Influence of Adrenal Steroids on Self-Stimulation Rates in Rats.* (30606)

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In behavior experiments where electrical stimulation is a source of positive reinforcement, activation of fiber systems associated with this phenomenon has been proposed to inhibit pituitary-adrenal response to stress (1,2). The lateral hypothalamus has been variably reported as a site of positive reinforcement for self-stimulation in rats. The hypothalamus has also been implicated in facilitatory and inhibitory mechanisms related to regulation of ACTH release. Thus, it appeared of interest to the investigator to determine whether any correlation existed between regulatory mechanisms for ACTH release and maintenance of stabilized self-stimulation rates in rats. The present study was designed to produce inhibition of acute release of ACTH by a single injection of corticosteroids and to produce chronic facilitation of ACTH release by bilateral adrenalectomy in rats. Effects of such alteration of ACTH release were assessed on animals self-stimulating for positive reinforcement.

Materials and methods. In 38 adult male Sprague-Dawley rats, bipolar electrodes† were stereotaxically aimed for unilateral placement in the lateral hypothalamic area. Electrodes had been previously soldered to a 2-prong Winchester plug which following implantation was attached to the skull with acrylic cement. All rats were maintained throughout the control and experimental period in a temperature regulated, light-controlled room and permitted Purina lab chow and water *ad libitum* except where noted below. Body weights were recorded 2-3 times a week and all rats handled and adapted to attachment of a plug-cable assembly and placement in a box(3) for a 3-5-day pre-experimental period. On the first day, rats were introduced to the procedure of self-stimulation by placement on the lever. Stimulation was effective only upon release of the lever and with repress.‡ All responses were recorded on a Gerbrands cumulative recorder. Stimuli were delivered at 60/second for a 0.25-0.5 second period, at voltages required to deliver 5-100 μA.§ Current levels were monitored on a calibrated oscilloscope. During the acquisition stage of 2 days, current levels were increased at 5-minute intervals in increments of 5-10 μA over a 2-hour period until rates exceeded 1000/hour. No attempt was made to determine the optimal current level. When rates exceeded 1000/hour rats were placed daily in the box for 10 minutes, and counts were determined for a consecutive 5-minute period. Control rates were recorded for 2 weeks prior to experimental testing. Parameters obtained during acquisition were maintained throughout the control period; any rat whose rate varied more than ± 200/hour was discarded from the series.

A total of 20 of 38 rats met the above criteria and were divided into 2 groups. For determination of effects of acute inhibition of ACTH release on self-stimulation rates, a 5-minute control record for 15 rats was taken in the a.m. of the experimental day. Rats were immediately injected subcutaneously with saline, hydrocortisone sodium succinate (100 μg/100 g), or dexamethasone 21-phosphate (10-100 μg/100 g) in a total 0.5 ml volume. Subsequent stimulation rates were recorded 2, 4, 6 and 24 hours post-injection. For assessment of effects of chronic facilitation of ACTH release, 5 rats were bilaterally

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† Stainless steel, 0.0063 inch diam. with interdistance of 0.5-0.7 mm, insulated to tips.

‡ Time delay switch was incorporated in circuit which cut current at predetermined level.

§ Stimuli were delivered through a set sine wave source consisting of a 6.3 V step-down transformer and a Variac to adjust amplitude of output voltage. Stimulator was constructed from design by P. J. Morgane (personal communication).

TABLE I. Alteration in Stabilized Self-Stimulation Rates in Rats After Acute Subcutaneous Injection of Hydrocortisone or Dexamethasone.

Rat No.	Site* stimulated	Stimulating current μA	Stimulating current Duration (sec)	Injection Compound	Injection μg/100 g BW	Self-stimulation rate/hr Rate pre-	Rate change post-inj (hr) 2	4	6	24
458	MFB-Fx	50	.25	saline	—	2570	— 190	+ 120	— 50	+ 130
474	LHA	70	.50	"	—	2320	+ 80	+ 60	— 160	+ 180
475	LHA-MFB	50	.50	"	—	3620	+ 90	— 100	+ 150	— 20
425	LHA-ZI	50	.50	dexametht	10	2520	+1220§	+1400	+ 82	+1120
411	LHA-MFB	38	.25	"	20	3362	— 130	+ 80	+ 420	+ 800
421	LHA-MFB	48	.50	"	20	2560	+ 420	+ 240	——	+1070
442	LHA	56	.25	"	100	2480	+1410	+ 160	+ 710	+ 970
477	MFB	58	.50	"	100	2530	+ 270	+ 200	+ 460	+ 280
423	C. Ped.	18	.25	"	10	2840	— 950	— 860	— 300	— 130
424	C. Ped.	16	.25	"	50	2500	— 790	— 150	— 926	— 440
408	C. Ped.	58	.50	"	100	3120	+ 540	+ 80	+1760	conv.‖
405	LHA	25	.25	hydrocort‡	100	1740	— 20	+ 276	— 130	— 280
406	MFB	52	.25	"	100	3330	— 102	+ 410	+ 370	+ 590
478	LHA-MFB	68	.25	"	100	1520	+ 118	+ 330	——	— 360
463	LHA-Fx	98	.50	"	100	2720	+ 140	+ 520	+ 740	+ 620

* MFB = median forebrain bundle; Fx = fornix; LHA = lateral hypothalamic area; ZI = zona incerta; C. Ped. = cerebral peduncle.
† Dexamethasone 21-phosphate.
‡ Hydrocortisone sodium succinate.
§ The underlined figures increased (+) or decreased (—) more than 190/hr in self-stimulation rates.
‖ Convulsions.

adrenalectomized under ether by the dorsal approach, and subsequently maintained on 0.9% saline drinking water. Stimulation rates were recorded 2 days post-surgery and at frequent intervals during each week of a total 2-month post-operative period. At the conclusion of all experiments, rats were administered pentobarbital, and microlesions were made at the electrode tips(4). Localization of stimulated sites was verified histologically.

Results. In Table I data are presented for effects of acute injection of steroids on self-stimulation rates. In 3 control rats, injected with saline, rates did not vary ± 190 for any of the 4 recorded periods post-injection. Dexamethasone, regardless of dose level, altered rates in 7 of 8 rats 2 hours post-injection, and in all 8 rats 6 hours post-injection. Rates were significantly increased in 5 rats with stimulating electrodes in the lateral hypothalamic area (LHA) and/or median forebrain bundle (MFB) following dexamethasone; rates were significantly decreased in 2 of 3 rats with electrodes in the cerebral peduncle. Following hydrocortisone injection, increased rates were observed only 4 or more hours following injection in all 4 rats.

Data on effects of bilateral adrenalectomy on self-stimulation rates are noted in Fig. 1. For purposes of simplicity, all data accumulated for each week have been averaged and graphed. In 4 rats with stimulating electrodes in the LHA and/or MFB, stimulation rates increased by the 5th week post-surgery. In these rats impedance was increased 2 days post-operation, requiring an increase of voltage amplitude to maintain current at the acquisition level. Body weights of all rats increased 50-81 g during an 8-week period with the exception of rat No. 488 in which a gain of 135 g was noted. This rat exhibited the highest increase in self-stimulation rates during the 5-8 week period. In 1 rat, with stimulating electrode in the cerebral peduncle, rates were decreased 1 week post-surgery; further decrements were recorded for the total 8-week period. This rat showed consistent convulsive behavior following 4-5 bar presses per session even with cur-

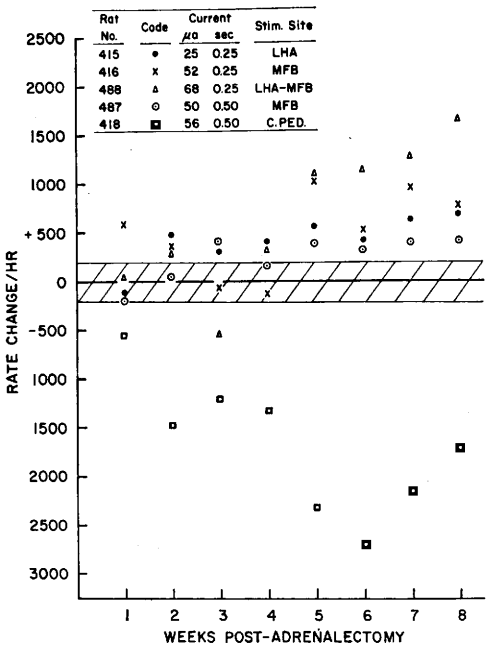

FIG. 1. Change in self-stimulation rates after bilateral adrenalectomy. Average rates per week are plotted. Barred area represents range of rate changes for all rats during the 2-week pre-adrenalectomy period. Abbreviations as in Table I. Values for current were constant through pre- and post-adrenalectomy period for all rats except No. 418; *cf.* Text.

rent decreased to 50% that established for the acquisition level. Behavior in this rat was similar to that of rat No. 408 in the acute steroid injection series with similar electrode placement (Table 1).

Discussion. Following acute injection of either hydrocortisone or dexamethasone and independent of the amount injected (dexamethasone), self-stimulation rates were increased 2-6 hours later in all rats with electrodes in the LHA/MFB region. In contrast, in 2 of 3 rats with electrodes in the cerebral peduncle decreased rates were noted; the third rat exhibited increased rates for the second and sixth hour post-injection; convulsions by 24 hours. In the chronic series, increased rates were recorded 5 weeks post-adrenalectomy in rats with stimulating electrodes in the LHA/MFB region. In 1 rat with electrode in the cerebral peduncle, rates decreased 1 week post-surgery, increased impedence was noted and convulsive behavior was consequent on brief stimulation. In the acute and chronic series for the 2 rats showing convulsive behavior, current was delivered over a 0.5 second interval. Olds(2) has suggested seizures may be more directly related to time over which current is delivered than level of current *per se.*

The conditioned-avoidance response in rats is unaffected by adrenalectomy(5,6,7). In the present study, the adrenal glands *per se* are also unessential for behavior associated with self-stimulation for positive reinforcement; rates were increased in chronic bilaterally adrenalectomized rats with electrodes in the LHA/MFB region. Although maintenance of self-stimulation rates was not determined for intact rats for a 2-month period, Olds(8) has reported consistency in rates for periods of several months.

Both hydrocortisone and dexamethasone are known to inhibit release of ACTH. The relative efficacy of the 2 steroids for such inhibition in conditions of stress is a reported ratio of 1:2, respectively, *i.e.,* dexamethasone is twice as active as hydrocortisone(9). The present data pertinent to the effects of acute administration of steroids on self-stimulation rates further indicate the greater effectiveness of dexamethasone. Thus following injection of this hormone, rates were altered earlier (2 hours)and changes were of greater magnitude than those observed following hydrocortisone injection. Acute steroid effects on inhibition of ACTH release are reported maximal at 4-8 hours post-injection(10), with recovery from inhibitory influences by 24 hours. In the present study, persistence of both steroid effects on self-stimulation rates continued through the 24-hour post-injection period. Differences in effects of corticosteroids on the inhibition of ACTH release and alteration in self-stimulation rates suggest that, for the latter phenomenon, systems may be involved other than, or in addition to, those directly related to the regulation of ACTH release.

The present experiments may be interpreted as evidence for a relationship between fiber systems for regulation of ACTH release and those involved in self-stimulation behavior for positive reinforcement. It is conceivable that activation or inhibition of either

system may induce a shift in autonomic predominance resulting in a decreased or increased threshold in the other. Such a thesis would be in conformity with that proposed by Gellhorn(11,12). It should be emphasized, however, that alteration in self-stimulation rates as effected by steroids may be a result of effects which are independent of those involved in the regulation of ACTH release. Indeed, the effects of corticosteroids on changes in brain excitability, behavior, and numerous physiological phenomena have been amply reviewed by Woodbury(13).

Summary. Self-stimulation rates in rats were altered 2 to 24 hours after a single subcutaneous injection of steroid in doses reported to inhibit acute release of ACTH. Dexamethasone exerted more profound effects than hydrocortisone. Self-stimulation rates were consistently increased in rats stimulating to electrodes in the LHA and/or MFB. Bilateral adrenalectomy produced increased rates of self-stimulation by the 5th postoperative week.

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Effect of Different Steroids on Lactating Rats.* (30607)

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We have recently reported(1) the effect of different steroids on prolactin secretion in organ co-culture. We found that estradiol and hydrocortisone stimulate prolactin release in doses of 1 and 10 μg/cc and were depressive at doses of 10 and 100 μg/cc while progesterone and its derivatives in doses higher than 1 μg/cc depress prolactin release. Nicoll and Meites(2) also observed an increase in prolactin production by rat pituitary cultured *in vitro* when estrogen was added to the medium.

There is no doubt that prolactin is necessary for milk secretion and maintenance(3,4), but estrogen, which promotes lactation when used at low doses for priming, was found to be a potent inhibitor of milk production in many species, including the rat, when the optimal dosage was surpassed(5,6).

Since various doses of estradiol showed a different influence on prolactin production by rat pituitary *in vitro,* we set out to test whether different doses of estradiol and other steroids act similarly on milk yield in lactating rats.

Materials and methods. Lactating rats of the "Sabra" Hebrew University strain, weighing 250 ± 20 g, each nursing 6 litter mates, were kept in individual cages and received Purina Lab Chow and water *ad libitum.*

Daily weights of dams and litters were recorded from day 7 to day 20 postpartum. Beginning on the 7th day, s.c. injections of different steroids dissolved in disacidified olive oil were given daily until the 20th day. With one dose of estradiol, (0.01 mg/kg/d) injec-

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