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Endocrinologic Basis of Electrochemical Vaginal Potential in Estrous Cycle of Rats

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ABSTRACT

Bruzzone ME, Angelo S, Zipper J, Wheeler RG (Dept of Biophysics and Physiology, School of Medicine, University of Chile, Santiago, Chile, and the International Fertility Research Program, Research Triangle Park, NC, USA). Endocrinologic basis of electrochemical vaginal potential in estrous cycle of rats.

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This sequel to an earlier report on the correlation of vagino-oral voltage measurements in rats with their estrous cycles deals with the endocrinologic activation of these voltages. The normally sinusoidal voltages, positive during estrus and negative during diestrus, were absent in spayed rats but were restored with a single intraperitoneal injection of 5 μg or more of 17β-estradiol or with 0.1 mg of adrenaline injected subcutaneously every 12 hours. Estrogen antagonists, drugs that inhibit catecholamine synthesis or adrenergic β blockers, totally inhibit the negative potential recorded during estrus. Vaginal estrous potentials in the rat reflect an important reproductive state possibly linked to sexual behavior (heat) or to ovulation. Triggering of the cyclic potential by adrenaline without estrogenic medication suggests that adrenaline belongs to the group of sexual-specific hormones.

INTRODUCTION

The variations of electropotentials associated with the reproductive functions have been studied since the initial works of Burr in 1935 (2, 2a, 3). No cyclic variations in any of the species studied (4, 7) were reported, however, until the work of Zipper and Angelo (12), who measured vagino-oral potentials in rats. Using KCl electrodes, Zipper and Angelo observed negative vaginal potentials during estrus that became positive during diestrus. In spayed animals, the positive potential became permanent. This potential is also positive throughout the gestational period in rats, becoming negative the first day after delivery.

These observations led us to study the possibility of an endocrinologic origin of these cyclic estrous potentials. The most important hormones offering cyclic variations in concentration during the estrous cycle in the genital tract of the rat are 17β-estradiol, progesterone and epinephrine (10). Other hormones and some inhibiting agents of the action of catecholamines were also studied.

MATERIALS AND METHODS

Female rats of the Sprague-Dawley strain were used, weighing 200 gm–250 gm. The rats were spayed 15 days before potentials were measured. Measurement of voltages was performed under tribromethanol anesthesia (Avertin, Winthrop Laboratories, New York, NY, USA). The ability to consistently measure changes in potential with the estrous cycle indicates that the anesthetic does not obscure potentials.

Nonpolarizable electrodes were made of three molar KCl in 2% agar, inside polyethylene tubes with an inner diameter of 0.85 mm and an outer diameter of 1.25 mm. This KCl electrode was connected through a 3 M KCl solution to a silver wire anodized in HCl. New electrodes were prepared for each measurement, and the system was checked for polarization in an NaCl solution each time before use.

Voltage measurements were obtained with a dual beam Tektronix 502 oscilloscope. The positive electrode was placed in the vagina and the negative one on the tongue (vagino-oral potentials). Twenty groups of 4–8 animals were formed. Voltage measurements were performed daily at 10 a.m. The hormones and chemicals used were bought at the Sigma Laboratories. The drug regimens used on 20

experimental groups of animals are shown in Table I.

RESULTS

On each of the voltage-versus-time graphs (Figs. 1–6), the average standard deviation of voltage for the plotted points is indicated by the length of the vertical bar on the graph. The graphs of vagino-oral potentials recorded from spayed animals taken for four or more consecutive days indicate that the one injection of more than 5 μg of 17β-estradiol, as well as 0.2 mg of adrenaline injected daily, reproduces the normal (–) curve of the estrous potential (Figs.

Table I. Drug regimen used on 20 groups of 4–8 animals.

Drug Treatment	Experiment No. 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
	1*			2*			3*		4*					5*		6*				
17β-estradiol																				
• 5 μg (one intraperitoneal injection)	X																			
• 10 μg (one intraperitoneal injection)		X					X	X	X	X	X	X			X		X		X	
Testosterone																				
• 1 mg (one intraperitoneal injection)			X						X	X				X						
Adrenaline																				
• 0.2 mg (two subcutaneous injections of 0.1 mg every 12 hours)				X			X			X										
• 0.2 mg/day (one subcutaneous injection of 0.1 mg every 12 hours)												X	X	X		X		X		
• 0.2 mg/day (one subcutaneous injection of 0.1 mg every 12 hours for eight days																				X
Progesterone																				
• 1 mg (one intraperitoneal injection)					X			X												
Noradrenaline																				
• 0.001 mg (one subcutaneous injection daily)						X														
Reserpine																				
• 1 mg daily (two intraperitoneal injections for two days)												X	X	X						
Propranolol																				
• 10 mg/kg/day (one intraperitoneal injection daily)															X	X				
• 10 mg/kg/day (one intraperitoneal injection daily) 96 hours before initiation of other treatment																	X	X	X	

*Figure no

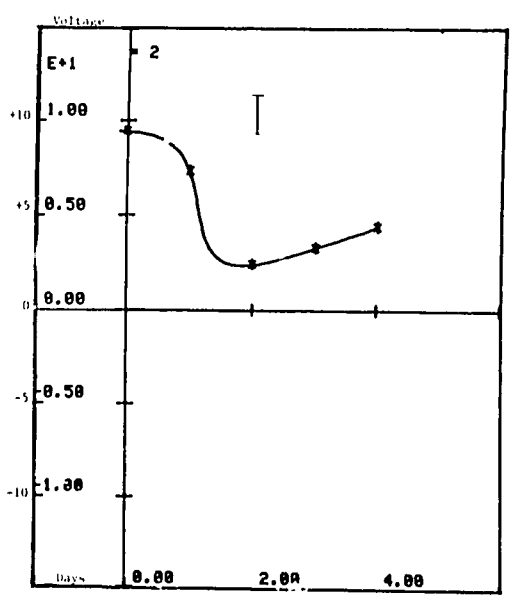

Fig. 1. Vagino-oral potentials with a trial of 17β-estradiol (5 μg, one intraperitoneal injection).

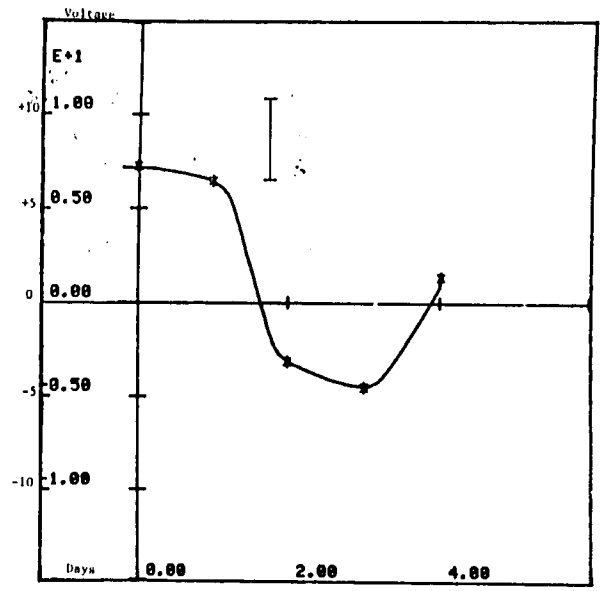

Fig. 2. Vagino-oral potentials with a trial of adrenaline (0.2 mg, two subcutaneous injections [0.1-mg injection every 12 hours]).

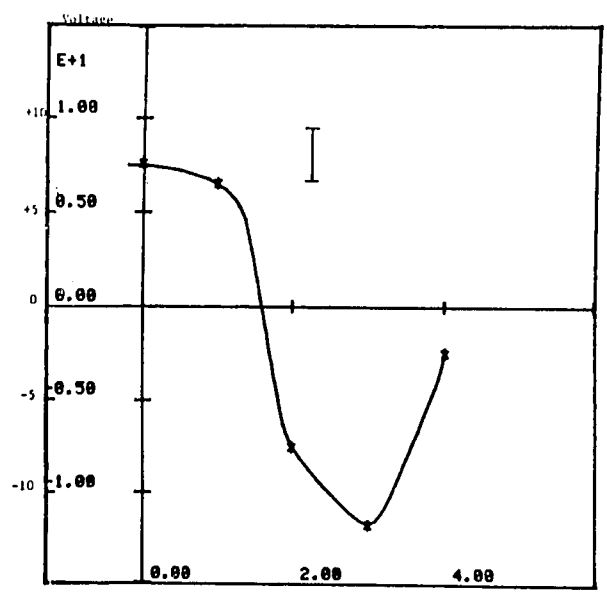

Fig. 3. Vagino-oral potentials with a trial of 17β-estradiol (10 μg, one intraperitoneal injection); adrenaline (0.2 mg/day, two subcutaneous injections every 12 hours).

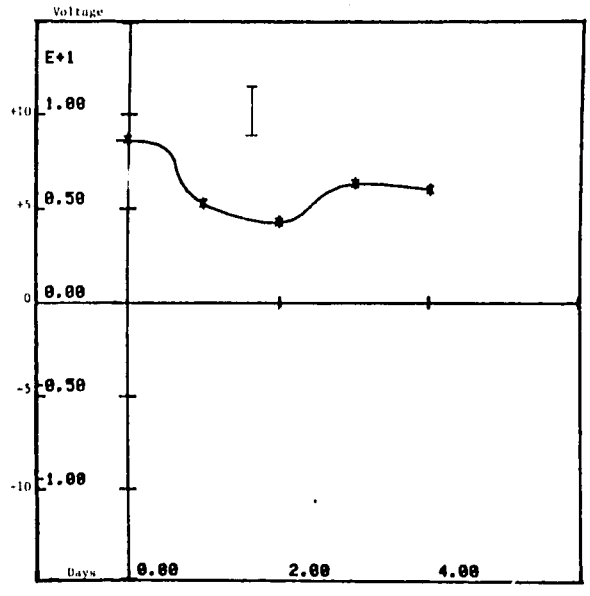

Fig. 4. Vagino-oral potentials with a trial of 17β-estradiol (10 μg, one intraperitoneal injection) and testosterone (1 mg, one intraperitoneal injection).

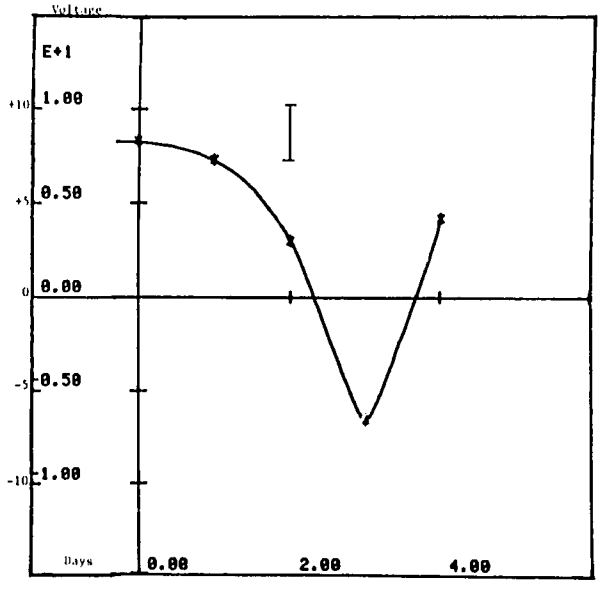

Fig. 5. Vagino-oral potentials with a trial of testosterone (1 mg, one intraperitoneal injection) and adrenaline (0.2 μg/day, one 0.1-mg injection every 12 hours).

1–2). The association of both drugs potentiates the detected voltages (Fig. 3). On the other hand, estrogen antagonists, drugs that inhibit the catecholamine synthesis or adrenergic β blockers, totally inhibit the negative potential recorded during estrus.

Testosterone blocks the action of estrogens but not that of adrenaline (Figs. 4–5).

Propranolol scarcely blocks the action of estrogens but markedly blocks that of adrenaline (Fig. 6).

DISCUSSION

The results described demonstrate that epinephrine (adrenaline) plays a determining role in the generation of vagino-oral cyclic potentials in the rat and acts as an intermediate link in the chain of events originated by 17β-estradiol. We find that adrenergic inhibitors block this potential and also other periferic effects of 17β-estradiol as has been described (1, 5, 6). We know from the initial works of Wurtman et al (10, 11) that uterine epinephrine is subject to fluctuations with the estrous cycle and *l*-norepinephrine is not. No other organ, including the heart, behaves in regard to epinephrine as does the uterus (8, 9). Uterine epinephrine binding is estrogen-dependent. To obtain epinephrine in other organs such as the spleen, heart and adrenal glands, a tenfold higher dose of 17β-estradiol is required.

The classic inhibition of myometrial activity induced by adrenaline (β adrenergic effect) is counteracted by propranolol (5). The principal uterine catecholamine is epinephrine. The uterus lacks phenylethanolamine-*N*-methyl transferase, so this tissue does not synthesize this amine. Even adrenalectomy does not prevent estradiol from increasing uterine epinephrine, which must originate in the

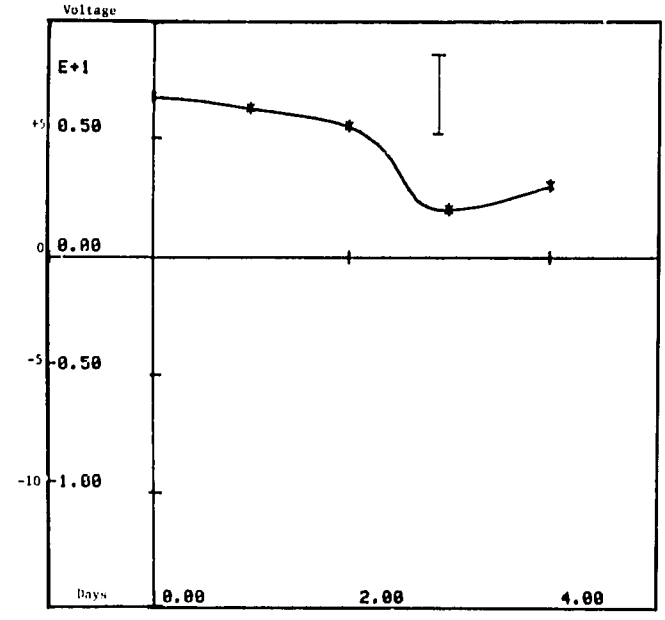

Fig. 6. Vaginal-oral potentials with a trial of adrenaline (0.2 mg/day, one subcutaneous 0.1-mg injection every 12 hours) and propranolol (10 mg/kg/day, one intraperitoneal injection daily).

chromafine cells found in different regions of the organism. During pregnancy, the concentration of epinephrine in relation to uterine weight decreases (10). This agrees with the vaginal potentials (+) recorded during this reproductive state.

The blocking of these cyclic potentials with chemicals that block the catecholamine synthesis, such as reserpine, or that counteract the action of estrogens, such as testosterone, or through adrenergic β blockers, such as propranolol, leads us to believe that adrenaline is the hormone that generates these potentials. We can also cite the fact that adrenaline activates uterine adenylcyclase and that some other effects of the adenosine monophosphate liberated through estrogens (6) are inhibited by propranolol, suggesting that there is an adrenergic mediation in the estrogenic action (11).

Vaginal estrous potentials in the rat reflect an important reproductive state possibly linked to sexual behavior (heat) or to ovulation. The fact that this potential is triggered by adrenaline without estrogenic mediation suggests that adrenaline belongs to the group of sexual-specific hormones.

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