References and Notes

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27 November 1967

Corticotropin Release: Inhibition by Intrahypothalamic Implantation of Atropine

Abstract. *Anterior hypothalamic implantations of crystalline atropine markedly inhibit the adrenocortical responses evoked by surgical stress, ether anesthesia, or intravenous injection of arginine vasopressin. Similar implants in nearby regions of the brain or sham implantations in the same region were ineffective. The data suggest that the hypothalamic control of pituitary corticotropin may have a cholinergic component.*

Although there is general agreement that the hypothalamus controls pituitary secretion of corticotropin (ACTH) by way of a neurohumoral agent, corticotropin-releasing factor (CRF), relatively little is known about the factors that regulate the production and release of CRF itself. Histochemical studies have demonstrated the presence of acetylcholinesterase (*1*) and monoamine oxidase (*2*) in the hypothalamus, which suggest the presence of cholinergic and monoaminergic transmission. Moreover, fluorescence techniques have shown that norepinephrine and other biologically active monoamines may be found in the hypothalamus (*3*).

The functional significance of such substances in the hypothalamus remains unknown, but, since some of them undoubtedly act as synaptic transmitters peripherally, one may logically expect them to play a similar role in the central nervous system; if so, one or more of these substances may play a significant role in mediation of the neural control of production or release (or both) of CRF.

We have investigated this possibility by implanting some of these substances or their blocking agents in various hypothalamic areas and by then observing the effect on the adrenocortical stress response. We now report results with the anticholinergic agent atropine. For all experiments we used female albino rats weighing 110 to 120 g. Small pellets (200 to 250 μg) of crystalline atropine were implanted stereotaxically under pentobarbital anesthesia; sham operations were similar except that the implantation cannula contained no atropine. The responses of the adrenocortical system to various stimuli were then assayed by determining the concentration of corticosterone in plasma or corticosteroid production by adrenal glands incubated in vitro in a manner described (*4*). The basal levels for these assays in animals receiving only pentobarbital anesthesia are 5.0 ± 1.8 μg/100 ml and 7.9 ± 0.8 μg/100 mg hour^{-1}, respectively.

The stress inherent in the implantation procedure results in a rather prolonged release of ACTH, as is indicated by either of the assay methods (Fig. 1). In contrast, implantation of atropine in the region of the anterior hypothalamus quickly terminates this response. Activity of the adrenocortical system is very low 30 minutes after implantation and can be considered basal at 60 minutes.

The effective site of the atropine implants is on the midline just rostral to the paraventricular nuclei (Fig. 2). Similar implants in nearby regions had no effect, so the possibility is eliminated that the anterior hypothalamic implants were effective because of systemic distribution of the atropine by the circulation. Other drugs (for example, norepinephrine, epinephrine, and *d*-amphetamine) implanted in the effective site did not inhibit release of ACTH.

This inhibitive effect of atropine was observed even when other noxious stimuli were combined with the stress of implantation; for instance, when ether was substituted for the pentobarbital, corticoid production was again basal within 60 minutes of implantation. Similarly, when 50 milliunits of arginine vasopressin was administered intravenously immediately before the implantation of atropine, a very marked (but not quite complete) inhibition of arginine vasopressin–induced release of ACTH was observed.

Thus it was apparent that atropine in the anterior hypothalamus could terminate an adrenocortical response that had been evoked by any of several different stimuli. Our data suggest the presence of a cholinergic step in the mediation of the adrenocortical stress response. This suggestion is consistent with recent observations (*5*, *6*) of release of ACTH after cholinergic stimulation of various hypothalamic areas. Krieger and Krieger (*6*) have also prevented the normal diurnal rise in corti-

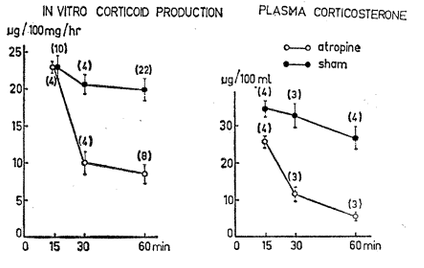

Fig. 1. Time courses of adrenocortical activities following implantations (of atropine or sham) in the anterior hypothalamus; implantation at zero time.

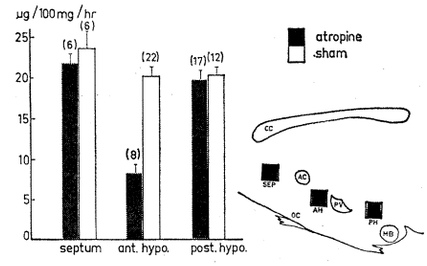

Fig. 2. Effective site of implantation. The histogram indicates corticoid productions in vitro 60 minutes after implantations were made in the three brain areas depicted in the accompanying midsagittal section of the rat brain. Abbreviations: *CC*, corpus callosum; *SEP*, septum; *AC*, anterior commissure; *OC*, optic chiasm; *AH*, anterior hypothalamus; *PV*, paraventricular nucleus; *PH*, posterior hypothalamus; *MB*, mammillary body.

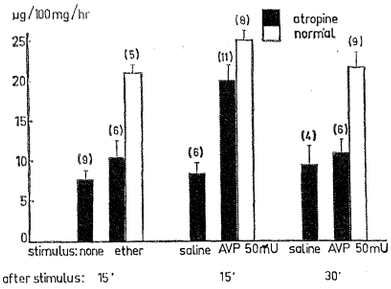

Fig. 3. Corticoid productions in vitro after noxious stimuli were administered to rats bearing atropine implants in the anterior hypothalamus. In all instances the stimuli were presented 45 minutes after implantation; the time intervals indicated are between stimulus and decapitation. Normal responses to the two stimuli are also indicated.

costeroid levels by systemic administration of atropine (*7*).

Although there is now some evidence of a central cholinergic mechanism that activates the adrenocortical system, Naumenko (*8*) has suggested that cholinergic agents stimulate this system indirectly by way of some peripheral effect. Our results are not in agreement with this view since the atropine implants are completely ineffective if made in hypothalamic areas other than the one we have described. The significance of involvement of a central cholinergic mechanism in the control of release of ACTH is also accentuated by the apparent lack of a comparable adrenergic mechanism, as is indicated by the demonstration that depletion of hypothalamic catecholamines does not modify stress-induced secretion of ACTH (*9*).

Further experiments were performed to determine whether the atropine could prevent the responses to stimuli presented 45 minutes after implantation, by which time the blockage was well established. Under these conditions the response normally observed 15 minutes after exposure to ether was completely abolished (Fig. 3); the same was true 30 minutes after injection of arginine vasopressin, but the 15-minute response to arginine vasopressin was only slightly though significantly diminished.

Thus in atropine-implanted rats arginine vasopressin, unlike ether, can still cause release of ACTH, although the magnitude and duration of the response are decreased. The different patterns of responses observed after ether and after arginine vasopressin may conceivably be ascribed to either differing degrees of intensity or different sites of action of these stimuli. Our data cannot conclusively distinguish between these two possibilities, but they seem to favor the latter.

The responses to ether and surgical stress were apparently mediated by way of the structures in the anterior-dorsal area of the hypothalamus, since these responses were consistently abolished by implantation of atropine in this region. Although our results do not indicate the precise location of the site of action of vasopressin, other results have indicated either a direct pituitary-stimulating effect (*10*) or an action on the tuberal part of the hypothalamus (*11*). The transient release of ACTH that we observed after injection of arginine vasopressin in atropine-implanted animals is consistent with the suggestion that arginine vasopressin acts on a structure distal to the site of the atropine implant.

Very few data are available from stimulation and ablation studies involving the specific area of the rat hypothalamus that contained our atropine implants; we know of only one instance in which the lesions employed occupied the same site and yet did not impinge upon the median eminence region (*12*). Such lesions inhibited secretion of ACTH in response to a wide variety of stimuli; this observation supports the contention that our atropine implants were inhibiting a stimulatory pathway rather than activating an inhibitory pathway.

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Physical Support with and without Optical Support: Reaction to Apparent Depth by Chicks and Rats

Abstract. *The locomotor reactions to apparent depth made by chicks and rats were measured in situations offering physical support with and without optical information for support. Chicks avoided an optical void though it provided physical support. Rats responded indifferently to the presence or absence of optical information for support as long as physical support was available.*

Although normally treated terrestrial animals of all species tested have exhibited depth perception on the visual-cliff apparatus (*1*)—a device designed to control for tactual, auditory, and olfactory cues, forcing animals to respond solely on the basis of visual cues—it is likely that the effectiveness of certain classes of visual stimuli for depth perception is greater for some animals than for others. Several obvious species differences point to this: all species do not attain the same level of optical proficiency; they may possess varying sensory anatomies, and may characteristically habituate in differently illuminated environments. For example, the day-active chick manifests correlated visual and locomotor abilities shortly after hatching, while the nocturnal rat requires a period of development in order to perform adequate visual-motor activities. Moreover, some structural differences suggest sensory dominance: some animals may react on the basis of nonvisual cues to a situation offering both visual and nonvisual information. Thus, the rat possesses vibrissae which may engender a haptic orientation in exploration. In these same terms, the fact that small birds give over a relatively high proportion of the head to the eyes (*2*) suggests that the locomotion of the chick may be predominately controlled by optical information. "Optical dominance" for the chick is further indicated by the fact that locomotor activity ceases when optical stimulation ceases, as in total darkness. If it can be generally assumed that the effectiveness of certain classes of stimuli varies with the anatomy, ecology, and biological requirements of the species, then a reasonable prediction is that some relevant differences between these two species will be reflected by different responses to physical support with and without