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Autonomic control of acid phosphatase exocrine secretion by the rat prostate*

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Summary. In vivo prostatic secretion was collected from retired breeder Sprague Dawley rats using a method for isolated perfusion of the rat prostatic urethra. Enzymatic acid phosphatase determination was performed on the collected effluent. Control acid phosphatase secretion was 24.2 ± 2.7 nm over 30 minutes. Intravenous phenylephrine 5 mg/kg stimulated a 10 fold increase in acid phosphatase secretion. The secretion seen with phenylephrine was dose dependent and could be blocked with prazosin, but not yohimbine, atropine, or propranolol. Intravenous β-adrenergic agonist isoproterenol caused no increase in the secretion of rat prostatic acid phosphatase. Intravenous administration of the cholinergic agonist pilocarpine also resulted in a dose dependent rise in acid phosphatase secretion. The stimulation seen could be blocked by atropine but not phentolamine or propranolol. The stimulation of acid phosphatase secretion seen with α_1 adrenergic or cholinergic agonists was not additive. Intravenous vasoactive intestinal peptide did not stimulate acid phosphatase secretion nor did it augment the secretion induced by α_1 adrenergic or cholinergic agonists. Release of acid phosphatase into rat prostatic exocrine secretion is under both α_1 adrenergic and cholinergic control.

Key words: Acid phosphatase – Prostate – Cholinergic agents – Vasoactive intestinal peptide – Adrenergic alpha receptor agonists – Adrenergic beta receptor agonists

Introduction

Two acid phosphatases (AP) are present within rat prostatic tissue: a lysosomal enzyme common to many other rat tissues and a secretory enzyme that is unique to the prostate [23, 24, 27]. The synthesis of the secretory AP is under androgen control and this enzyme appears in rat prostatic tissue between 28 and 35 days of age. After that age there is a rapid growth in prostatic weight and a rapid increase in the amount of tissue secretory AP; the specific activity of AP (nm/mg protein) remains relatively stable [24]. The distribution of secretory AP throughout the rat prostate is uniform [9]: there is little difference between the tissue levels in the ventral, lateral, or dorsal lobes. The uniformity of distribution allows secretory AP to be a marker in prostatic fluid for total prostatic protein [13] secretion rather than as a specific lobe marker.

The present study employed a model of in vivo isolated perfusion of the rat prostatic urethra to collect the prostatic exocrine secretion. The influence of autonomic pharmacologic agents on the secretion of AP into the prostatic urethra was then investigated.

Materials and methods

Animals

Sprague Dawley rats aged 1 year (400–450 g, Sasco, Madison, Wisconsin) were placed under nembutal anesthesia for removal of the prostate or collection of their prostatic fluid, after which they were sacrificed by nembutal overdose. Animals for collection of the prostatic fluid underwent ligation of the vasa deferentia, seminal vesicles, and coagulating glands at the level of the dorsolateral prostate. A 0.025" ID silastic tube for perfusion inflow was ligated in place at the bladder neck so as to exclude all urine from the bladder and ureters. A similar tube for perfusion effluent collection was placed at the level of the external sphincter, brought out the urethra and sutured in place. The distance between the inflow and outflow tube tips (8 mm) was held constant by a 4-0 silk suture through the tips of each tube. A Minipuls II peristaltic pump (Gilson, Middleton, Wisconsin) delivered a constant perfusion of 4 ml/h 0.15 NaCl in Tris buffer pH 7.6 to the inflow tube. Protease inhibitors EDTA (10 mM), phenylmethansulfonyl fluoride (1 mM), L-1-(tosylamido)-2-phenyl-

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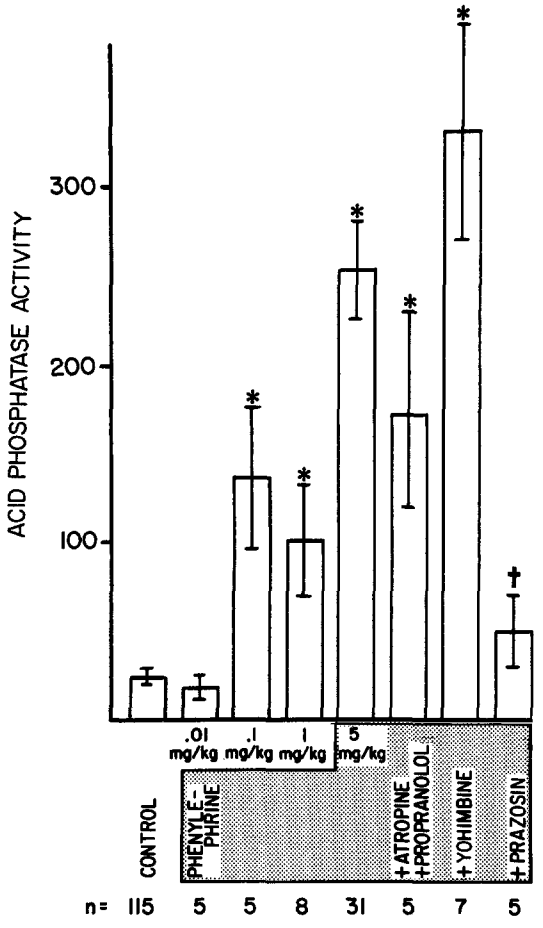

Fig. 1. Secretion of acid phosphatase in response to IV alpha adrenergic stimulation. Atropine = 0.2 mg/kg IP; propranolol = 0.2 mg/kg IP; yohimbine = 5 mg/kg IP; prazosin = 5 mg/kg IP; I = ± SEM. * $P < 0.01$ from control, + $P < 0.01$ from phenylephrine 5 mg/kg

ethyl chloromethyl ketone (0.03 mM), ethylmaleimide (0.05 mM) and soybean trypsin inhibitor (10 mg/l) were added to the perfusate. Collection of the effluent via the urethral catheter was made at 15 minute intervals. A 45 minute equilibration period was allowed before collection of a 15 minute baseline control determination for each rat. Agents were then administered and the next 30 minutes of collection following the administration of each agent was compared to 2 times the 15 minute baseline control.

Tissue homogenization

Some animals had total tissue AP determined on prostatic lobes before and after agent administration. These prostatic lobes were dissected out, weighed, and frozen to −20 °C. Tissue homogenization was then performed in 4 mls perfusion buffer with protease inhibitors using a Polytron tissue homogenizer (setting 4, 4 bursts of 10 seconds each). The samples were centrifuged at 2,000 rpm for 10 minutes and determined on the supernatant.

Drugs

Agents administered intravenously (IV) included: phenylephrine (Sigma, St. Louis, MO), isoproterenol (Winthrop-Breon, NY, NY), pilocarpine (Sigma), and vasoactive intestinal peptide (Bachem, Torrance, CA). Intraperitoneal (IP) agents administered 1 hour before IV agents were given included: atropine sulfate (Elkins-Sinn, Cherry Hill, NJ), phentolamine (Ciba, Summit, NJ), prazosin (Pfizer, NY, NY), yohimbine (Sigma) and propranolol (SoloPak, Elm Grove Village, IL).

Acid phosphatase determination

All prostatic effluent or prostatic homogenate specimens were maintained frozen −20 °C until the assay was performed. Samples were thawed and assayed as described in Sigma Bulletin No. 104. Units are expressed as nm p-nitrophenol hydrolyzed/h. Significance between groups was determined by the Student t test.

Results

Baseline secretion of AP activity into the urethral effluent over 30 minutes was 24.2 ± 2.7 SEM nm p-nitrophenyl phosphate hydrolyzed/h ($N = 115$). Alpha adrenergic stimulation via administration of 5 mg/kg IV phenylephrine to the animals resulted in a 10 fold increase in secretion of AP activity (see Fig. 1). The stimulation seen with the α-adrenergic agent was dose dependent. The concomitant administration of the β blocker propranolol and the anticholinergic atropine did not alter the secretion of AP induced by phenylephrine. This indicated that no reflex β adrenergic or cholinergic processes were involved. The α_1 blocker prazosin effectively obliterated the response to phenylephrine, but the α_2 blocker yohimbine did not, indicating that the response to IV phenylephrine was primarily through α_1 receptors in the prostate.

The administration of the β adrenergic agonist isoproterenol 0.04 mg/kg IV resulted in the secretion of 46.2 ± 11.3 SEM nm AP activity over 30 minutes, a value not significantly different from control.

The cholinergic agonist pilocarpine released AP into the rat prostatic urethra (see Fig. 2). Pilocarpine 5 mg/kg IV resulted in a 6.8 fold increase in AP secretion over 30 minutes. Higher doses of pilocarpine led to death of the animals. The stimulation seen with pilocarpine was completely blocked with atropine, but not with the α-adrenergic blocker phentolamine or the β-adrenergic blocker propranolol, indicating that no reflex adrenergic forces were responsible for the cholinergic response.

The AP secretion induced by cholinergic stimulation or α-adrenergic stimulation was not additive. Figure 2 shows that the combination of phenylephrine

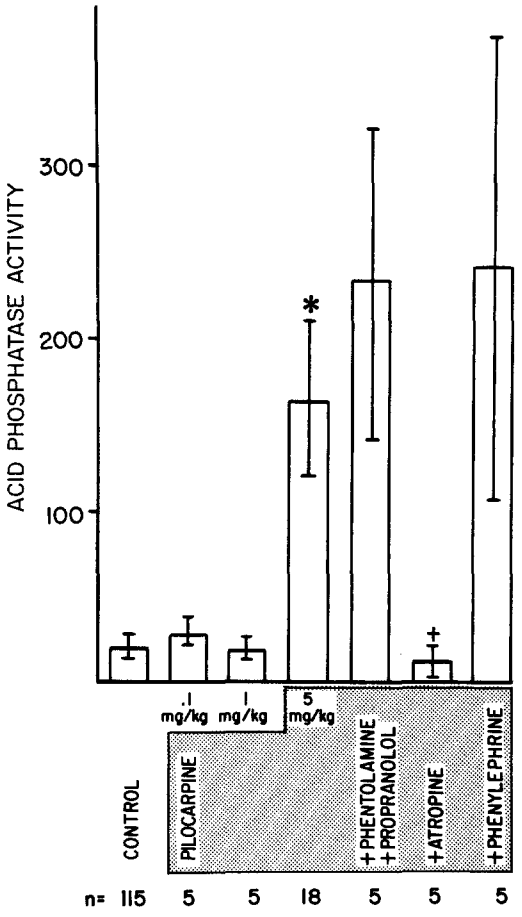

Fig. 2. Secretion of acid phosphatase in response to IV cholinergic stimulation. Phentolamine = 1 mg/kg IP; propranolol = 0.2 mg/kg IP; atropine = 0.2 mg/kg IP; phenylephrine = 5 mg/kg IP; I = ± SEM. * $P < 0.01$ from control, + $P < 0.01$ from pilocarpine 5 mg/kg IV

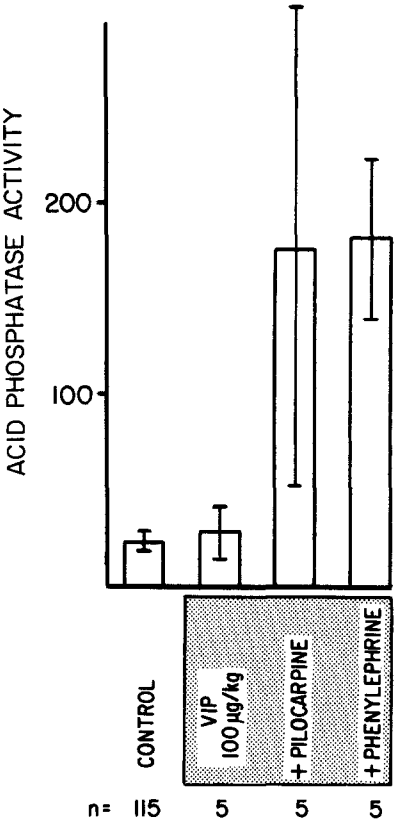

Fig. 3. Secretion of acid phosphatase in response to IV vasoactive intestinal peptide (VIP). Phenylephrine = 5 mg/kg IV; pilocarpine = 5 mg/kg IV; I = ± SEM

and pilocarpine stimulated to the same degree as phenylephrine alone.

Vasoactive intestinal peptide (VIP) 100 µg/kg IV did not stimulate secretion of AP by the rat prostate (Fig. 3). The combination of VIP and pilocarpine stimulated AP secretion to the same degree as pilocarpine alone. The combination of VIP and phenylephrine stimulated AP secretion to the same degree as phenylephrine alone. Thus, VIP did not synergistically augment AP secretion by either α-adrenergic or cholinergic agents.

The acid phosphatase content of the rat prostatic lobes was determined in 5 control rats and in 5 additional rats after 5 mg/kg IV phenylephrine. Table 1 shows the total tissue AP activity for the ventral prostate and dorsolateral complex. The distribution of AP within control prostatic tissue was uniform: the amount of AP/g prostatic tissue was the same in the ventral or dorsolateral prostate. The ventral prostates contained about twice as much AP because they weighed almost twice as much as the dorsolateral

Table 1. Tissue acid phosphatase activity of retired breeder rats

Lobe	Acid phosphatase activity	Acid phosphatase/g prostate
Ventral		
Control	14,370 ± 150	24,210 ± 1,900
Phenylephrine	14,090 ± 360	24,190 ± 1,300
Dorsolateral		
Control	7,460 ± 650	26,280 ± 1,610
Phenylephrine	8,490 ± 910	21,810 ± 1,540

prostate. Stimulation with phenylephrine did not demonstrably deplete the lobes of AP. The amount of AP secreted over 30 minutes following 5 mg/kg IV phenylephrine was 254 ± 27 nm or approximately 0.5% of the total glandular AP. This could not be detected as a reduction in whole gland AP.

Ventral prostatectomy was performed in 6 rats and their prostatic secretion collected after phenylephrine stimulation. Control secretion of AP in the ventral prostatectomy rats was 36.4 ± 12.7 SEM nm. After 5 mg/kg IV phenylephrine, the rats secreted 128.2 ± 33.4 SEM nm AP over 30 minutes. This indicated that the AP secreted into the prostatic urethra in response to phenylephrine was evenly divided between the ventral prostate and the dorsolateral prostate. Removal of the dorsolateral prostatic complex was performed but the animal preparations showed excessive urethral bleeding and the integrity of the neural and vascular supplies to the remaining ventral prostate could not be assured.

Discussion

Secretory AP's are synthesized by the rough endoplasmic reticulum of androgen dependent prostatic acinar epithelial cells [22, 25, 26] and packaged into secretory granules in the Golgi apparatus [15]. There is controversy over whether the AP's are released into the acinar lumen by merocrine secretion [8], apocrine secretion [1], or by both [14]. The whole process of synthesis, packaging, and cellular secretion takes longer than two hours [8, 11] and there is no evidence that the prostatic cell performs these steps under direct neurologic control.

Smith et al. [19–21] have shown in the dog that cellular secretion of fluid and electrolytes but not protein is under cholinergic control. With the protein AP's in the acinar and ductular lumens, an outpouring of fluid by the gland should wash the AP's down the prostatic ducts and into the urethra. Hence, the cholinergic control of AP secretion seen in our rat model may well be as a result of cholinergic control of fluid and electrolyte secretion. With such a cholinergic control, AP secretion appears in the urethra early and, then, should diminish as the fluid output becomes isotonic [13].

The prostatic periacinar and periductular smooth muscle is innervated by α_1 adrenergic fibers. Contraction of this smooth muscle propels the luminal contents which contain AP into the urethra [7]. The dual α_1 adrenergic and cholinergic control of prostatic secretion has previously been proposed for the dog [3] and the human [10].

In the rat, only Farnsworth and Lawrence [6] have previuously recorded collecting rat prostatic secretion in vivo. Their findings suggested that stimulation of prostatic fluid volume secretion was mediated primarily through an α-adrenergic pathway and could be blocked with phenoxybenzamine. Alpha-adrenergic stimulation was most effective in inducing volume secretion in their study. In the present rat study, secretion of AP activity was clearly provoked by either cholinergic or α_1 adrenergic agents and no reflex pathways were involved.

Modulation of adrenergic and cholinergic control of acid phosphatase exocrine secretion by the prostate by a number of substances will ultimately be elucidated. High on the list of probable modulators will be the prostaglandins (PG). These ubiquitous metabolites of arachidonic acid are very potent modulators of many different physiologic processes. The prostate synthesizes PG but their physiologic role is unknown. In our model PG may have a local feedback role as well as a generalized systemic feedback role in controlling exocrine secretion. PG are known to modulate noradrenergic synaptic transmission in a very complex way. For example, PGE inhibits prejunctional noradrenergic synaptic transmission but stimulates postjunctional synaptic transmission in vas deferens smooth muscle contraction. In cholinergic transmission, PGE_1 inhibits the response to acetylcholine, while PGF_2 facilitates acetylcholine release. Furthermore, PG seem to increase blood flow to exocrine organs, through the prostate has not been explored. Certainly, the role of PG in controlling prostatic secretion will be a major field of investigation.

The cholinergic stimulation of secretion by some exocrine glands is known to be synergistically augmented by VIP [17, 18]. Nerves supplying the glands contain both acetylcholine and VIP [17]. Rat prostatic epithelial cells in culture are known to contain membrane bound VIP receptors [4]. In the present study, however, no synergistic action of VIP on secretion could be demonstrated, apparently indicating no role for VIP in prostatic secretion. However, if remains possible that in the present model is not sensitive enough to detect modest synergistic effects of VIP.

The present study employed retired breeder rats that were no longer sexually active. Synthesis of AP diminishes in aged and sexually inactive rats [2, 24] and total prostatic fluid secretion decreases with age [12]. A further change with age is the increase in interstitial smooth muscle which might alter the propulsion of luminal contents into the urethra. The sensitivity the smooth muscle fibers to sympathomimetics may also increase with age [16]. Whether the neurologic control of AP secretion varies with age will need to be addressed in further studies.

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