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Regulation of Prostate Secretion in the Rat.* (30185)

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Over 30 years ago, Moore and Gallagher (1) undertook the bioassay of androgenic substances by measuring the volume of ejaculate released on electrical stimulation of control and treated guinea pigs. Farrell(2) modified the procedure of Eckhard(3) for cannulating the prostatic urethra of dogs and replaced the electrical stimulus by administration of the parasympathomimetic drug, pilocarpine. Thereupon, his group undertook an extensive pharmacological study of this reaction. Farrell concluded that "pharmacologically, the secretory innervation of the prostate is chiefly parasympathetic in nature, although its secretion is also affected to some extent by the sympathetic drugs, epinephrine and ergotamine. However, considering the physiologic fact that the pelvic nerve, or the sacral parasympathetic, does not augment secretion when stimulated as the sympathetics do, we must consider the prostate as being most analogous to the sweat glands."

Although pilocarpine is widely used in assaying anti-androgenic activity and as a means of collecting prostatic fluid for bio- and immuno-chemical studies, little more has been done to elucidate the neurological basis of the secretory process. The mechanism of ejaculation has been studied by Potts(4). No work has been done on the rat except the histo-chemical demonstration by Risley and Skrepetos(5) of cholinesterase-positive receptor, or possibly effector, cells in the prostatic urethra of this species.

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The present report presents our efforts to define the pathways of normal and pilocarpine-stimulated release of prostatic secretion.

Materials and methods. Rats weighing 300-400 g were anesthetized with intraperitoneal sodium pentobarbital, (50 mg/kg, initially, then 5-10 mg/kg as needed). In lieu of an airway, each animal was tracheotomized.

Using an inverted Y abdominal incision, the genitourinary system was exposed. The ductus deferens, coagulating gland, and seminal vesicle on each side were ligated at their junctions with the urethra. The pubic bones were separated to expose the urethra. After the body of the bladder was opened and laid back against a saline-soaked sponge to collect urine, a 6-9-in. piece of PE-90 or PE-100 polyethylene tubing was inserted through the neck of the opened bladder to a point in the urethra proximal to the openings of the prostatic ducts. A second piece of tubing was inserted through a perforation in the urethra to a point distal to the prostatic ducts. Both tubes were secured in place by ligatures tied around the tube-containing parts of the urethra. The character, rate and volume of flow of secretion in these tubes could be observed readily. To determine rate of flow, the leading edge of the column of fluid was marked at known times by tying colored threads around the tubing. Then, following each experiment, flow rates were determined by plotting the measured advances of fluid against time. Knowing the internal diameter of the tubing to be 0.86 mm it was possible to convert the linear advances of the fluid into volumes, since 1 cm of tubing contains 5.7 mm^3. Slow advances of retrograde secre-

TABLE I. Retrograde Secretion of Prostatic Fluid.

Blocking agent	Days	Total dose, mg/kg	No. animals	Rate of secretory flow mm/hr*	% Control
None	–	—	45	6.5 ± .5	100.0
Atropine	–	.3– .5	6	2.8 ± .9	43.0
Dibenzyline	–	12 –15	4	3.1 ± .8	48.3
"	–	21 –26	2	1.7 ± .6	26.3
"	–	37 –40	3	1.1 ± –	17.5
Reserpine	2	.5– 3.0	5	1.8 ± .3	28.1
"	2	3.5– 5.5	6	1.6 ± .5	24.6
"	2	7.5–10.0	7	.9 ± .5	14.0
"	1	.5– 3.0	3	4.4 ± .2	67.5
"	1	3.5– 5.5	4	3.1 ± .1	48.3
"	1	6.0– 7.0	4	1.7 ± .5	26.3

* Mean ± S.E.

tion are reported in mm^3/hr, more rapid antegrade flow in mm^3/17 min.

All intravenous injections were made into the left renal vein; doses were adjusted to body weight. Pilocarpine (1 mg/ml), bretylium (5 mg/ml or 0.4 mg/ml), epinephrine (1:10,000), and norepinephrine (200 μg/ml) were administered in normal saline; dibenzyline (0.5 mg/ml) was given in 10% alcoholic saline. Reserpine (2 mg/ml) in saline, was given intraperitoneally.

Results. Tonic retrograde secretion. Prostatic secretion is not a discontinuous process occurring only at times of sexual or artificial stimulation. In the rat, there is continuous retrograde flow into the bladder, the rate in any animal being very constant and unaffected by the level of anesthesia except at nearly lethal levels. In animals ranging in weight from 300 to 400 g and with prostates of variable size, there was no obvious correlation between flow rate and either body weight or gland size. It was thus possible to establish the control rate for each rat during an initial 1-2-hour period preceding administration of drugs (Table I). In contrast to retrograde secretion, antegrade secretion was never observed in the absence of exogenous stimulation.

To determine the nature of the tonic stimulus, various blocking agents were employed. Atropine (0.3-0.5 mg/kg) produced a 60% decrease in the flow over a 2.5 hour period (Table I). Dibenzyline (7.5-44.0 mg/kg) caused an immediate 80% fall in secretion which lasted for at least 2 hours (Table I). Pretreatment of animals for 1-3 days with reserpine also produced a marked depression of secretion to about zero at higher dose levels (Table I).

Administration of epinephrine or norepinephrine to the rat produced an immediate spurt of prostatic secretion which was greatest during the first 5 minutes after injection and continued at a diminishing rate for 10-15 minutes. Unlike the clear product of tonic stimulation, this was opalescent. It was delivered, not into the bladder, but into the distal tube (antegrade).

In the absence of a blocking agent, epinephrine (67 μg/kg) evoked as much as 91.2 mm^3 flow in surving animals (Table II). Most of this group died with tachycardia and pulmonary edema. Following death, rapid antegrade flow continued, accompanied by an equal rate of flow toward the bladder. The lethal effect of epinephrine was completely eliminated by treatment with dibenzyline 2 hours before the catecholamine injection. Antegrade secretion was largely or completely blocked by doses of 15-42 mg/kg of this andrenolytic agent (Table II).

Norepinephrine (120-150 μg/kg) was well tolerated but far less effective than epinephrine in stimulating antegrade flow (Table II). Both reserpine and especially bretylium (Table II), at the higher doses used in this study, potentiated the effect of norepinephrine on prostatic antegrade secretion.

Cholinergic stimulation (pilocarpine). Within one minute after injection of pilocarpine, there was secretion of clear prostatic fluid into the prostatic urethra (Table II). Flow was at its maximum for one minute and ceased after 5 minutes. Following this, the gland was refractory to pilocarpine for the remainder of the test period, although still responsive to epinephrine or norepinephrine. Maximum response was elicited by 1.8 mg/kg. The pilocarpine effect, both on prostatic secretion and salivation, was completely blocked by atropine (0.3-0.5 mg/kg) within 15 minutes and up to 2.5 hours (Table II) without in any degree affecting sensitivity of the prostate to epinephrine or norepinephrine.

TABLE II. Antegrade Secretion of Prostatic Fluid.

Stimulus	Dose/day	Block	Treatment period, days	Total dose, mg/kg	No. rats	Rate of secretory flow mm³/17 min*	Control
None	—	—	—	—	45	0	
Pilocarpine	1.8 mg/kg	0		—	4	10.3 ± 1.1	100.0
"	1.8 "	Dibenzyline		15 –30	4	1.8 ± .5	17.8
"	1.8 "	"		39 –42	3	0	0
"	1.8 "	Reserpine	1	.5– 4	5	10.8 ± .9	105.5
"	1.8 "	"	1	4.9– 6.6	4	3.4 ± 2.1	33.4
"	1.8 "	"	3	.5– 3.0	6	7.4 ± 2.3	72.2
"	1.8 "	Bretylium	—	5.4	7	5.7 ± 1.5	55.5
"	1.8 "	"	—	12.5	10	7.4 ± 1.6	72.2
"	1.8 "	Atropine	—	.3– .5	6	0	
Epinephrine	67 μg/kg	0	—	—	5	91.2†	
"	134 "	0	—	—	2	‡	
"	134 "	Dibenzyline	—	15 –30	5	1.5 ± .9	
"	134 "	"	—	39 –42	3	0	
Norepinephrine	120–150 "	0	—	—	5	14.8 ± 2.8	100.0
"	120–150 "	Reserpine	1–3	.5– 2.0	5	18.3 ± 3.9	123.1
"	120–150 "	"	1–3	3 – 6	5	26.2 ± 4.2	177.0
"	120–150 "	Bretylium		5.4	7	19.1 ± 2.4	128.8
"	120–150 "	"	—	12.5	3	42.8 ± 4.6	288.5

* Mean ± S.E. † All but 2 expired. ‡ All expired.

Mediation of the pilocarpine response by catecholamines. To determine if pilocarpine acts directly upon the prostatic acini or depends upon mediation by catecholamines, antiadrenergic agents were tested. Dibenzyline (15-42 mg/kg) blocked not only endogenous and exogenous epinephrine and norepinephrine but also the response to pilocarpine (Table II). Salivation was unaffected.

With reserpine, the pilocarpine effect was also depressed (Table II). Effectiveness of reserpine was dependent both on dose and duration of administration.

Within 5 minutes after bretylium injection, there was a severe fall of response to pilocarpine (Table II). This inhibition was brief, waning within 15-20 minutes.

Discussion. It appears from these studies that the mechanisms controlling prostatic secretion in the rat are very similar to those in the dog. There is tonic stimulation by atropine-insensitive elements of a slow but constant liberation of fluid into the bladder. Farrell(2) found this spontaneous flow in the dog and saw that it could be increased by stimulation of the hypogastric nerve. He noted that the effect of hypogastric stimulation was blocked by both atropine and ergotamine, although the latter did not depress spontaneous flow.

In the rat, exogenous stimulation of the prostate with either catecholamines or pilocarpine produces an increased flow of secretion which is directed into the prostatic urethra rather than the bladder. There are distinct differences in the effects of the two types of stimuli: Epinephrine and norepinephrine evoke an output of relatively long duration. The effect is suggestive of a direct response to the substance administered which diminishes as the drug is either locally consumed or systemically inactivated. Secretory flow can be sustained or reinitiated by successive treatments with these compounds. In contrast, the response to pilocarpine resembles the "cholinergic trigger" postulated by Burn and Rand(6) for release of adrenergic substances in the post-ganglionic sympathetic fibers or their termini. The effect is transitory and is followed by a long refractory period.

From the finding that both tonic and pilocarpine-stimulated secretion is blocked by atropine or dibenzyline and that dibenzyline, but not atropine, interferes with response to the catecholamines, it appears that both the flow seen in the absence of exogenous stimuli and the antegrade output produced by pilocarpine are mediated by catecholamines. It is evident, too, that tonic stimulation is pro-

vided by cholinergic (muscarinic) elements. Reserpine and bretylium were used to further test these conclusions.

McCoubrey(7), and also Potter and Axelrod(8) suggested that bretylium keeps the norepinephrine in the cells where it is made and/or bound. They also suggested that it inhibits the uptake of catecholamines from the circulation (from the adrenal as well as exogenous sources). On the other hand, Gokhale *et al*(9) said that bretylium releases catecholamines from their site of production or binding. Either mechanism would raise the circulating level available to the receptor but would by-pass the effect of cholinergic stimuli and, in effect, suppress the action of such stimuli. Consequently, pilocarpine could not act as well, but exogenous catecholamines, normally taken up and bound by cells responsive to pilocarpine, would be left free to act upon effector cells. In addition, according to Gokhale *et al*(9), the stores of catecholamines are released in active form to act on the receptors. This second phenomenon, if true, is in contrast to the effect of reserpine. The latter drug also depletes catecholamines but by a mechanism that permits destruction of the compounds by the action of monoamine oxidase.

The observed effects of reserpine and of bretylium are wholly compatible with the foregoing conclusions. Depletion of adrenergic substances with reserpine lowers the secretory response both to tonic stimulation and to exogenous cholinergic (pilocarpine) agents. Bretylium also interferes with the acceleration of antegrade flow by pilocarpine. Finally, both drugs potentiate rather than depress the action of administered norepinephrine.

It is concluded that the catecholamines, probably norepinephrine, are released by tonic or exogenous cholinergic stimuli to directly activate the liberation of secretory fluid by the rat prostate.

Summary. Employing rats cannulated to permit measurement of retro- and antegrade prostatic secretion, an attempt was made to determine the nature of secretory control (stimulation). A steady, tonically-stimulated retrograde flow was noted which could be blocked by atropine and the antiadrenergic drugs, dibenzyline and reserpine. Prolonged antegrade secretion, evoked by either epinephrine or norepinephrine, was suppressed by dibenzyline and potentiated by pretreatment with reserpine or bretylium. A definite but transient, atropine-sensitive antegrade response to pilocarpine was followed by a prolonged refractory period to the parasympathomimetic agent. During this time the gland was readily stimulated by catecholamines. It was found that dibenzyline, reserpine, and, to a limited extent at the doses employed, bretylium suppressed the action of pilocarpine. These findingss are interpreted as evidence that catecholamines mediate the pilocarpine effect on prostatic secretion.

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