orescence is consistent with the presence of protein breakdown products within the cells and not with serotonin (*9*).

The specific localization of [^{3}H]morphine in the myenteric plexus by autoradiography opens a new approach to the study of narcotic analgesic agents and their antagonists. The predominant localization and specific binding of morphine on the satellite cells which is reversed by prior incubation with naloxone suggests that the site of action of morphine in the myenteric plexus preparation is associated with the satellite cells rather than the larger ganglionic cells. The large neurons showed neither biogenic amine fluorescence nor morphine localization. The noradrenergic fluorescence associated with nerve fibers impinging on the "gliocytes" suggests an association between the binding of morphine and the presence of norepinephrine in these fibers. It was not necessary to treat the animals or the isolated tissue with inhibitors, precursors, or depleting agents for the fluorescence to be observed.

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10. [^{3}H]Morphine was checked for purity prior to incubation and after incubation by thin-layer chromatography on silica gel G with a solvent system of methanol, benzene, and water (75 : 10 : 15 by volume). The chromatogram showed a single spot with an R_F value corresponding to a standard pure nonradioactive morphine with no additional radioactive spots.
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Induction of Tyrosine 3-Monooxygenase in Adrenal Medulla: Role of Protein Kinase Activation and Translocation

Abstract. *The transsynaptic induction of tyrosine 3-monooxygenase (TH) in rat adrenal medulla is preceded by an early increase in the ratio of cyclic adenosine monophosphate (AMP) to cyclic guanosine monophosphate, an activation of cytosol cyclic AMP–dependent protein kinase, and a subsequent translocation of protein kinase catalytic subunits from cytosol to subcellular particles. As a result of this translocation, nuclear protein kinase activity increases during the induction of TH. Transection of splanchnic nerve reverts these events and prevents the induction of TH. Thus, adrenal medulla activation and translocation of cyclic AMP–dependent protein kinase may act as a long-range messenger for the genetic regulation of TH synthesis.*

In chromaffin cells of adrenal medulla, the expression of the genetic code can be regulated transsynaptically. A suitable model for studying this regulation is the transsynaptically elicited increase of tyrosine 3-monooxygenase—also called tyrosine hydroxylase (TH)—in rat adrenal medulla (*1*). Since Axelrod (*1*) proposed that this process requires new RNA synthesis, we have assumed that the release of acetylcholine triggers a sequence of biochemical events that mediate the transfer of information from the chromaffin cell membrane to the nucleus. In fact, we have shown that messenger RNA (mRNA) synthesis increases 6 hours after the transsynaptic stimulus (*2*) and that a few hours later the synthesis of TH is enhanced (*3*).

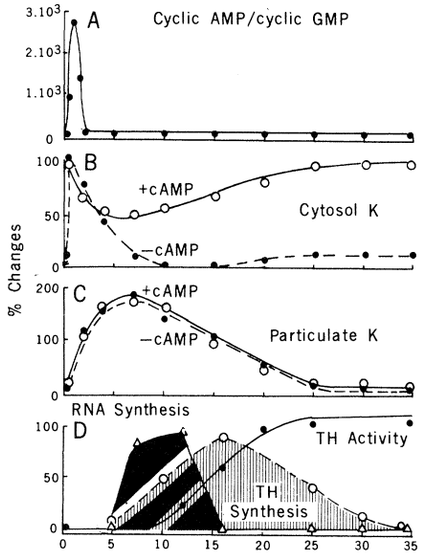

Fig. 1. Sequence of molecular events taking place in chromaffin cells during the transsynaptic induction of TH elicited by exposure to 4°C for 2 hours. The adrenal medullas were dissected stereomicroscopically (*13*). (A) Percent of change in the ratio of cyclic AMP to cyclic GMP concentrations. (B) Activity of cytosol kinase (*K*). (C) Activity of particulate kinase (*K*). (D) Synthesis of RNA, TH molecules, and activity of TH measured in vivo. For details on the methods, see (*6*).

The temporal sequence of the events leading to the induction of medullary TH elicited by a persistent activation of nicotinic receptors, with 2 hours of exposure to 4°C as the stimulus, is shown in Fig. 1. This transsynaptic activation of nicotinic receptors increases the ratio of cyclic adenosine monophosphate (AMP) to cyclic guanosine monophosphate (GMP). This increase is due to an elevation of cyclic AMP and to a decrease of cyclic GMP (*4*). The ratio increases from about 10 (100 percent) to 280 (Fig. 1A). This change in the cyclic AMP content acts as a short-range specific intracellular effector that releases catalytic subunits from cyclic AMP–dependent protein kinase in cytosol (Fig. 1B) and triggers the translocation of the catalytic subunit of this kinase from cytosol to the particulate fraction (Fig. 1C). This translocation acts as a long-range messenger for the regulation of the expression of the genetic code; thus the synthesis of mRNA (*2*), including the mRNA for TH, is increased. Finally, new enzyme protein is formed (Fig. 1D) and TH activity is increased (Fig. 1D) for several days (*3*). Results similar to those shown in Fig. 1 have been obtained with the use of reserpine, carbamylcholine, or aminophylline as an inducing stimulus (*4, 5*). Our data (Fig. 1) show that, concomitant with cold expo-

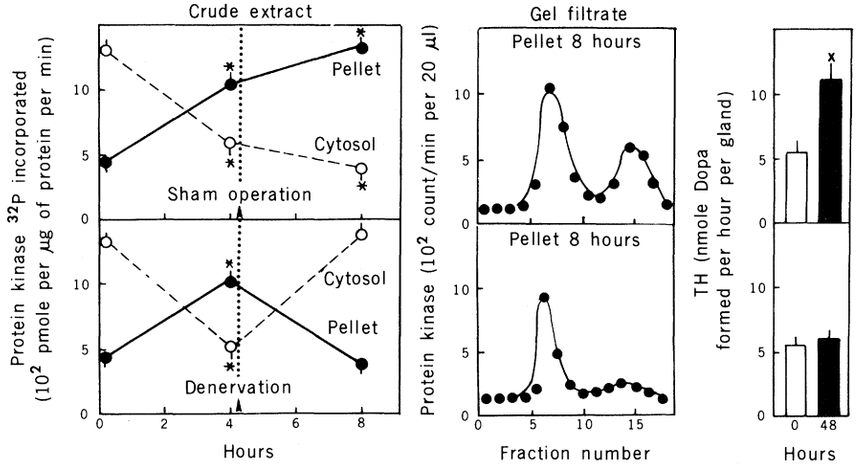

Fig. 2. Effect of splanchnic nerve transection of protein kinase and TH activities in adrenal medulla of rats receiving reserpine (16 μmole/kg, intraperitoneally). Left panel shows the protein kinase activity of cytosol and pellet extract in the intact medulla (left panel, upper part) or in the contralateral medulla in which transection of the left splanchnic nerve was performed 4 hours after reserpine (left panel, lower part). The protein kinase activity was measured as stated in (*6*). Middle panel shows a Sephadex G-200 elution profile of protein kinase extracted from pellets 8 hours after administration of reserpine. Sephadex G-200 column (0.2, 50 cm) equilibrated with 0.5*M* NaCl, 10 m*M* EDTA, and 10 m*M* potassium phosphate buffer (*p*H 6.5) was used in these experiments. The protein kinase was eluted with the same buffer in fractions of 80 μl. Upper part: intact adrenal medullas; lower part: contralateral denervated adrenal medullas. Right panel shows TH activity measured 48 hours after reserpine; upper part: intact adrenal; lower part: contralateral denervated adrenal.

sure, the ratio of cyclic AMP to cyclic GMP in the adrenal medulla increases about 28-fold. The duration of this increase is independent of the duration of the stimulus; even though the rats are kept in the cold for 2 hours, the ratio returns to baseline levels within 90 minutes. It is significant that, when the medullary cyclic AMP content has returned to baseline values, the activity of the cyclic AMP–dependent protein kinase in the cytosol resulting from centrifugation of the homogenate at 100,000*g* (*6*) is decreased, whereas that of its catalytic subunits which is independent of cyclic AMP is increased (Fig. 1B). In the cytosol, the increase in the activity of catalytic subunits of protein kinase lasts about 4 hours, whereas the decrease of the cyclic AMP-dependent protein kinase lasts for about 18 hours (Fig. 1B). In the extract of the particulate fraction obtained with 0.2 percent Triton and 0.5*M* NaCl (*6*), the increase in protein kinase activity also lasts for about 18 hours (Fig. 1C). Hence, in the pellet extract the increase in phosphorylating activity due to catalytic subunits of protein kinase (Fig. 1C) lasts as long as the decrease in the activity of cyclic AMP–dependent protein kinase in cytosol (Fig. 1B). The increase in the protein kinase activity associated with the low-molecular-weight protein of particulate fractions was completely obliterated by the addition of purified regulatory subunits (*5*). This finding suggests that catalytic subunits of the cyclic AMP–dependent protein kinase are translocated (transferred) from the cytosol to the cellular organelles of the chromaffin cells. The mechanisms that regulate protein kinase translocation have not yet been elucidated. In our experiments, we have attempted to define the nature of this translocation and the relation between translocation of protein kinase and induction of TH. It had been reported (*7*) that, when the adrenal is denervated at 4 hours after reserpine injection, the delayed induction of TH is abolished (see confirmation in Fig. 2, right panel). Since at this time the cyclic AMP content is back to normal (*5*), we have selected this experimental protocol to evaluate whether protein kinase translocation is a critical event in the transsynaptic induction of TH.

The increase of protein kinase activity in the pellet extract of medullary homogenates from rats treated with reserpine was reversed by denervation, and this decline was associated with an increase in cytosol protein kinase (Fig. 2). This finding supports the view that protein kinase translocation into the particulate fraction is a critical step in the chain of events that elicit the transsynaptic induction of medullary TH. The total amount of protein kinase in the homogenate of one adrenal medulla measured with the use of the histone as phosphate acceptor was 11 ± 0.7 and 10.5 ± 0.5 pmole per minute in rats treated with saline or reserpine, respectively. Only the distribution of the enzyme in the subcellular particles was changed. Moreover, prior treatment with cycloheximide failed to reduce the increase in protein kinase activity elicited by reserpine. In these extracts neither the degradation rate of adenosine triphosphate (ATP) nor the histone phosphatase activity was changed. Moreover, appropriate recombination experiments failed to prove that the increase in protein kinase activity in pellets was due to an increase in an activator or to a decrease in an inhibitor (*8*) of the protein kinase.

The chromatographic profiles (Sephadex G-200) of pellet extract of adrenal medullas excised from rats injected with reserpine 8 hours before are presented in Fig. 2 (middle panel). The elution profile from pellet extract of denervated (Fig. 2, lower part of middle panel) and intact medullas (Fig. 2, upper part of middle panel) consisted of two peaks of protein kinase activity. In the pellet extracts from intact medullas of rats treated with reserpine, the protein kinase activity associated with the high-molecular-weight protein was similar to that obtained from pellet extracts of the contralateral denervated medulla. However, the enzyme activity of the low-molecular-weight protein was severalfold greater in the intact side (Fig. 2, upper part of middle panel) than in the denervated side (Fig. 2, lower part of middle panel).

In our experiments the homogenates and pellet extracts were prepared according to the precaution recommended by Keely *et al.* (*9*) to avoid nonspecific protein aggregation. Hence the data show that a low-molecular-weight catalytic unit was translocated from the cytosol into the pellet. To decide whether the nucleus was a site for protein kinase translocation, we measured the protein kinase activity in nuclei isolated from medullas of rats treated with saline or reserpine. Nuclei were isolated by the two-step procedure of Yasmineh *et al.* (*10*); in each experiment about 100 adrenal medullas were used. In the nuclear fraction the ratio of monoamine oxidase activity to DNA was decreased. It was 3.0 in the original homogenate and became 0.21 and 0.19 in the nuclear fraction of homogenates from rats treated with saline and reserpine, respectively. It has been reported that the protein kinase activity in nuclei is not dependent on cyclic AMP (*11*). Also in the adrenal medulla, the nuclear protein kinase was not stimulated by cyclic AMP. The protein kinase activity of nuclei 7 hours after treatment with reserpine (a histone mixture from calf thymus was used as phosphate acceptor protein) was 144 ± 26 pmole per milligram of protein per minute; that of saline-treated rats 65 ± 11 ($N = 3$; $P < .05$). The properties of the translocated en-

zyme with respect to the molecular nature of the endogenous substrate or substrates remain to be determined.

Our findings extend to the transsynaptic induction of TH, the concept proposed for liver and ovary (*11, 12*) that protein kinase translocation may be a mechanism involved in transferring information to the nucleus. Moreover, since denervation reversed the decline of cytosol protein kinase and the increase in activity of the enzyme in the pellet, we suggest that protein kinase translocation is part of the mechanism whereby the sustained activation of nicotinic receptors coordinates the long-range reactions involved in the expression of the genetic code. Since adrenal denervation fails to abolish the increase in cyclic AMP elicited by cold exposure in adrenal cortex (*13*) but does abolish the increase of cyclic AMP and the induction of TH in the adrenal medulla, we can exclude a direct participation of corticosteroids in eliciting the increase of cyclic AMP in the medulla, the activation and translocation of protein kinase, and the transsynaptic induction of TH. However, we cannot rule out an indirect permissive role of corticosteroid in the regulation of TH biosynthesis.

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6. Cyclic AMP and cyclic GMP concentrations were measured by activation of a specific protein kinase after separation on alumina and Dowex columns (*13*). The baseline value of cyclic AMP was 25 ± 1.5 pmole per milligram of protein and that of cyclic GMP was 3.1 ± 0.28 pmole per milligram of protein (N = 15). To determine the kinase activity, four medullas were homogenized with 80 μl of 0.5M NaCl, 10 mM EDTA, 5 mM aminophylline, 10 mM potassium phosphate buffer (*p*H 6.5), modified from Corbin *et al.* (*14*). After centrifugation at 100,000g the cytosol was collected, and the pellet was homogenized again, in a mixture of 80 μl of 0.5M NaCl, 10 mM potassium phosphate buffer, 10 mM aminophylline, and 0.2 percent Triton × 100 (*p*H 6.5), and centrifuged again for 20 minutes at 2 × 10^4g. The protein kinase reaction was started by adding 10 μl of [γ-^{32}P]adenosine triphosphate (500 μM), 100 mc per mmole to an incubation mixture containing (in a volume of 140 μl) 10 μl of cytosol or pellet extract, 10 μl of 0.1M magnesium acetate buffer (*p*H 6), 40 μl of 0.5M sodium acetate buffer (*p*H 6.0), 10 μl of 0.3M NaF, 10 μl of 65 mM aminophylline, 20 μl of calf thymus histone mixture (2 mg/ml), 30 μl of water, and 10 μl of cyclic AMP or water. After 5 minutes at 30°C, the incubation was terminated by pipetting 50 μl portions on filter paper discs and the samples were washed (*4*). In some experiments, we have characterized the kinase activity with regard to the specificity for various histone or proteins. The enzymes in the medullas in the natural state or after exposure at 4°C have different specificities for different substrate proteins (*5*); since these kinases express their maximal catalytic rates in the presence of histones, we have provisionally used calf thymus histone mixture to measure the phosphorylating activity in the cytosol and insoluble portion (pellet) of the medulla. Under these conditions, the baseline cytosol kinase activity was 163 ± 9 pmole per milligram of protein per minute in the presence of 0.7 μM cyclic AMP and 55 ± 4 pmole per milligram of protein per minute in the absence of cyclic AMP. The kinase activity in the pellet extract was 70 ± 10 pmole per milligram of protein per minute in the presence of cyclic AMP and 69 ± 7 pmole per milligram of protein per minute in the absence of cyclic AMP. Proteins were measured by the method of Lowry *et al.* (*15*). The values of RNA synthesis (*2*), TH synthesis (*3, 4*), and TH activity (*4*) in Fig. 1D have been previously reported.
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Recognition and Sexual Selection in *Drosophila*: Classification, Quantification, and Identification

Abstract. Drosophila pseudoobscura *females show a positive bias toward mating with males whose proportion in the population is low. They can perform this discrimination even when three strains of males are present. The olfactory recognition required for this discrimination entails a hierarchically ordered recognition system and a natural unit of olfactory strength.*

"This leads me to say a few words on what I have called Sexual Selection. This form of selection depends, not on a struggle for existence in relation to other organic beings or to external conditions, but on a struggle between individuals of one sex, generally the males, for the possession of the other sex. The result is not death to the unsuccessful competitor, but few or no offspring" (*1*).

Thus did Darwin state the nature of evolution resulting from competition for mates. Such competition requires two kinds of information: a recognition of different classes of potential mates and a bias in selecting a mate from one class rather than another. Petit, Spiess, Ehrman, and others have shown that, in drosophilids and in some other insect species, one type of mate selection bias depends on the proportion of the two classes (that is, genetic strains) in a population, with a positive bias for the strain in least abundance (*2*). This "rare male advantage" is mediated by odor in *Drosophila pseudoobscura* and possibly in other species. A recognition system mediated by odor raises several questions with regard to the type of neural networks required to organize and quantify olfactory information and about the nature of olfactory cues.

Drosophila mating behavior has been extensively studied and appears to involve a complex of auditory, chemical, tactile, and visual cues that govern the interaction between males and females, leading to persistent courtship by the males and to final acceptance (or repeated rejection) of a male by a female (*2, 3*). During this interaction in *D. pseudoobscura*, males are indiscriminately active while females are discriminatingly passive. Each female must classify potential mates on at least three levels. Level 1 recognition determines whether the male is of the right species. Because this is a right or wrong binary choice under heavy selection pressure, it is undoubtedly genetically programmed. It also appears to involve more than one sensory modality; several studies have been conducted concerning the features required for species recognition (*4*). Level 2 recognition has also been the subject of several studies; it involves assessment of the "vigor" of the courting male (*2*). Some genetic strains are more readily accepted as mates than others. For example, there are mutant strains of males that are virtually incapable of competing with wild-type males for mates, even with females of their own genetic strain. The recognition mechanism here may involve assessment of the male's execution of the mating ritual, as judged against an internal (genetically programmed) or external (learned) standard. Level 3 recognition controls the rare male advantage. Three separate kinds of information are required for it. (i) The population must be classified into the various strains present. (ii) The classified strains must be quantitated to measure