had no effect on capsular motility *in vivo* or *in vitro*. These data suggest that inhibition of capsular motility by higher concentrations of prostaglandin E_1 and E_2 may be mediated by cyclic AMP and confirm the presence of α- and β-adrenergic receptors in rabbit testicular capsules. Moreover, the data affirm the importance of adrenergic stimulation in regulating spontaneous contractions of testicular preparations *in vivo*.

THE TUNICA albuginea of the testicular capsule contains smooth muscle cells as a major component.[1,2] These cells produce autorhythmic contractions that are thought to facilitate sperm transport and testicular circulation in rabbits and man.[2,3] Prostaglandin biosynthesis occurs at a low level in both rat[4–7] and rabbit testes,[8] and endogenous prostaglandin-like (PG-like) compounds appear to be important modulators of rabbit capsular contractions *in vitro*.[9,10] Exogenous $PGF_{1\alpha}$ in all concentrations tested stimulated contractions in isolated testicular capsules. PGE_1 and PGE_2, however, stimulated contractions in lower concentrations, but inhibited contractions at higher concentrations.[9,11–13]

PGE_1 and PGE_2 both stimulate cyclic AMP accumulation in lung, heart, aorta, uterus, fibroblasts, intestinal smooth muscle and other tissues of various animals.[14–17] The PGE compounds, in general, are far more potent than the PGF compounds in modulating cyclic AMP biosynthesis.[18,19] Factors that increase cyclic

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AMP concentrations in various smooth muscle preparations appear to reduce motility. These facts suggest that PGE-induced inhibition of rabbit testicular capsular contractions may follow increased cyclic AMP formation. In addition, stimulation of β-adrenergic receptors results in an increased cyclic AMP concentration and induces relaxation in rabbit intestinal smooth muscle[16,17] and rat myometrium;[15] β-adrenergic blocking agents antagonize the effect of catecholamines on these tissues and on adipose tissue.[20] Phosphodiesterase inhibitors (which block the conversion of cyclic AMP to 5′-AMP) also cause an accumulation of cyclic AMP and relaxation in smooth muscle. Furthermore, exogenous N^6,$O^{2'}$-dibutyryl cyclic AMP mimicks the effect of drugs that increase cyclic AMP levels.[17] Exogenous dibutyryl cyclic AMP stops spontaneous spike discharges that are associated with normal contractility, resulting in some hyperpolarization and a tension decrease in guinea pig taenia coli. These data show that cyclic AMP suppresses membrane activity of smooth muscle.[21]

The present investigation was undertaken to determine if a relationship exists between cyclic AMP and the response of rabbit testicular capsular contractions to prostaglandins. Interactions between prostaglandins, a phosphodiesterase inhibitor (theophylline), and dibutyryl cyclic AMP were investigated. In addition, rabbit testicular capsular preparations were tested for the presence of both α- and β-adrenergic receptors.

In this investigation, preparations both *in vivo* and *in vitro* were used, since previous work in our laboratory[13] has shown that several steroids inhibit capsular motility *in vitro* but not *in vivo*. That study also showed contractions to begin more rapidly *in vivo* and persist despite changes of the bathing medium.

MATERIALS AND METHODS

Testicular capsular contractions of 35 mature male rabbits of mixed breeding were recorded by a method modified from Davis and Langford[2] previously used in this laboratory.[13] For experiments *in vivo*, the rabbits were anesthetized by i.v. injections of sodium pentobarbital (Haver–Lockhart Laboratory, 22–65 mg/kg). After the animals were restrained on an animal board, an incision was made through the scrotal sac to expose one of the testes. The intact testis was drawn into a water-jacketed muscle warmer and a ligature, attached to the superior pole of the testis, was connected to a sensitive myograph transducer (Statham Co.). The opposite pole of the testis was secured to the base of the muscle warmer with a ligature, and the scrotal sac was sealed around the bottom of the muscle warmer with a circular wire tie as previously reported.[13] Isometric contractions of the testicular capsule were subsequently recorded with the myograph transducer and a Gilson minipolygraph. Unoxygenated Tyrode's solution (20 ml) at 35° was the bathing medium. Agonists were introduced into the bathing media with manual mixing.

For experiments *in vitro*, the contralateral testis of the experimental animal was excised and suspended in a water-jacketed muscle warmer (20 ml total volume). The testis was bathed in continuously oxygenated Tyrode's solution maintained at 35°. Bubbling action during aeration continuously mixed the bathing media. Contractions were measured isometrically. Baseline tension was essentially the same for the preparations *in vivo* and *in vitro*.

Log dose–response curves (LDR curves) for PGE_1, PGE_2, $PGF_{1\alpha}$, and $PGF_{2\alpha}$ on rabbit capsular preparations *in vitro* were generated by introducing aliquots of less than 75 μl prostaglandin, dissolved in 95% ethanol, directly into the bathing medium. For comparative purposes, arachidonic acid was dissolved in 95% ethanol and added to the bathing medium. Ethanol had little effect on testicular capsular motility in volumes of less than 100 μl.[11] All treatments were repeated a minimum of five times in order to establish reproducibility. Points on the graphs represent the mean $\pm$ the standard error of the mean. A standard *t*-test was used to compare mean responses of preparations *in vivo* and *in vitro*.

Theophylline (K & K Laboratories, Inc.), a phosphodiesterase inhibitor, was dissolved in distilled water and added to the bathing media of preparations both *in vivo* and *in vitro* in aliquots of less than 100 μl. Dibutyryl cyclic AMP, cyclic AMP and adenosine (Sigma Chemical Co.) were dissolved in Tyrode's solution and added to the testicular bathing medium of preparations *in vivo*. Propranolol (Ayerst Laboratories, Inc.) was dissolved in distilled water and added to the bathing media in aliquots of 20 ml.

The response of testicular capsules to PGE_1 was determined *in vivo*. After a thorough rinse with Tyrode's solution (until baseline was re-established) and pretreatment with cyclic AMP, the response of the capsule to the same concentration of PGE_1 was again determined.

RESULTS

The testicular preparations responded to exogenous prostaglandin with an increase in over-all tonus and subsequent development of rhythmic contractions. Induced tonus increases and testicular motility were easily eliminated by changing the testicular bathing medium. LDR curves for the effect of authentic prostaglandins on rabbit testicular preparations *in vitro* (Fig. 1) indicated that 0·68 nM, 10·2 nM, 112 nM and 113 nM of PGE_1, PGE_2, $PGF_{1\alpha}$ and $PGF_{2\alpha}$, respectively, were required to effectively stimulate capsular contractions to obtain 50 per cent of the maximum re-

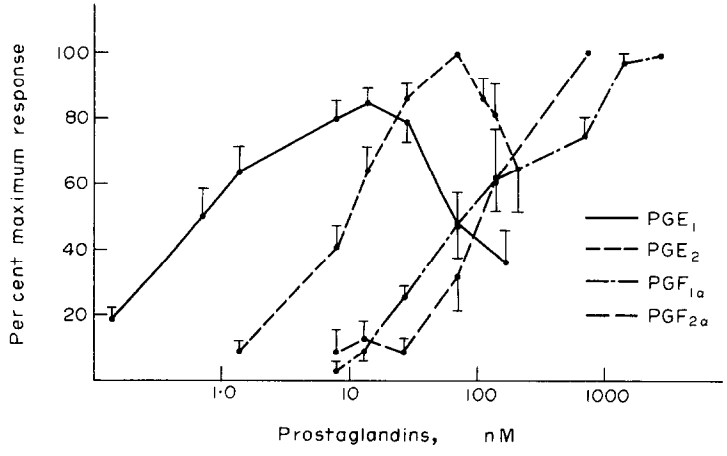

FIG. 1. LDR curves for the response of rabbit testicular capsules *in vitro* to: (a) PGE_1; (b) PGE_2; (c) $PGF_{2\alpha}$; and (d) $PGF_{1\alpha}$. The vertical bars denote standard error of mean values. Responses were plotted as a per cent of the maximum response generated by each prostaglandin tested.

sponse for that compound (effective dose or ED_{50}). Differences between maximal responses occurred in the order $PGF_{2\alpha} = 1{\cdot}6\ PGF_{1\alpha}$, 3·8 PGE_2 and 3·8 PGE_1 (means of five trials). The maximum response to $PGF_{2\alpha}$ was indistinguishable from that to epinephrine ($P > 0{\cdot}2$). Maximum responses were achieved with 14·1 nM PGE_1 and 70·1 nM PGE_2; higher concentrations of the prostaglandins yielded less than a maximum response. $PGF_{1\alpha}$ or $PGF_{2\alpha}$ stimulated capsular motility in all concentrations tested. No diminution of response was seen within 0·5 hr. Arachidonic acid at 1·05 mM final concentration had no effect on the testicular capsule (data not shown).

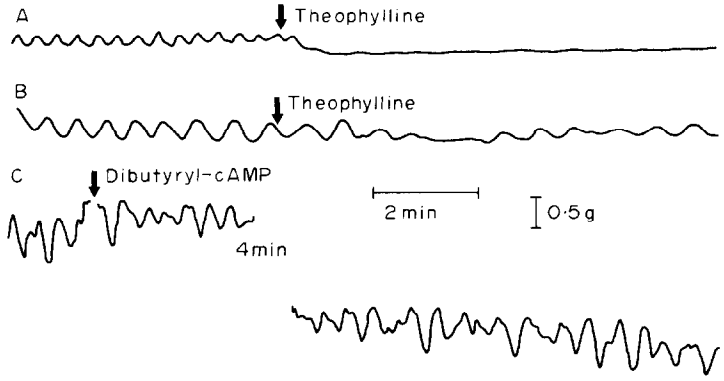

FIG. 2. Response of rabbit testicular capsular contractions to theophylline and dibutyryl cyclic AMP: (A) response of spontaneously rhythmic capsular contractions *in vitro* to 0·28 mM theophylline (final concentration) in the bathing medium; (B) response of spontaneously active testicular capsular contractions *in vivo* to 0·28 mM theophylline (final concentration) in the bathing medium; and (C) response of spontaneously active testicular capsular contractions *in vivo* to 2 mM dibutyryl cyclic AMP in the bathing medium (the break in the curve represents a 4-min lapse in time during which a continuous decrease in tonus occurred).

Addition of theophylline to the bathing media of preparations *in vivo* and *in vitro* (final concentration of 0·28 mM) slightly inhibited over-all tonus and strongly inhibited testicular capsular motility by reducing the rate and amplitude of the contraction (Fig. 2, A and B). Rhythmic contractions of preparations *in vivo* were more resistant to inhibition by theophylline than preparations *in vitro*. Final concentrations equaling 2 mM of either dibutyryl cyclic AMP (Fig. 2, C) or cyclic AMP gradually reduced over-all tonus of preparations *in vivo* without significantly reducing the amplitude of the autorhythmic contractions. Autorhythmically contracting rabbit testicular preparations responded with an increase in over-all tonus and amplitude of contractions to the successive addition of 0·16 nM and 1·6 nM PGE_1 respectively (Fig. 3, A). However, pretreatment of preparations *in vivo* with 0·5 mM cyclic AMP yielded a reduction in over-all tonus, amplitude and rate of contraction in response to successive additions of 0·16 nM and 1·6 nM PGE_1 into the bathing medium respectively (Fig. 3, B). Adenosine at 1 mM abolished contractions *in vitro* and *in vivo* (data not shown).

Epinephrine initiated tonus increases in preparations both *in vivo* and *in vitro*; the mean tonus increases in response to epinephrine are represented as LDR curves (Fig. 4). No significant difference existed between the two preparations ($P > 1{\cdot}0$), indicating that epinephrine stimulated over-all tonus of capsular smooth muscle equally *in vivo* and *in vitro*. The ED_{50} for the response of the capsular preparations was approxi-

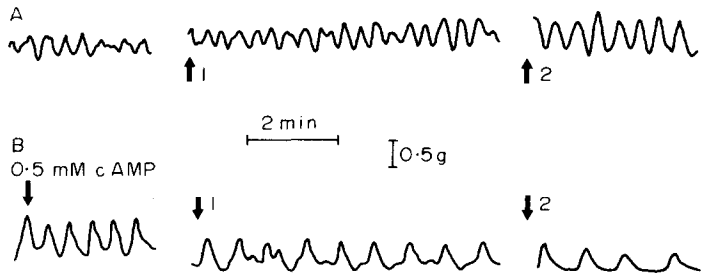

FIG. 3. Potentiation of the inhibitory response of the rabbit testicular capsule to PGE_1 by cyclic AMP: (A) response of spontaneously active rabbit testicular preparation *in vivo* to the successive addition of a final concentration of: (1) 0·16 nM PGE_1 and (2) 1·6 nM PGE_1 in the bathing medium; and (B) response of spontaneously active rabbit testicular preparations *in vivo* after treatment with a final concentration of 0·5 mM cyclic AMP to the successive addition of a final concentration of: (1) 0·16 nM PGE_1 and (2) 1·6 nM PGE_1 in the bathing medium.

mately 125 nM. Epinephrine was effective in approximately the same concentration range as $PGF_{1\alpha}$ or $PGF_{2\alpha}$, however, PGE_1 and PGE_2 were effective in smaller concentrations (cf. Figs. 1 and 4). The maximum response to epinephrine was consistently larger than the greatest response to any prostaglandin tested.

Propranolol (3·4 mM) in the bathing media of spontaneously contracting preparations *in vivo* (Fig. 5 B) or in inactive, rinsed testicular preparations *in vitro* (data not shown) elicited no change in motility or tonus generated by capsular smooth muscle. Pretreatment of inactive, rinsed preparations *in vitro* with propranolol did not alter the response of the testicular capsule to epinephrine (Fig. 5, A). However, propranolol (8·5 μM) pretreatment of active testicular preparations *in vivo* eliminated the normal inhibitory response of the capsule to isoproterenol (4·0 μM) (Fig. 5, B).

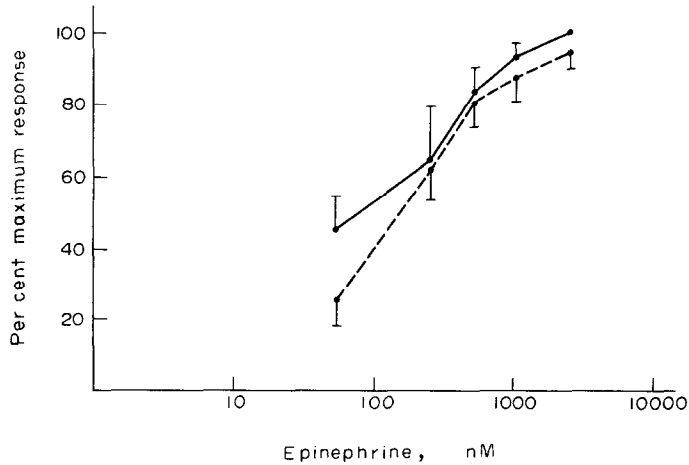

FIG. 4. Similarity of the LDR curves for epinephrine on rabbit testicular contractions *in vivo* and *in vitro*. The broken line represents contractions *in vivo* and the solid line represents contractions *in vitro*. There was no significant difference between the two preparations. The vertical bars denote standard error of mean values.

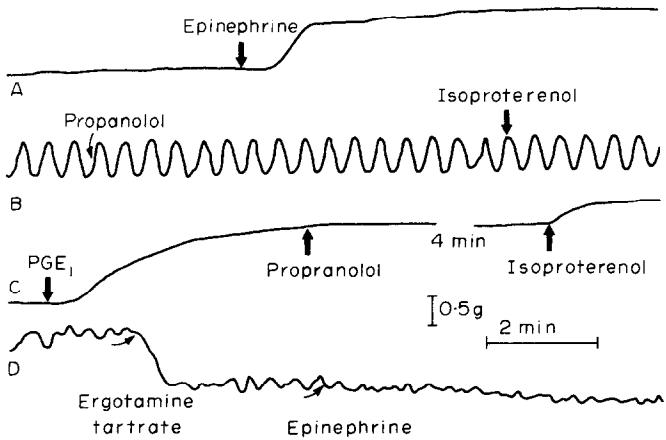

FIG. 5. Effect of α- and β-adrenergic blocking agents on the response of rabbit testicular capsular contractions to epinephrine and isoproterenol: (A) response to 0·55 μM epinephrine by an inactive rabbit testicular capsular preparation *in vitro* that had been pretreated with 3·4 μM propranolol; (B) response of spontaneously contracting rabbit testicular capsular preparations *in vivo* to 3·4 μM propranolol followed by the addition of 4·0 μM isoproterenol; (C) response of an inactive rabbit testicular preparation *in vitro* to 28 nM PGE_1 followed by 3·4 μM propranolol and then by 4·0 μM isoproterenol (the break in the trace represents 4 min); and (D) response of a spontaneously contracting rabbit testicular preparation *in vivo* to 9·5 μM ergotamine tartrate followed by 5·5 μM epinephrine.

Tonus increases induced with PGE_1 (28 nM) *in vitro* were unaffected by propranolol, and subsequent addition of isoproterenol yielded a slight increase in tonus. Ergotamine tartrate (9·5 mM) decreased over-all tonus in spontaneously contracting preparations *in vivo* and subsequent addition of epinephrine initiated a slight decrease in over-all tonus and motility.

DISCUSSION

The spontaneous contractility of isolated rabbit testicular capsules follows the release of PG-like material into the bathing medium.[9,10] These spontaneous contractions are not abolished by bretylium, atropine or tetrodotoxin.[22] However, DMPP (dimethyl-4-phenylpiperazinium iodide) causes a contracture, probably by post-junctional stimulation of autonomic ganglia. Blockade of adrenergic transmitter release with bretylium abolishes this DMPP-induced contracture, and tyramine restores it.[22] These data suggest that prostaglandins and adrenergic stimulation may be important regulators of testicular capsular motility or tonus. The present paper demonstrates that $PGF_{1\alpha}$ and $F_{2\alpha}$ stimulated the capsule at all concentrations, while PGE_1 and E_2 caused a biphasic response. Threshold for stimulation occurred in the order $E_1 < E_2 < F_{1\alpha} = F_{2\alpha}$, while the order of maximum response was $PGF_{2\alpha} > PGE_1 = PGE_2$.

That high concentrations of E-type prostaglandin inhibit spontaneous and induced capsular motility equally *in vitro* and *in vivo* has been established;[9,13] this effect is not seen with F-type prostaglandins.[12] This difference in action may be due to the greater potency of PGE's over PGF's in increasing cyclic AMP biosynthesis.[18,19] Moreover, compounds which increase cyclic AMP concentrations in

other tissues elicited an inhibitory response in rabbit testicular capsular preparations. Theophylline, a phosphodiesterase inhibitor, reduced over-all tonus and motility, as did isoproterenol, a β-adrenergic stimulating agent.[23] Dibutyryl cyclic AMP and cyclic AMP also inhibited testicular capsular tonus but did not abolish motility. Similarly, sub-threshold concentrations of cyclic AMP, when added to the testicular bathing media of spontaneously contracting preparations *in vivo*, potentiated the inhibitory response of the capsule to PGE_1. These data suggest that PGE_1- and PGE_2-induced inhibition of testicular capsular motility may be mediated by cyclic AMP. In this respect, PGE_1 has been reported to increase cyclic AMP concentrations in rat testes.[18,24] That cyclic AMP mainly inhibited capsular tonus, while theophylline inhibited both tonus and motility, may be explained by differences in intracellular cyclic AMP concentrations, although it is possible that these agents act through different mechanisms. However, the inhibitory responses of the testicular capsule to β-adrenergic agents and higher concentrations of PGE_1 and PGE_2 strongly suggest that endogenous cyclic AMP is an important modulator of rabbit testicular capsular motility.

Isolated rabbit testicular capsules responded with an increase in tonus to epinephrine.[22] Data presented in this paper indicate that epinephrine stimulated preparations *in vivo* and *in vitro* equally, and that epinephrine was effective in approximately the same concentration range as $PGF_{1\alpha}$ and $PGF_{2\alpha}$, with an efficacy equal to that of $PGF_{2\alpha}$. However, the capsule responded with a higher amplitude of contraction to epinephrine than to the other prostaglandins. The presence of stimulatory α-adrenergic receptors and inhibitory β-adrenergic receptors was suggested in isolated strips of rabbit testicular capsules.[22] Our data corroborate the existence of α- and β-adrenergic receptors in the testicular preparations *in vitro* and *in vivo*. The present data show that the increase in tonus induced by epinephrine was prevented by pretreatment with ergotamine tartrate *in vivo* (an α-adrenergic blocking agent), but not by propranolol (a β-adrenergic blocking agent). However, propranolol effectively prevented the β-agonist, isoproterenol, from inhibiting capsular contractions. Pretreatment of rabbit testicular capsules *in vitro* with ergotamine tartrate eliminated the capsule's response to epinephrine, but did not prohibit the capsule's response to prostaglandins.[23]

Inhibition of tonus in spontaneously contracting testicular capsules *in vivo* by ergotamine tartrate suggested an influence of sympathetic innervation on these autorhythmic contractions. Histochemical data indicating the presence of autonomic innervation of the testicular capsule corroborate this conclusion.[25] Moreover, the greater lability of contractions *in vitro* as compared to the preparation *in vivo* after steroid treatment or changes of the bathing medium suggests the importance of innervation to contractions of the intact preparation.[13]

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