rhizoids. The pattern is identical to that described for normal secondary rhizoid formation in fern gametophytes (*5*).

These observations support the hypothesis that asymmetry of cell division is a key factor in cellular differentiation (*1*). In our example, the differentiation of a cell into a rhizoid is associated with the size of the cell in which the nucleus resides. A treatment that replaces the normal, unequal division of a fern spore with an equal division drastically changes the differentiation of one of the daughter cells. In an other instance, extensive twinning seems to be induced in spores of the fern *Anemia phyllitidis* by treatment with allogibberellic acid (*6*). Spore germination in *Anemia*, however, normally occurs with a symmetrical first division that produces equal cells; the rhizoid does not originate until one of the equal cells divides asymmetrically (*7*). The effect of allogibberellic acid could be the result of the suppression of the second cell division combined with some enlargement of each of the initially equal cells.

The most interesting feature of the system is an apparent coupling between cytokinetic asymmetry and cellular differentiation, which can be controlled by a simple technique. One may produce for comparative purposes populations of spores in which either (i) rhizoid differentiation occurs normally or (ii) essentially no rhizoids are produced and all cells are protonemal.

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8. Supported by a grant from the Syracuse University Senate Research Committee.

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[^{3}H]Morphine Localization in Myenteric Plexus

Abstract. *Preferential binding of ^{3}H-labeled morphine to satellite cells, but not to large neurons in the myenteric plexus, is demonstrated autoradiographically. Microfluorometric spectra of the plexus show nerve fibers that contain norepinephrine and impinge on satellite cells. Cells containing serotonin occur occasionally on longitudinal muscle outside the myenteric plexus.*

To demonstrate that narcotic analgesic drugs act at a specific anatomical site, it is important that their localization be correlated with various pharmacological, physiological, and biochemical parameters. There is considerable evidence suggesting interactions of morphine with one or more neurotransmitters, but such evidence regarding specific neurotransmitters is often confusing, contradictory, and incomplete. Acetylcholine, catecholamines, and indolealkylamines have all been implicated in the pharmacological effects of morphine (*1*). While a major site of action of narcotic analgesics is considered to be within the central nervous system, the complexity of the brain and neurotransmitters has made the elucidation of the mode of action of such analgesics a formidable task. To circumvent some of the problems associated with the study of brain a simpler peripheral system was proposed as a model (*2*). This system, consisting of isolated guinea pig ileum with the myenteric plexus attached to the longitudinal muscle layer, is useful for the study of narcotic drugs and their antagonists.

The myenteric plexus preparation serves as a model of the central nervous system because it contains neurons, satellite cells, glial elements, and nerve fibers. It has a large surface area, which facilitates the diffusion of drugs into the tissue from incubation media and, because it is stripped from the circular muscle of the ileum, it is sufficiently thin for use in cellular autoradiography and fluorescence microscopy. Experiments with isolated myenteric plexus preparations have demonstrated that the agonist and antagonist actions of narcotic analgesic agents are dose-dependent and stereospecific. Morphine and other narcotic agonists inhibit the contraction of ilia smooth muscle, and such inhibition is reversed by narcotic antagonists (*3*).

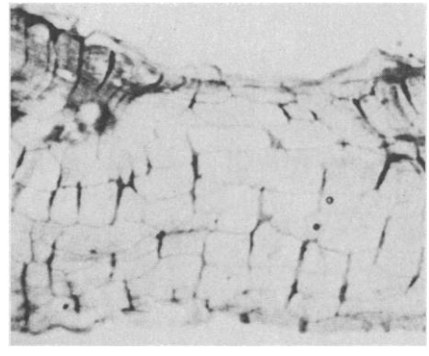

Fig. 1. Guinea pig ileum preparation. The longitudinal muscle strip contains the myenteric plexus which has a honeycomb appearance. Stained with 0.01 percent toludine blue (×16).

Using combined autoradiography, fluorescence microscopy, and microspectrofluorometry we have identified those cells within the myenteric plexus preparation that became labeled with [^{3}H]morphine; we have characterized the biogenic amines by their excitation and emission spectra, and have correlated the localization of morphine and putative neurotransmitters within the same preparation.

Female guinea pigs (150 to 200 g) are starved for 24 hours prior to decapitation. The ilia are cut 2 cm from the cecum to a length of 20 to 30 cm, flushed with ice-cold Tyrode's solution, and divided into segments of 4 to 5 cm in length. A flushed segment is then pulled over a 2-ml pipette for isolation of the longitudinal muscle layer with the attached myenteric plexus. The external longitudinal muscle layer is separated from the internal circular muscle at one end of the segment by gentle rubbing with a Q-tip wetted with Tyrode's solution. The separated longitudinal muscle is then grasped with a small tweezer and stripped free from the circular muscle along the entire length of the intestinal segment. The isolated longitudinal muscle strip with the myenteric plexus attached is placed in a petri dish containing ice-cold Tyrode's solution for dissection of 4- to 6-mm sections, and examined for the presence of plexus under the dissecting microscope. The attached plexus on the longitudinal muscle is identified by its honeycomb appearance (Fig. 1). These sections are then incubated in Tyrode's solution containing 0.1 μg/ml of [^{3}H]morphine (1 μc/ml) for 1 minute at 25°C, washed three times for 1 minute in nonradioactive Tyrode's solution to remove the unbound [^{3}H]morphine, and mounted directly on Formvar-coated microscope slides without adhesive or media. The sections are dried in air and then stored over Drierite for 24 hours. Sections prepared in this manner may be coated directly with NTB-3 Kodak liquid emulsion for autoradiography. For the combined cytopharmacological method, a Formvar-coated slide is cemented at one end to a cover slip previously coated with NTB-3 emulsion. Both slide and cover slip are of the same optical glass

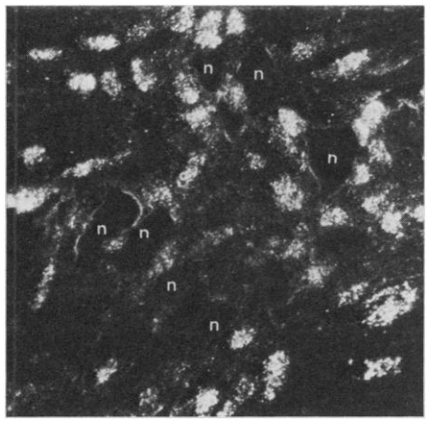

Fig. 2. Autoradiogram of myenteric plexus preparation incubated with [^{3}H]morphine (1 μc/ml) for 1 minute and washed three times with Tyrode's solution (*10*). The autoradiogram was photographed with dark-field illumination so that the silver grains appear white (*n*, neuron) (×320).

composition and have the same coefficient of expansion (*4*). After 90 days of exposure at −15°C the slide and cover slip are separated in a manner that permits photographic development of the emulsion while the tissue on the slide is left untouched by the photographic solutions. The dry sections are then treated with formaldehyde to induce formation of the fluorophore. The autoradiogram and the "fluorogram" are photographed, and after the microfluorometric spectrum of the fluorophore has been recorded the section is stained (*4*).

Autoradiograms of isolated myenteric plexus preparations that have been incubated in Tyrode's solution containing [^{3}H]morphine and then washed with nonradioactive Tyrode's solution demonstrate a specific localization of [^{3}H]morphine on small satellite cells (Fig. 2). [^{3}H]Morphine was found to be localized on cells surrounding the ganglionic neuron as well as on cells within the nerve fiber network. Because the autoradiograms from the unwashed plexus preparation revealed that the [^{3}H]-morphine was distributed throughout the entire plexus, including the longitudinal muscle layer, we inferred that the [^{3}H]morphine distribution seen after washing was a reflection of binding. Incubation of the plexus preparation with unlabeled naloxone, followed by transfer of the plexus to a Tyrode's solution containing [^{3}H]morphine, resulted in autoradiograms showing no [^{3}H]morphine bound to these cells. The satellite cells or "gliocytes" can be differentiated from other neuronal elements in the plexus by their small size, their spherical to elliptical shape, their location around the large neurons within the plexus junctions, and by their location within the network of nerve fibers (*5*).

The control preparations incubated without [^{3}H]morphine exhibited the specific green fluorescence characteristic of varicosities associated with nerve endings (Fig. 3, A and B). The nerve endings were shown to contain norepinephrine by microfluorometric spectroscopy (*6*). Both norepinephrine and dopamine, when treated with formaldehyde, have fluorescence spectra with an excitation peak at 410 nm and an emission peak at 490 nm. However, when the tissue sample is treated with HCl vapor (*7*) there is a shift in the norepinephrine excitation peak from 410 to 390 nm, and an additional excitation peak occurs at 320 nm. This new spectrum is identical with the observed spectra of a standard norepinephrine sample treated in the same manner (Fig. 4). No such change in the excitation spectrum occurs with a standard sample of dopamine.

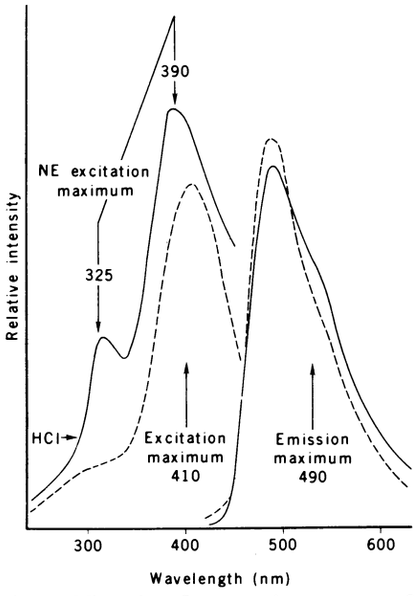

Fig. 4. Microphotofluorometric spectrum of green-fluorescing nerve fiber varicosities in the myenteric plexus. The relative intensity, uncorrected, and the spectra were compared with biogenic amine standards and were found to be consistent with norepinephrine.

Cells with yellow fluorescence are seen occasionally on the longitudinal muscle indicating the presence of serotonin. Such serotonin-containing cells are identified by their short-lived yellow fluorescence on exposure to ultraviolet light; when examined by microspectrofluorometry, their excitation peak occurs at 400 nm and their emission peak at 520 nm. It is doubtful that these cells are neuronal in nature because they are only observed outside the myenteric plexus proper; they are more likely to be mast cells or fibroblasts within the intestinal wall. The presence of serotonin in neuronal cells within the myenteric plexus has been postulated on the basis of experiments in which animals were treated before decapitation with biogenic amine depleting agents, monoamine oxidase inhibitors, or massive amounts of biogenic amine precursors (*8*). In our study the animals received no such treatment nor were we able to show the presence of serotonin in neuronal cells or to demonstrate a correlation between serotonin and morphine. Other cells adjacent to the myenteric plexus are occasionally observed to have a yellow-orange fluorescence which did not fade upon prolonged exposure to ultraviolet light. The microfluorometric spectra of these cells showed an excitation peak of 350 nm and a broad emission maximum at 480 to 555 nm. This yellow-orange flu-

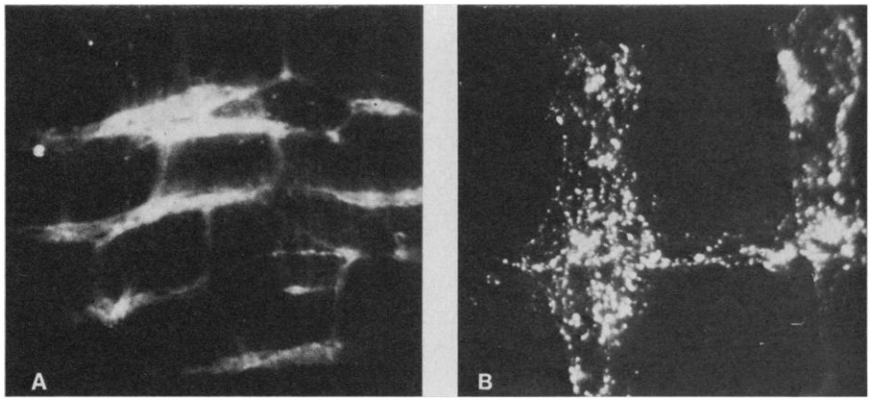

Fig. 3. (A) Fluorogram of myenteric plexus preparation showing nerve fiber fluorescence surrounding the whole plexus. The fluorogram was photographed through a Zeiss microscope illuminated with a mercury H BO 200-watt lamp with an activation filter (Zeiss No. 1) and barrier filters (Zeiss 53 and 47) (×32). (B) Fluorogram of myenteric plexus showing fluorescent varicosities surrounding the neurons of the plexus (×125).

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*).

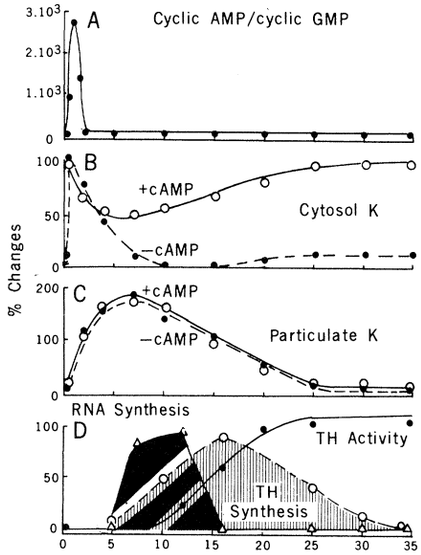

Fig. 1. Sequence of molecular events taking place in chromaffin cells during the transsynaptic induction of TH elicited by exposure to 4°C for 2 hours. The adrenal medullas were dissected stereomicroscopically (*13*). (A) Percent of change in the ratio of cyclic AMP to cyclic GMP concentrations. (B) 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-