Brain-Dependent ACTH Secretion from Multiple Heterotopic Pituitaries.* (31127)

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There is considerable evidence that pituitary ACTH secretion is stimulated by a neurohumor (CRF). There have been ample demonstrations that the integrity of the hypothalamic-hypophyseal portal circulation is essential for optimal ACTH secretion. For example, if the pituitary is transplanted to a heterotopic site, adrenal secretion of corticosteroids is markedly diminished(1); whereas transplantation of a pituitary under the median eminence of a hypophysectomized rat restores normal adrenocortical activity(2). If the pituitary stalk is sectioned and a permanent barrier placed so that revascularization of the connections between the hypothalamus and pituitary is prevented, the ACTH secretory response to stress is markedly diminished; whereas if revascularization occurs this response may be restored(3).

Whether CRF can reach sufficient concentration in the general circulation to stimulate pituitary ACTH secretion remains controversial. A few studies provide evidence that such high peripheral CRF levels may be attained. Wise, Van Brunt and Ganong(4) found that adrenocortical secretion of 17-hydroxycorticoids was appreciable in chronically stalk-sectioned dogs bearing a permanent block between the hypothalamus and pituitary. Several laboratories have reported an ACTH-releasing material in the peripheral blood of hypophysectomized animals(5-7). Critchlow *et al* showed that a CRF preparation injected i.v. into hypophysectomized rats with one pituitary transplanted under the kidney capsule increased circulating corticosterone levels(8). However, none of these studies demonstrate that endogenous CRF can stimulate ACTH secretion after passing through the general circulation.

Ganong(9) has proposed an experimental model for such a demonstration of systemically circulating CRF. He suggested that the hypophysectomized animal bearing a heterotopic pituitary have the median eminence destroyed by a lesion to determine if the ACTH secretion of the transplanted pituitary is brain-dependent. There are two difficulties with this experimental design. First, the single transplanted pituitary secretes so little ACTH that the adrenal weight(10) and adrenocortical function(1) of hypophysectomized animals bearing such transplants are virtually indistinguishable from those of non-transplanted hypophysectomized animals. Also, the predictability of the endocrine effects of any given hypothalamic lesion is rather poor. Therefore, we modified Ganong's plan in two respects to give what we consider a more sharply defined experiment.

It was felt that the hypophysectomized rat bearing multiple heterotopic pituitaries might be a suitable model to determine whether heterotopic pituitary ACTH secretion is dependent upon a neurohumor of brain origin transported through the general circulation. Our recent findings that hypophysectomized rats with multiple pituitaries transplanted to the kidney capsule have nearly normal adrenal weight and significant, though subnormal, corticosterone secretion suggest that heterotopic pituitaries do secrete ACTH although the quantity produced by a single pituitary may be very small. Furthermore, ACTH secretion by the heterotopic pituitary does not appear to be autonomous since it can be suppressed by exogenous corticoids(11). Ganong's proposal was further modified by choosing forebrain removal instead of a lesion in the median eminence because it was felt that this would be a more certain means of ablating the source of CRF.

Materials and methods. Adult, male Sprague-Dawley rats weighing approximately 120 g were hosts for pituitary transplantation in all experiments. Because 10 pituitaries transplanted into adult hosts had been found

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TABLE I. Adrenal Weight and Function After Brain Operations in Hypophysectomized Rats with Heterotopic Pituitaries.

Group (No. animals)	Adrenal wt, mg/100 g	Corticosterone secretion rate, μg/hr	Plasma corticosterone conc, μg %	Adrenal corticosterone conc, μg/g
Intact (8)	14.3 ± .7*	44.0 ± 5.2	31.4 ± 3.2	29.7 ± 3.8
Hypophysectomized (9)	7.1 ± .5	.6 ± .1	1.2 ± .2	6.0 ± 1.7
Hypophysectomized, transplanted, decortication (8)	11.6 ± 1.0	8.5 ± 1.6	16.8 ± 3.3	20.7 ± 5.4
Hypophysectomized, transplanted, forebrain removal (10)	13.1 ± 1.4	1.8 ± .4†	4.1 ± .4‡	14.7 ± 4.3

* Mean value ± S.E.
† Significantly different from mean value of hypophysectomized controls, $p < .01$.
‡ Significantly different from mean value of hypophysectomized controls, $p < .001$.

previously to be effective in maintaining adrenal weight and adrenocortical secretion(11), 5 whole pituitaries from 14-16-day-old donors of unselected sex were transplanted under each renal capsule. After operation the animals were housed in groups of 4-5 and fed Purina Laboratory Chow and water *ad lib.* Four weeks after transplantation, the recipient animals were hypophysectomized by the parapharyngeal approach. One control group of non-transplanted rats was hypophysectomized on the same day and another group of non-transplanted rats was left intact. All animals were maintained in the laboratory under similar conditions. Two weeks following hypophysectomy, half of the hypophysectomized-transplanted animals were subjected to forebrain removal as previously described (12) and the other half were decorticated. The latter operation was performed to provide a control for the blood loss sustained from the brain removal. The essential difference between the two groups was that the hypothalamus and thalamus were present in the decorticate animals whereas they were absent in the animals with complete forebrain removal. Four hours after operation, blood was taken from the left adrenal vein at laparotomy under ether anesthesia for determination of corticosterone secretion, as previously described(12). All adrenal venous blood specimens were promptly centrifuged and the plasma was frozen for subsequent determination of corticosterone by the method of Mattingly(13). Blood was also obtained from the abdominal aorta for measurement of circulating corticosterone concentration. The adrenal glands were weighed on a torsion balance and frozen for later determination of corticosterone concentration(11). The heads were fixed in 10% formalin, decalcified and the hypothalamic-hypophyseal region was serially sectioned and examined histologically for completeness of hypophysectomy and the extent of brain removal. Incomplete hypophysectomy was found in one animal and forebrain removal was inadequate in another. Data from these animals were discarded.

Results and discussion. The pooled results of 3 brain removal experiments are shown in Table I. The adrenal weight and corticosterone measurements in adrenal effluent, aorta plasma and adrenal gland of all hypophysectomized-transplanted animals were significantly above values found in non-transplanted, hypophysectomized controls as previously described(11). The absolute adrenal corticosterone secretion rates found in decorticate-hypophysectomized-transplanted animals were approximately 15 times those of hypophysectomized controls, but only ⅕ of that of intact controls, which is a nearly identical relationship to that reported previously. The absolute values of all secretion rates are somewhat higher in the present studies, which we attribute to use of a new spectrofluorometer in our laboratory. The group of hypophysectomized-transplanted animals with complete forebrain removal had corticosterone secretion rates and circulating corticosterone levels significantly lower than those of decorticate-hypophysectomized-transplanted animals. These results indicate that the adrenocortical secretory activity of animals with heterotopic pituitaries is dependent upon hypothalamic-thalamic integrity. It is probable that some

CRF circulates systemically to affect ACTH secretion. It is also suggested that this neurohumor has a relatively short half-life because 4 hours after removal of the hypothalamus and thalamus, corticosterone secretion was markedly reduced. However, after complete forebrain removal none of the corticosterone measurements decreased to levels found in hypophysectomized control animals. It has been our experience that within 4 hours after acute hypophysectomy, the adrenal corticosterone secretion rate falls to minimal values similar to those observed in chronically hypophysectomized rats. Therefore, it is possible that a small portion of the ACTH secretion of heterotopic pituitaries is not brain-dependent or that CRF has a longer half-life than ACTH. It is nonetheless clear that the major portion of ACTH secretion of heterotopic pituitaries is under the stimulatory influence of some subcortical portion of the brain.

Summary. Hypophysectomized rats with multiple heterotopic pituitaries have nearly normal adrenal weight and significant, though subnormal, corticosterone secretion. Removal of the entire forebrain, but not decortication, in these animals reduced corticosterone secretion nearly to values found in hypophysectomized control animals. These results indicate that heterotopic pituitary ACTH secretion is dependent on some subcortical portion of the forebrain.

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Instrumental Acquisition in Rats After Twelve Exposures to Deep Hypothermia.* (31128)

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When a mammal is being cooled the intensity of most of its body processes decreases. However, some processes increase initially but decline when hypothermia progresses further. An example of this is oxygen consumption, which in many mammals reaches a peak at a body temperature of 30°C (8). Further lowering of the body temperature diminishes and eventually stops all physiological processes. For various processes the critical temperature of cessation is found at different body temperatures. While

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