Spinal Cord and Adrenocorticotropin Release.* (26165)

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Earlier investigations have provided evidence that adrenocorticotropin (ACTH) release is under control of one or more chemical mediators delivered to adenohypophyseal tissue *via* the hypophyseal portal venous system. Assuming this to be the case, the location of site or sites of origin and the mechanism controlling release of these chemical mediators, often referred to as corticotropin releasing factors (CRFs), becomes important. The hypothalamus, and in particular the median eminence, has been proposed by several workers as the site of release. However, sites of origin of CRFs located more peripherally have also been proposed. These proposals have been made on the basis of experimentally induced increases in ACTH release following stimulation of denervated structures. To account for the increase, blood borne chemical mediators such as epinephrine, or products of tissue destruction, are considered to be carried from a peripheral site to the pituitary *via* systemic circulation(1,2,3, 4,5).

The apparently conflicting experimental results, methodological shortcomings and deficiency of adequate controls of previous experiments in cord-sectioned animals prompted a reinvestigation of the problem. The observations reported here offer no support for a peripherally elaborated CRF since application of stimuli below the level of cord section failed to increase rate of release of ACTH.

Methods. Adult male rats (Sprague-Dawley) weighing 225-275 g, were used following one to 2 weeks acclimatization in this laboratory. The ACTH-releasing effect of a given procedure was evaluated by means of adrenal ascorbic acid depletion(6). The rats were sacrificed by application of a large surgical forceps at the level of the neck. This procedure decapitated the rat and prevented exchange of blood between head and trunk. The left and right adrenal glands were removed and ascorbic acid concentrations were determined separately, averaged, and expressed in milligrams adrenal ascorbic acid per 100 g fresh adrenal tissue. The assumption is made in these experiments that changes in adrenal ascorbic acid concentration of the experimental animal reflect increased rate of release of ACTH from the adenohypophysis.

Adrenal ascorbic acid depletion was studied in unanesthetized intact and in unanesthetized cord-sectioned rats after electrical stimulation and after injection of U.S.P. Corticotropin Reference Standard. Cord-sectioned rats were prepared under ether anesthesia. The spinal cord was sectioned at T_2 and a short probe destroyed spinal segments T_1 to T_3. The operated rats were caged individually in a room regulated at 30°C $\pm$ 1.0.

Unanesthetized rats were prepared for electrical stimulation by placing electrodes consisting of a length of soft copper wire either on both hindpaws or on both forepaws. An electrical stimulus believed sufficient to activate all nerve fibers in the vicinity of the electrodes was applied. The stimulation consisted of a square wave current flow having peak value of 4 to 7 milliamperes and a pulse duration of 3.2 milliseconds delivered at 100 pulses per second. The square wave current was turned on for 10 seconds, then off for 20 seconds, by a timer for a total of 12 minutes. Current flow was monitored on a cathode ray oscilloscope.

Results. Effect of spinal cord transection on adrenal ascorbic acid concentration and on adrenal weight. Adrenal ascorbic acid concentrations were determined at 4, 24, and 48 hours and at 7 days after operation (Table I). Four hours after spinal cord transection, adrenal ascorbic acid concentration was less than normal. At 24 hours, concentration was higher but still less than normal. At 48 hours, concentration was not significantly different from that of normal animals receiving

* This research was sponsored by the U.S.A.F. through the AFOSR of the Air Research and Development Command.

TABLE I. Adrenal Ascorbic Acid Concentration after Spinal Cord Transection.

Time after cord transection	
Intact rats*	450 ± 9 (7)†
4 hr	298 ± 9 (3)
24 "	328 ± 10 (10)
48 "	430 ± 12 (12)
7 days	432 ± 10 (5)

* All rats were sacrificed by neck clamp.
† Mean ± stand. error of mean (No. of rats).
All results expressed as mg adrenal ascorbic acid per 100 g fresh adrenal tissue.

no experimental treatment. Rats maintained for one week after cord-section exhibited normal adrenal ascorbic acid concentration.

Adrenal weight showed an increase after cord section. Adrenal weight of intact 250 g rats was 18.49 ± 0.8 (9) mg. Two days after cord section adrenal weight was 24.0 ± 0.8 (7) mg and at 7 days, 26.4 ± 0.8 (7) mg. *Effect of injected ACTH on adrenal ascorbic acid.* Dose response to injected ACTH was compared in 24-hour hypophysectomized and 2- and 7-day cord-sectioned rats. The members of the 3 groups had similar body weights. The rats were wrapped in a soft towel and the solutions in acid-saline (1 ml volume) were injected into a saphenous vein. One-half, 1.0 and 2.0 milliunits of U.S.P. ACTH Reference Standard, were employed (Table II). Response to ACTH was the same in the 3 groups. The sensitivity of the adrenals of 2- and 7-day cord-sectioned rats equals that of the 24-hour hypophysectomized rat.

TABLE II. ACTH Dose Response in 24-Hr Hypophysectomized Rats and in 2- and 7-Day Cord-sectioned Rats.

Exp. treatment	24-hr hypox rats	Cord-sectioned rats 2-day	Cord-sectioned rats 7-day
Acid saline	434 ± 12* (12)†	430 ± 13 (9)	427 ± 17 (9)
.5 mU ACTH	384 ± 8 (16)	380 ± 10 (13)	392 ± 12 (8)
1.0 " "	356 ± 9 (13)	370 ± 13 (8)	379 ± 23 (8)
2.0 " "	314 ± 7 (13)	317 ± 10 (11)	325 ± 17 (8)

* Mean ± stand. error of mean.
† No. of rats.
All results expressed as mg adrenal ascorbic acid per 100 g fresh adrenal tissue.

Comparison of response to forepaw stimulation and hindpaw stimulation in cord-sectioned and in intact rats. Two important aspects of the electrical stimulation procedure were tested with intact rats: 1) the possibility that forepaw and hindpaw stimulation may induce different amounts of ACTH release, 2) the possibility that sham stimulation may elicit ACTH release in absence of electric current flow. The parameters of the electrical stimulus chosen resulted in moderate ACTH release in the intact rat. Application of this electrical stimulus to forepaws or to hindpaws of intact rats resulted in equivalent adrenal ascorbic acid depletion. Sham hindpaw or sham forepaw stimulation did not result in significant ascorbic acid depletion (Table III).

TABLE III. Adrenal Ascorbic Acid Concentration after Sham Stimulation or Electrical Stimulation of Forepaws or Hindpaws of Intact Rats.

Untreated	427 ± 16 (6)*
Sham forepaw stimulation	422 ± 10 (6)
Forepaw electrical stimulation	260 ± 18 (6)
Untreated	424 ± 23 (6)
Sham hindpaw stimulation	419 ± 20 (6)
Hindpaw electrical stimulation	254 ± 10 (6)

* Mean ± stand. error (No. of rats).
All rats sacrificed by neck clamp.
All results expressed as mg adrenal ascorbic acid per 100 g fresh adrenal tissue.

TABLE IV. Adrenal Ascorbic Acid Concentrations after Sham Stimulation or Electrical Stimulation of Forepaws or Hindpaws of 1-Week Spinal Cord-Sectioned Rats.

Untreated	432 ± 10 (5)*
Sham forepaw stimulation	404 ± 23 (6)
Forepaw electrical stimulation	310 ± 19 (5)
Untreated	444 ± 14 (8)
Sham hindpaw stimulation	435 ± 11 (9)
Hindpaw electrical stimulation	410 ± 18 (9)

* Mean ± stand. error (No. of rats).
All rats sacrificed by neck clamp.
All results expressed as mg adrenal ascorbic acid per 100 g fresh adrenal tissue.

The results obtained above in intact rats may be compared with results obtained in one-week cord-sectioned rats (Table IV). Ascorbic acid concentrations in untreated groups and in sham stimulated groups were similar to those obtained in intact rats. However, in contrast to the results obtained in electrically stimulated intact rats, only fore-

paw stimulation elicited significant adrenal ascorbic acid depletion in the cord-sectioned rat. Consequently intact spinal cord paths are essential for mediation of ACTH release following electrical stimulation of the periphery.

Discussion. The results indicate that intact neural paths in the spinal cord are essential for mediation of ACTH release following hindpaw electrical stimulation. No ACTH release was observed following hindpaw stimulation even though considerable directly and reflexly elicited muscular activity and elevation of blood pressure occurred during stimulation. This evidence supports the view that CRF is released by neural structures located in the brain plus cervical cord portion of the central nervous system, probably the hypothalamus, rather than from the periphery. These conclusions conflict with the interpretation of experimental results supplied by others(1,2,3,4,5). It is believed that this discrepancy is the result of methodological shortcomings and the failure of previous investigators to carry out sham control experiments to define the effective factor in stimulation procedures leading to ACTH release.

The results of indirect assays of blood ACTH levels by measuring activity of the adrenal of chronic preparations must be interpreted with caution. It is possible that altered adrenal metabolism under the peculiar conditions existing in certain chronic preparation may be misleading. Precautions have been taken in these experiments to avoid some of the common failures: 1) only spinal cord rats with adrenal ascorbic acid levels similar to unoperated controls were used, 2) responsiveness of the adrenals of the spinal rats was defined with a 3 point dose-response curve to U.S.P. Reference Standard ACTH. 3) capacity to release ACTH was established by demonstrating ascorbic acid depletion following stimulation above the level of cord transection.

After hindpaw stimulation in cord-sectioned rats adrenal ascorbic acid depletion was absent. After forepaw stimulation, ascorbic acid depletion occurred but was less than that observed in intact rats. The possibility was considered that the decreased depletion following forepaw shock was due to a reduction in adrenal sensitivity to ACTH in the cord-sectioned rats and that this reduction masked a small response to hind-paw stimulation. Sensitivity of adrenals of cord-sectioned rats to exogenous ACTH was investigated. Dose response to injected ACTH demonstrated that adrenals of 2- and 7-day cord sectioned rats responded similarly to adrenals of 24-hour hypophysectomized rats.

Other evidence obtained recently also supports the view that there is a depression in ACTH release in cord-sectioned rats which is independent of adrenal gland function. The difficulty of interpreting data obtained in chronic preparations in which altered adrenal sensitivity to ACTH may occur was eliminated by measuring blood ACTH levels in adrenalectomized rats. Furthermore, since the view is held that depression of ACTH release in cord-sectioned preparations is due to lack of a supporting secretion from the adrenal medulla, the adrenal medulla was eliminated. Under these conditions blood ACTH levels were depressed after cord-section in comparison to levels in control and sham operated adrenalectomized rats.

Summary. Spinal cord section results in early adrenal ascorbic acid depletion. Recovery of normal levels occurs after 48 hours and is maintained for at least one week. Cord-sectioned rats (2- or 7-day), exhibiting normal initial adrenal ascorbic acid levels, respond to injected ACTH with ascorbic acid depletion similar to that observed in 24-hour hypophysectomized rats. Adrenal ascorbic acid depletion response was employed as an index of blood ACTH levels. Intact rats respond to electric current flow through either forepaws or hindpaws with equivalent ACTH release. Cord-sectioned rats respond to forepaw stimulation with ACTH release, but do not respond to hindpaw stimulation. Sham stimulation of intact or cord-sectioned rats does not release ACTH.

Marion D. Lipscomb rendered valuable technical assistance.

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Received Sept. 20, 1960. P.S.E.B.M., 1960, v105.

Pharmacologic Demonstration in Man of Increased Thresholds to Tooth Pain Following Carisoprodol and other Analgesics. (26166)

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Most methods for evoking experimental pain in humans trigger at the same time adjacent sensory fibers which interfere with precise perception and measurement of pain. Brashear(1) has observed that thermal, mechanical or chemical stimulation of a normal human tooth triggers pain only. No other sensation is experienced. Only pain fibers enter the pulp accompanying larger vessels and form an almost complete mantle around the arteries. The practicability of an algesimetric method based upon this neural structure is enhanced by constant conditions of stimulation afforded by a suitable electronic stimulator(2,3,4).

Methods. With a Burton "vitalometer,"* current was delivered to the base of the upper right incisor for 2 seconds, and increased by small increments at 5 to 10 second intervals until unequivocal recognition of pain was reported by the subject. One threshold measurement was made at each hourly trial. The contacting electrode was kept moist by frequently dipping it in 70% alcohol. Twenty normal men and women, 22 to 52 years of age, participated. Neither observer nor subjects had knowledge of the assignments of placebo or drug. One week elapsed between tests. On test days subjects reported to the laboratory at 9:00 a. m., when a control pain threshold record was obtained. Each subject then received 2 capsules of drug or placebo immediately after this test. Every subject received placebo 3 times during the series of trials. No foods or drinks were consumed until after all threshold tests had been completed. One hour after the drugs were ingested a second tooth pulp pain threshold was determined. A final threshold measurement was made 2 hours later (11:00). With initial readings (9:00 a. m.) as baseline, changes in tooth pulp threshold were calculated, and means computed for one-hour and 2-hour intervals. Biometric tests for significance of differences between groups are based upon "F" values derived by the analysis of variance(5).

Results. Carisoprodol at a single oral dose of 350 mg in gelatin capsules produced a statistically significant increase in pain threshold (130 ± 12.7% at one hour; 132 ± 12.4% at 2 hours) (Fig. 1). A single dose of 30 mg of codeine phosphate orally also raised pain threshold (128 ± 8.3% at one hour; 130 ± 10.1% at 2 hours). Placebo response did not differ significantly from baseline thresholds (96 ± 4.2% 104 ± 4.5%). Meprobamate in a single oral dosage of 800 mg failed to show any analgesic effect; the threshold value of 100 ± 7.4% did not differ significantly from placebo or baseline responses. In a single oral dose, acetylsalicylic acid at 1200 mg/person elevated pain threshold significantly at one hour (121 ± 14.5%). This response, however, was of relatively short duration since a statistically significant increase was not found at 2 hours. With 600 mg of acetylsalicylic acid, pain threshold was elevated (109 ± 9.2%), but not significantly. An oral dose

* Burton Mfg. Co., Santa Monica, Calif.