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Adrenal Sensitivity to ACTH as a Function of Time after Hypothalamic Lesion and after Hypophysectomy.* (25517)

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The usually available criteria for endogenous discharge of ACTH are indirect and are based on corticotropic stimulation of the adrenal cortex. When studying hypophysiotropic activity of preparations of corticotropin releasing factor (CRF) in animals with "effective" hypothalamic lesions, one should therefore know the sensitivity of the adrenals to ACTH. Considered "blocked" in their ACTH discharge when they show no evidence of adrenocortical stimulation upon exposure to stress, these animals might simply be less sensitive to ACTH than normal. Adrenocortical sensitivity to ACTH has been studied at various time intervals ranging from 24 hr to 60 days after stereotaxic placement of a lesion in the median eminence and demonstration of its effectiveness to inhibit stress-induced ACTH release. For comparison, adrenal sensitivity to ACTH was also studied in hypophysectomized animals.

Materials and methods. A. *After hypothalamic lesion.* Two hundred sixty animals (rats, male, body weight 230-250 g, Holtzman Farms, Houston) were used. A lesion of the bulbar part of the median eminence was made (Fig. 1), using a high frequency generator, the electrodes being placed with a modified Krieg-Johnson stereotaxic instrument. Brains of *all* animals were preserved at autopsy and a description of lesions from frozen sections is available for each animal. Eighteen to 19 hours after placement of the lesions, all animals were tested for block of stress-induced ACTH discharge as previously described(1). Only animals with "effective" lesions (60 out of 260) were saved for experiments reported here. Twenty-four hours after lesioning, 3 groups of animals were administered 0.5, 1.0, 1.5 mU ACTH USP Reference Standard (I.V., in 0.2 ml of 0.01 N HCl in saline). Adrenocortical response to 1 mU ACTH USP Reference Standard administered as above was investigated at 2, 4, 7, 9, 20, 30 and 60 days following placement of hypothalamic lesion. Persistence of inhibition of stress-induced ACTH-release(3) was reevaluated 6 hours before injection of ACTH in all animals used after fourth post-lesion day. In all cases adrenocortical stimulation produced by ACTH was assessed by measurement of peripheral plasma free corticosteroids 15 minutes after injection of the solution of adrenocorticotropin(2,3). The animals were then sacrificed and weight of adrenal glands recorded.

B. *After hypophysectomy.* One hundred fifty-six animals (rats, male, body weight 175-200 g, Holtzman Farms) were hypophysectomized. Three groups were administered 0.25, 0.5, 1.0 mU ACTH USP Reference Standard (as above), 24 hours later. Animals of other groups were similarly injected with 1 mU

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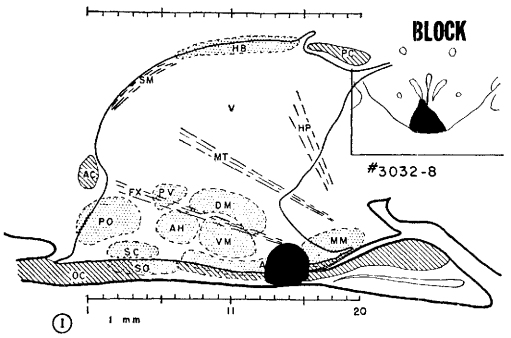

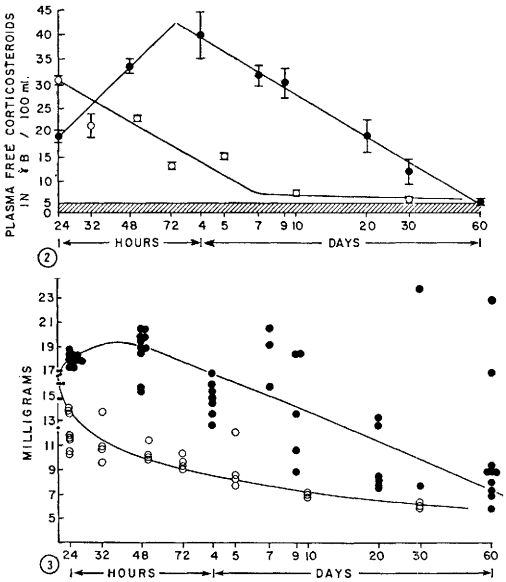

FIG. 1. Typical hypothalamic lesion (see text).

FIG. 2. Adrenocortical response to 1 mU ACTH at various time intervals after hypothalamic lesion (●) or hypophysectomy (○). Vertical bars through each circle represent 1 stand. error. (No. of animals/group can be calculated from Fig. 3.) Stippled area represents background reading in fluorometric method for plasma corticosteroids(2).

FIG. 3. Adrenal weights (left gland) after hypothalamic lesion (●) or hypophysectomy (○). (•), absolute controls of same experimental series.

ACTH, 32 and 52 hours, 3, 5, 10 and 30 days after hypophysectomy. Adrenocortical stimulation following ACTH was assessed as above (2,3), and adrenal weights recorded.

Results. Table I shows response of animals to multiple doses of ACTH, 24 hours after hypophysectomy and 24 hours after placement of an effective lesion. The animal with the hypothalamic lesion has an adrenal sensitivity to ACTH which is slightly lower than that of the hypophysectomized. Fig. 2 shows adrenal response of hypophysectomized and lesioned animals when injected with 1 mU ACTH USP Standard at various intervals after hypophysectomy or hypothalamic lesion. In the rat with hypothalamic lesion there is an increased response to ACTH for a few days (as compared to that observed at 24 hours post lesion). Subsequently, the response to ACTH decreases exponentially to completely disappear at 60 days after the lesion. After hypophysectomy, the adrenal response to 1 mU ACTH decreases rapidly to become asymptotically nil after 10 days. However 30 days post-hypophysectomy, there is still indication of a minute response to 1 mU of ACTH as judged by measurement of the plasma corticosteroid levels; this was confirmed by measurement of concentration of corticosterone in adrenals of the same animals (not reported here). Fig. 3 shows weights of adrenals as a function of time after hypophysectomy or hypothalamic lesion. Atrophy of the adrenal gland is clearly observable as early as 24 hours after hypophysectomy. It progresses linearly as a function of time. The adrenal of animal with effective hypothalamic lesion increases in weight in the first 48 hours; it then decreases in weight as a linear function of time. There are however considerable variations around mean adrenal weight calculated for each group, even though the responsivity to ACTH of these adrenals is quite constant within each group as exemplified by the small standard error of mean responses (Fig. 2).

Discussion. The progressive decrease of adrenal sensitivity to ACTH in animals with effective hypothalamic lesions, makes it desirable to use only an acute preparation as a

TABLE I. Adrenocortical Response to Multiple Doses of ACTH 24 Hr after Hypothalamic Lesion (A) or Hypophysectomy (B).

	No. animals	Plasma B, γ/100 ml, after stress	mU ACTH	Plasma B, γ/100 ml, after ACTH
A	4	12.6 ± 1.3*	.5	12.7 ± 1.7
	10	6.7 ± 1.8	1.0	19.4 ± 2.3
	5	13.6 ± 2.8	1.5	27.1 ± 2.7
B	6		.25	19.1 ± 1.7
	6		.5	24.0 ± .5
	6		1.0	30.9 ± 1.9

* Stand. error of mean.

test for CRF. In view of the increased response to ACTH, 2-4 days post-lesion, this period would seem to be an even better time schedule (than 1 day after lesion) for CRF assays. Since, 24 hours post-lesion, the animal has a sensitivity to CRF similar to that of morphine-nembutal blocked animal(see 1) which in turn has an adrenocortical sensitivity to ACTH similar to that of the 24-hour hypophysectomized rat, it is probable that 24 hours after hypothalamic lesion a greater sensitivity of the pituitary to CRF compensates for a submaximal adrenal response to ACTH. The increased adrenocortical response to the standard 1 mU dose of ACTH, as observed in early days after hypothalamic lesion, may be related to increased adrenal weight. This transient increased adrenal weight is likely related to trauma involved in surgical procedure at lesioning; similar adrenal weights were noted in animals of the same experimental series submitted to sham hypothalamic lesion.

It is interesting that up to 30 days after hypothalamic lesion, circulating free corticosteroids are still detectable in peripheral plasma (4). These levels, however, should not be necessarily considered as true correlates of concentration of any circulating ACTH; they may simply reflect impaired conjugation mechanisms. Taken in conjunction with maintenance of adrenocortical response to ACTH, still significant 30 days after the hypothalamic lesion and considerably higher than that observed at the same time following hypophysectomy, these data would indicate some paralytic secretion of adenohypophysial ACTH after placement of the hypothalamic lesion. This residual secretion appears to diminish exponentially with time (Fig. 2) and to have practically disappeared by the 60th day post lesion (no adrenocortical response to exogenous ACTH). In animals with this type of hypothalamic lesion, the corresponding hypocorticoidism is therefore unable to elicit the well known reciprocal hyper-secretion of ACTH.

Through latest time interval studied here, the adrenal gland of the hypothalamic animal remains statistically heavier than that of the hypophysectomized; variations around mean adrenal weight of each group are however considerable (Fig. 3). Since large adrenals as late as 60 days post hypothalamic lesion, are unable to respond to exogenous ACTH by increased corticoidogenesis, it is difficult to visualize weight maintenance solely on the basis of circulating ACTH. Two likely substances of hypophysial origin which might explain this adrenotrophic effect after hypothalamic deafferentiation of the pituitary are prolactin and growth hormone. This hypothesis will be discussed in another report.

Summary. The adrenocortical response to injected ACTH USP Standard has been studied as a function of time in rats after hypophysectomy or placement of a lesion in median eminence of the hypothalamus. In either case, there is an exponential decrease in sensitivity to ACTH of the adrenal gland, as a function of time. This can be observed in the presence of large adrenal glands, in animals bearing a hypothalamic lesion. The progressive decrease of adrenal sensitivity to ACTH in the lesioned animal makes it desirable to use only an acute preparation as a test for CRF.

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