cytes enclosed vigorously. Therefore, any participation of lymph gland cells in phagocytosis may reasonably be dismissed. Rather, we suggest that these cells are exclusively responsible for the initiation of histolysis. In this view, the organ would prepare larval tissues for the subsequent steps in histolysis, such as phagocytosis. The means by which the lymph gland cells recognize their target tissue and the nature of the signals triggering the release of lytic enzymes remain to be clarified.

Various roles have been attributed to the lymph gland (*7*). The present study would appear to rule out phagocytosis (*3, 15*). Nor did we find any support for melanin deposition in imaginal disk development of the eye, stimulation of imaginal primordia during metamorphosis, formation of the mesodermal parts of the imaginal appendages (*16*), and larval hematopoiesis (*17, 18*), thus confirming our previous conclusions (*8*). However, it is interesting to note that Gateff (*18, 19*) found, in a hereditary malignant blood neoplasm of *D. melanogaster*, *l(1)mbn*, that the lymph gland cells attack and histolyse the imaginal disk epithelia in situ. Moreover, when parts of such lymph glands were injected into normal adult hosts, various tissues were attacked, including flight muscles. These observations allow us to suggest that mutant lymph gland cells, in addition to having uncontrolled proliferation and possibly disturbed target specificity, may have increased histolytic potential.

In *Calliphora*, cells similar to the lymph gland cells of *Drosophila* were observed by Crossley (*20*) and by Zachary and Hoffmann (*21*). They also noted a strikingly large number of dense bodies with a strongly positive reaction in the acid phosphatase test. The "hematopoietic" organs in *Calliphora* (*20, 21*) or other Diptera [*Chironomus*, *Musca*, and *Phaonia* (*22*)] which release cells just before pupariation, could be organs analogous to the lymph gland of *Drosophila*.

Concerning the initiation of histolysis, our observations do not support the view of Pérez (*1*), who stated that hemocytes penetrate intact muscles. Nor do we agree with the concept of an autonomous autolysis of muscles during metamorphosis, as proposed for *Lucilia* (*2*), *Drosophila* (*3*), *Sarcophaga* (*4*), and *Calliphora* (*5, 6*). We propose that lymph gland cells are responsible for initiating histolysis of transient larval tissues before these are fragmented and phagocytized. Our observations point to a prehistolytic or clastic action of a specific type of cell as an indispensable first step in histolysis. Thus the term "lymph gland" should be changed according to the function of the organ upon confirmation of our prediction that similar histolytic organs will be identified in other holometabolous insects.

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Brainstem Catecholamine Neurons Are Target Sites for Sex Steroid Hormones

Abstract. *Sex steroid hormones and catecholamines have physiological interactions in the brain. By the combined use of autoradiography and fluorescence histochemistry, steroid hormone target sites and catecholamine neurons were visualized simultaneously in the same tissue preparation. By this dual localization method, [^{3}H]estradiol and [^{3}H]dihydrotestosterone target sites were identified in nuclei of many catecholamine cell bodies in the brainstem, and catecholamine nerve terminals were observed near certain steroid hormone target neurons. These results suggest close anatomical interrelations between steroid hormone sites of action and catecholamine sites of production and action in the brain.*

Sex steroid hormones and catecholamines have physiological interactions in the central nervous system (CNS) (*1*) that appear to play a significant role in the regulation of a number of physiological processes, including ovulation (*2, 3*), copulatory behavior (*4*), and blood pressure (*5*). It is not known, however, where and how sex steroids and catecholamines interest in the CNS. In order to answer these questions, we developed a formaldehyde-induced fluorescence (FIF)-autoradiography technique that locates catecholamines and sex steroids simultaneously in the same tissue section (*6*).

Through autoradiography alone, estrogen and androgen target sites in rat brain have been located (*7*). Comparison of these steroid hormone target sites with catecholamine mapping data (*8*) suggests anatomical overlap between sex steroid target sites and catecholamine neuronal systems. In the present investigation, the FIF-autoradiography technique was used to locate concentrations of the female sex hormone 17β-estradiol and the male sex hormone 5α-dihydrotestosterone in relation to brain catecholamine systems in male and female rats. Estradiol was selected because its influence on CNS catecholamines is widely acknowledged; and dihydrotestosterone was chosen because it is an active testosterone metabolite that is not converted to estrogen and thus acts as a true androgen (*9*).

Two types of morphological relations between steroid hormone target sites and catecholamine neurons are described: (i) sex steroid hormones are concentrated in nuclei of catecholamine neurons and (ii) steroid hormone target neurons are surrounded by catecholamine terminals. This suggests that sex steroids directly influence catecholamine neurons by exerting genomic effects at nuclear sites (*7*) and that catecholamine neuronal systems influence steroid hormone target neurons by direct innervation of steroid hormone target neurons.

Six male and six female 60-day-old Holtzman rats were gonadectomized; the male rats were also adrenalectomized and

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maintained on salt water. After 48 hours, each animal was injected intraperitoneally with pargyline (30 mg/kg), a monoamine oxidase inhibitor, and 2 hours later each of three male and three female rats was injected intravenously with 17β-[2,4,6,7-^{3}H]estradiol (0.5 μg/100 g) (115 Ci/mmole) or [1,2,4,5,6,7-^{3}H]dihydrotestosterone (123 Ci/mmole). All animals were killed 1 hour after injection of the radioactively labeled hormone. Brain tissue was placed on brass mounts and frozen in liquid propane (− 180°C), and 6-μm frozen serial sections were cut at − 35°C in a Harris wide-range cryostat. The sections were freeze-dried with a cryosorption pump, exposed to formaldehyde vapor (80°C) for 1 hour, and dry-mounted on emulsion-coated (Kodak NTB-3) slides (*6*). After photographic exposure for 8 to 12 months, the slides were developed and examined by using a Zeiss photomicroscope with fluorescence attachments (mercury lamp HBO 200W/4; BG-12 excitation and Zeiss "50" emission filters). Sections were observed first under ultraviolet light to locate fluorescent catecholamine neuronal elements and then under a combination of ultraviolet and tungsten light in order to view, simultaneously, catecholamine neurons with cytoplasmic fluorescence and neurons with nuclear concentrations of silver grains representing steroid hormone target sites. After photographic recording, sections were stained with methyl-green pyronin and studied as conventional autoradiograms. Cells were considered to be labeled with tritiated steroid hormone if nuclear concentrations of silver grains were four to five times greater than extranuclear levels (*10*).

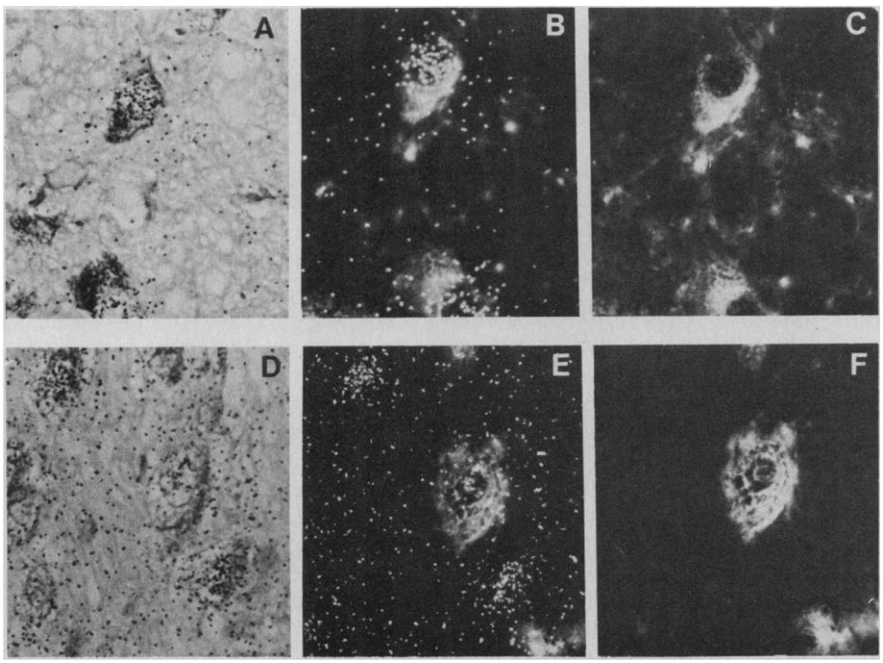

Fig. 1. FIF-autoradiograms showing that catecholamine neurons near the nucleus reticularis lateralis of the medulla (group A1) concentrate [^{3}H]estradiol (A to C) but not [^{3}H]dihydrotestosterone (D to F). Sections (A) and (D) are shown in tungsten light; (B) and (E), in ultraviolet and tungsten light; and (C) and (F), in ultraviolet light. Note that noncatecholamine neurons in this region concentrate [^{3}H]dihydrotestosterone (D and E).

Fluorescent catecholamine neurons in several cell groups of the brainstem showed labeling with the steroid hormones (Figs. 1 and 2). The localization patterns were similar for both sexes but different for the two sex steroids. In the medulla, catecholamine neurons with concentrations of [^{3}H]estradiol were found in the region of the nucleus (n.) reticularis lateralis (group A1) and the n. tractus solitarii (group A2); in the pons, they were found adjacent to the n. olivaris superior (group A5), in the locus ceruleus (group A6), and in the vicinity of the lemniscus lateralis (group A7). Some 50 to 80 percent of the fluorescent catecholamine neurons in these groups contained [^{3}H]estradiol, with the exception of the locus ceruleus, where approximately 25 percent of the catecholamine neurons contained [^{3}H]estradiol. The catecholamine cell bodies in all these areas are reported to contain primarily norepinephrine (*8*). Epinephrine is also found in some of these regions (*11*): in the medulla, adjacent to the n. reticularis lateralis (group C1) and in the vicintiy of the n. tractus solitarii (group C2). The cell bodies of most reported dopamine neurons in the midbrain (groups A8, A9, and A10) and in the diencephalon (group A11) did not concentrate [^{3}H]estradiol (*8, 12*); however, a small number of labeled dopamine neurons were present in the n. arcuatus and n. periventricularis of the hypothalamus (group A12). Information is not yet available on dopamine neurons in the dorsal part of the hypothalamus (group A13) and in the n. periventricularis of the hypothalamus rostral to the n. arcuatus (group A14).

Catecholamine neurons that contained [^{3}H]dihydrotestosterone were located in the pons at the dorsolateral corner of the fourth ventricle (group A4), adjacent to the n. olivaris superior (group A5), in the

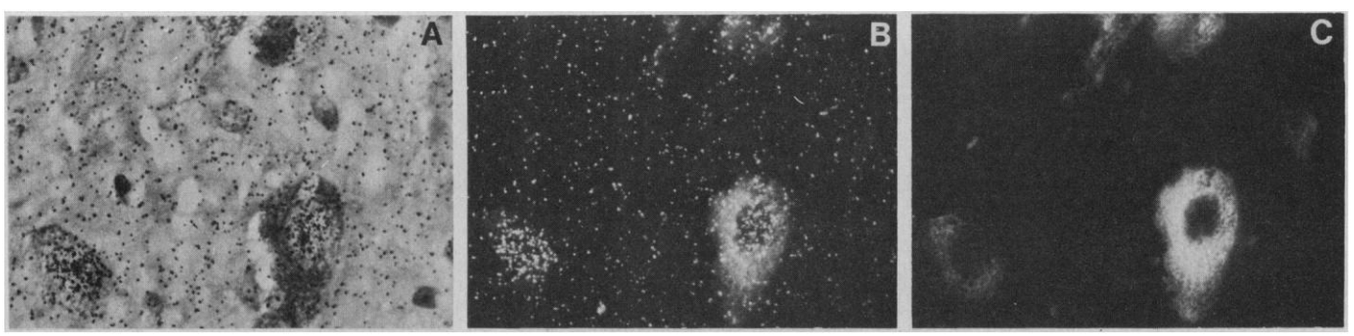

Fig. 2. FIF-autoradiograms showing [^{3}H]dihydrotestosterone concentration in nuclei of catecholamine neurons located adjacent to the nucleus olivaris superior of the pons. Section (A) is shown in tungsten light; (B), in ultraviolet and tungsten light; and (C), in ultraviolet light.

locus ceruleus (group A6), and in the region of the lemniscus lateralis (group A7). Some 50 to 80 percent of the catecholamine neurons in these cell groups contained [^{3}H]dihydrotestosterone. No [^{3}H]dihydrotestosterone labeling was observed in catecholamine neurons of groups A1 and A2 in the medulla. As with [^{3}H]estradiol, [^{3}H]dihydrotestosterone was concentrated in a small number of dopamine neurons in the n. arcuatus and n. periventricularis of the hypothalamus. Other dopamine cell groups were not labeled with [^{3}H]dihydrotestosterone.

In addition to the concentration of steroid hormones in nuclei of catecholamine cell bodies, there was a second morphological relation between catecholamine neurons and sex steroids. In many brain areas, both [^{3}H]estradiol and [^{3}H]dihydrotestosterone target neurons were surrounded by numerous fluorescent catecholamine terminals, suggesting that the target neurons receive significant catecholaminergic innervation. In the midbrain, this type of relation was found in the ventrolateral portion of the substantia grisea centralis; in the diencephalon, it was found in the n. paraventricularis, n. dorsomedialis, n. periventricularis, and the ventral portion of the n. interstitialis striae terminalis.

Our studies revealed both similarities and differences between the distribution of [^{3}H]estradiol and [^{3}H]dihydrotestosterone in catecholamine neuronal systems. In the medulla, catecholamine neurons in the region of the n. reticularis lateralis [particularly its caudal portion (group A1)] and in the n. tractus solitarii (group A2) contain [^{3}H]estradiol but not [^{3}H]dihydrotestosterone. This suggests that certain catecholamine neurons in groups A1 and A2 are directly influenced by estradiol but not dihydrotestosterone. These neurons, therefore, may play a functional role in physiological processes that are selectively modulated by estradiol. The differential distribution of [^{3}H]estradiol and [^{3}H]dihydrotestosterone is noteworthy since both hormones are metabolites of testosterone. Depending on the availability of enzymes for conversion of testosterone to estradiol or dihydrotestosterone, testosterone could affect both [^{3}H]estradiol- and [^{3}H]dihydrotestosterone-concentrating catecholamine neurons and thus could have more widespread effects on catecholamine systems than either of its metabolites alone.

In several instances, [^{3}H]estradiol and [^{3}H]dihydrotestosterone are concentrated in neurons located in the same catecholamine cell group. For example, in the pons the majority of catecholamine neurons in groups A5 and A7 concentrate [^{3}H]estradiol as well as [^{3}H]dihydrotestosterone. Therefore, it is probable that certain catecholamine neurons in these cell groups contain receptors for both hormones and thus are capable of responding to estradiol as well as to dihydrotestosterone. In addition, both types of steroid hormone target neurons are found in some of the same dense catecholamine terminal fields. This suggests that certain estrogen and androgen target neurons are innervated, and therefore influenced, by the same catecholamine neuronal systems. Our results also indicate that estradiol and dihydrotestosterone are more widely associated with reported noradrenergic neuronal systems than with dopaminergic systems.

It has been known for many years that there is a functional link between gonadal hormones and catecholamine metabolism and action in the CNS. Our histochemical studies prove that a number of the catecholamine neurons in the lower brainstem are targets for certain sex steroids and indicate that certain sex steroid target neurons are heavily innervated by catecholamine neurons. These discoveries may be important in understanding the many physiological and psychological processes in which catecholamines have been ascribed a functional role. These include the neuroendocrine regulation of reproduction, thermoregulation, blood pressure regulation, vomiting, feeding, drinking, and emotional behavior such as aggression and depression (*13*). Sex steroid hormones have been reported to influence many of the same processes (*14*). These observations, along with our discovery of a direct morphological association between steroids and catecholamine systems, indicate a close interaction between steroid hormones and catecholamines in the control of many endocrine, autonomic, and behavioral events.

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