Development of Choline Acetyltransferase (CAT) in the Sympathetic Innervation of Rat Sweat Glands

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It has been postulated that the developing sympathetic innervation of rat eccrine sweat glands changes from adrenergic to cholinergic under the influence of its target. In agreement with previous evidence that the sympathetic innervation of *adult* rat sweat glands is cholinergic, we found that choline acetyltransferase (CAT)-immunoreactive nerve fibers are present in adult glands, and that gland-rich chunks of adult footpads contain CAT enzyme activity. We were therefore interested in determining when CAT activity is first expressed in the developing gland innervation. Low levels of acetylating activity were observed in rat footpads as early as postnatal day 4, when sympathetic fibers first contact the glands. A greater than fourfold increase in CAT specific activity occurred between postnatal days 11 and 21. Neonatal treatment of rats with the adrenergic neurotoxin 6-hydroxydopamine (6-OHDA) eliminated most of the CAT activity in 14 and 19 d footpads. In contrast, the acetylating activity observed prior to day 11 was unaffected by neonatal 6-OHDA treatment, and only slightly reduced by the selective CAT inhibitor, naphthylvinylpyridine. These results indicate that the sympathetic fibers that innervate rat sweat glands do not acquire detectable levels of CAT activity until a full week after they contact the glands.

A minority of sympathetic neurons are cholinergic. The best characterized cholinergic sympathetic neurons innervate eccrine sweat glands in the footpads of the cat. Muscarinic agonists mimic the ability of sympathetic nerve stimulation to elicit sweating in cat footpads, and sympathetic nerve stimulation-evoked sweating is blocked by muscarinic antagonists (Foster and Weiner, 1970; Langley, 1922). In contrast, adrenergic agonists do not elicit sweating, and adrenergic antagonists do not inhibit nerve stimulation-evoked sweating except at very high concentrations. Moreover, stimulation of the sympathetic nerves that innervate cat sweat glands results in the appearance of ACh in the venous effluent of the footpads (Dale and Feldberg, 1934). AChE is present in the nerve terminals around the sweat glands (Hellmann, 1955), and the sympathetic ganglia that innervate the sweat glands contain a population of intensely AChE-positive neurons (Sjöqvist, 1963a, b).

Rats, like cats, have sweat glands concentrated in their footpads (Ring and Randall, 1947), and there is evidence that the sympathetic innervation of rat sweat glands is also cholinergic. Injection of muscarinic agonists into rat footpads elicits sweating, while adrenergic agonists have at best weak and inconsistent effects (Hayashi and Nakagawa, 1963; Stevens and Landis, 1984). Spontaneous and nerve-evoked sweating in rat footpads is

blocked by muscarinic antagonists and is unaffected by adrenergic antagonists (Stevens and Landis, 1984). Finally, we show here that the sympathetic innervation of rat sweat glands contains choline acetyltransferase (CAT) activity and that intact footpads can synthesize and store ACh.

The sympathetic innervation of *adult* rat sweat glands thus appears by several criteria to be cholinergic. However, it has been shown that when sympathetic fibers first arrive at the sweat glands early in postnatal development, they exhibit several cytochemical traits characteristic of noradrenergic sympathetic neurons. These include catecholamine fluorescence, tyrosine hydroxylase (TH) and dopamine- β -hydroxylase (DBH) immunoreactivity, and small granular vesicles after permanganate fixation (Landis and Keefe, 1983; Siegel et al., 1982). The developing fibers are also sensitive to the adrenergic neurotoxin 6-hydroxydopamine (6-OHDA), suggesting that they possess a norepinephrine (NE) uptake system (Yodkowski et al., 1984). These noradrenergic properties gradually decrease as the gland and its innervation mature during the first few weeks of postnatal life. Neither catecholamine fluorescence nor small granular vesicles are detectable in the adult innervation, although faint TH and DBH immunoreactivity can still be seen in some fibers. The fibers also appear to retain a catecholamine uptake system: They become fluorescent after loading with α -methylnorepinephrine.

As the developing sweat gland innervation loses its adrenergic properties, it begins to acquire the AChE activity and vasoactive intestinal polypeptide (VIP)-like immunoreactivity that are characteristic of the adult innervation. These findings suggest that the neurons innervating rat sweat glands undergo a transition from noradrenergic to cholinergic under the influence of their target tissue. However, the time of onset of cholinergic function in the sweat gland fibers remains to be established. Hence, it is possible that the transition from noradrenergic to cholinergic begins before the fibers reach the sweat glands. It is also possible that the sweat gland neurons initially express cholinergic as well as noradrenergic traits and that the observed cytochemical changes simply reflect an increasing predominance of the cholinergic over the noradrenergic phenotype. The presence of cholinergic properties in the newly arrived innervation would favor these latter possibilities. The fact that AChE activity does not appear in the fibers until a week after they have reached the sweat glands suggests that the fibers have not yet differentiated cholinergically when they first arrive at the target; however, AChE is known to be present in some noncholinergic neurons and, hence, is not a definitive marker for cholinergic function. The aim of the present study was to define the time of onset of cholinergic function in the neurons that innervate rat sweat glands by assaying the development of CAT activity in the gland innervation.

Materials and Methods

Litters of newborn rats and adult rats (CD strain) were obtained from Charles River, Wilmington, MA. Rats were killed by CO₂ inhalation,

Received May 28, 1985; revised July 25, 1985; accepted July 26, 1985.

We would like to thank Drs. Eve Wolinsky and Robert Baughman for their helpful advice during this work. This research was supported by U.S. Public Health Service Grants NS15549, NS02253, and NS07112, and a grant from the Dysautonomia Foundation.

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Figure 1. Immunocytochemical localization of CAT. CAT-immunoreactive fibers cut in cross (*arrowhead*) or tangential (*arrow*) section are evident in the sweat glands of adult rats. *a*, Brightfield micrograph: the cells of the secretory tubule are barely visible due to low background level of endogenous peroxidase activity. *b*, Normarski interference contrast micrograph: the cells of the tubule are clearly visible. Each fiber visualized with the light microscope represents a bundle of 8–12 axons, so individual varicosities cannot be discerned. $\times 320$.

and the anterior and medial footpads from each hindfoot were removed. Footpads taken from animals aged 11 d or older were further dissected to remove the dermis and epidermis, so that only the subcutaneous portion of the footpad (containing primarily sweat glands, arteries, arterioles, and connective tissue) was used. It was not possible to do this in footpads of younger animals, because the developing sweat glands are too closely associated with the epidermis, from which they arise.

Some litters of rats were injected with 6-OHDA in order to destroy the sympathetic innervation of the footpads (Yodkowski et al., 1984). In initial experiments, 6-OHDA (100 mg/kg, dissolved in 0.9% NaCl containing 1 mg/ml ascorbic acid) was injected subcutaneously daily on postnatal days 1–7 and again on day 12. However, this injection schedule resulted in rather high (25%) mortality rates. In subsequent experiments, rats were injected on postnatal days 1, 3, 5, 8, and 13. This protocol resulted in improved survival rates and appeared equally effective in destroying sympathetic fibers; irides of rats treated with 6-OHDA according to either protocol were devoid of fluorescent fibers when examined by glyoxylic acid histochemistry.

For determinations of CAT activity, footpads were homogenized extensively in ground-glass homogenizers (Kontes) in 50 mM sodium phosphate buffer, pH 6.8, containing 200 mM NaCl and 0.5% Triton X-100. CAT activity was assayed in the crude homogenates according to the method of Fonnum (1975). The final assay mixture (total volume = 30 μ l) contained 10 mM choline bromide, 0.1 mM eserine sulfate, 0.17 mg/ml bovine serum albumin, 135 μ M [3 H]acetyl coenzyme A (final specific activity = 176 Ci/mol), and 15–20 μ l of tissue homogenate. The reaction was run for 20 min at 37°C. In some experiments, naphthylvinylpyridine (PVN, Calbiochem) was added to some reaction tubes, usually at a final concentration of 0.3 mM. Stock solutions of PVN were made immediately before use by dissolving the compound in CO₂-saturated distilled water. Experiments in which PVN was used were conducted in a darkened room.

[3 H]ACh synthesis was measured in intact footpads using modifications of previously described methods (Hildebrand et al., 1971; Mains and Patterson, 1973). Footpads were preincubated for 15 min in 75 $\times$ 100 mm borosilicate glass culture tubes containing 50 μ l of choline-deficient L15 medium (Grand Island Biological Co.) supplemented with 6 mg/ml glucose. The medium was then replaced with 50 μ l of medium to which varying concentrations of [3 H]choline had been added, and the footpads were incubated for 15–60 min. The medium was removed, 50 μ l of L15 (containing 1 mg/liter choline) was added, and the footpads were incubated for an additional 15 min. All incubations were carried out at 37°C in a shaking metabolic incubator under oxygen. At the end of the incubation period, the footpads were transferred to small plastic tubes containing 21 μ l of pH 1.9 electrophoresis buffer (4% formic acid and 16% acetic acid in water) containing 0.3% sodium dodecyl sulfate, and unlabeled ACh and choline standards. [3 H]ACh and [3 H]choline were extracted from the footpads into the buffer by freezing and thawing the sample tubes twice and then crushing the footpads in the buffer. The extraction buffer was then removed from the tube and analyzed by high-voltage paper electrophoresis using a modification (Mains and Patterson, 1973) of the method of Hildebrand et al. (1971).

For immunocytochemical studies, a mouse antiserum raised against CAT purified from pig brain (Eckenstein et al., 1981) was used. Binding of the primary antiserum was visualized with a double cycle peroxidase–anti-peroxidase procedure as follows (F. Eckenstein, personal communication; Ordronneau et al., 1981). Six-week-old rats were anesthetized with ether and perfused for 10 min with 4% paraformaldehyde buffer in 0.1 M phosphate buffer, pH 7.3, containing 15% by volume of a saturated aqueous solution of picric acid. The footpads were removed and fixed for an additional 50 min in the same solution. The pads were rinsed several times with 0.1 M phosphate buffer and then equilibrated with 30% sucrose in phosphate buffer. Twenty micron cryostat sections were cut. The sections were incubated overnight at room temperature in mouse anti-CAT (the gift of Felix Eckenstein) diluted 1:1000 in incubation buffer containing 0.1 M Tris buffer, pH 7.3, 150 mM sodium chloride, 1% bovine serum albumin, and 5% Triton X-100. The sections were rinsed several times with incubation buffer and then incubated in affinity-purified goat anti-mouse immunoglobulins (Boehringer-Mannheim) diluted 1:40 in incubation buffer containing 10% rat serum. After rinsing in incubation buffer, the sections were incubated in a mouse monoclonal antibody to HRP–peroxidase complex (PAP; Sternberger-Meyer) diluted 1:80 in incubation buffer containing rat serum. The sections were then cycled a second time through the goat anti-mouse antiserum and mouse PAP. The sections were reacted with diaminobenzidine and peroxide in 0.1 M Tris-HCl, pH 7.2, mounted on gelatin-coated slides, dehydrated, and mounted in Permount (Fisher). No staining was observed if the primary antiserum was omitted or if pre-immune mouse serum was substituted for the primary antiserum.

Except where noted, chemicals were obtained from Sigma. Isotopes were obtained from New England Nuclear.

Results

In footpad sections from adult rats, CAT immunoreactivity was evident in nerve fibers in the sweat glands (Fig. 1). No staining was detected in the sensory fibers of the dermis or epidermis, nor associated with the arteries and arterioles present in the connective tissue between the glands. The immunoreactive fibers coursed between the coils of the secretory tubule in a pattern identical to that observed after histochemical staining for AChE or immunocytochemical staining for VIP (Landis and Keefe, 1983; Siegel et al., 1982).

In agreement with a previous preliminary report (Woodward and Uno, 1981), we found that homogenates of adult rat footpads contain significant levels of choline acetylating activity (Table 1A). This activity could be due either to CAT or to carnitine acetyltransferase, a mitochondrial enzyme that is present in many tissues and can also acetylate choline (White and Wu, 1973). We therefore examined the effect of a selective CAT inhibitor, PVN, on acetylation in rat footpad homogenates. PVN caused a 91% reduction in acetylating activity; a similar reduc-

Figure 2. Development of choline acetylating activity in rat footpads. Each point represents the mean $\pm$ SEM of activity determinations in 3 or 4 homogenates, each containing footpads pooled from 1 or 2 rats. Aliquots of each homogenate were assayed in duplicate in the presence and absence of 0.3 mM PVN. Inset: Development of [3 H]ACh synthesis in intact rat footpads *in vitro*. Two footpads from each animal were incubated in separate tubes in the presence of 2.5 μ M [3 H]choline for 45 min, and the [3 H]ACh synthesis rates for the two footpads were averaged. Each point is the mean $\pm$ SEM of the average synthesis rates in three animals.

tion in activity was observed when choline was left out of the assay mixture (Table 1A). These results indicate that most of the choline acetylating activity present in rat footpads is due to CAT rather than to other acetylating enzymes.

We also examined the ability of intact chunks of rat footpads to synthesize and store [3 H]ACh when incubated with [3 H]choline. Table 1B shows that intact footpads do accumulate significant levels of 3 H-ACh. Moreover, [3 H]ACh accumulation was reduced 50% by 5 μ M hemicholinium-3, a blocker of high-affinity choline uptake. This finding suggests that the sympathetic fibers that innervate the sweat glands are similar to other cholinergic fibers insofar as they possess a high-affinity uptake mechanism for choline.

The time course of development of acetylating activity in rat footpad homogenates is shown in Figure 2. Acetylating activity was again assayed in the presence and absence of PVN in order to determine what proportion of the activity was due to CAT. Low levels of acetylating activity were observed in the footpads as early as postnatal day 4, when sympathetic fibers first reach the sweat glands. PVN consistently produced small (about 15%) decreases in the activity of both 4 and 7 d footpads. However, no [3 H]ACh synthesis was detectable in 7 d pads (Fig. 2, inset). It is therefore unclear from these results whether 4 and 7 d footpads contain any CAT activity.

A marked increase in acetylating activity occurred around postnatal day 11. Activity then rose steadily until day 21. In 11 day footpads, PVN caused a 46% inhibition of acetylating activity; by 21 d, the level of inhibition had increased to 69%. Thus, CAT accounts for an increasingly large proportion of total

acetylating activity as the pads mature. If we assume that the PVN-sensitive component of acetylating activity is equal to the level of CAT activity present in the footpads, then CAT activity increased approximately fourfold between postnatal days 14 and 21. Both the PVN-insensitive acetylating activity and total protein content of the footpads only rose twofold during the same period, suggesting that a specific induction of CAT occurs in the developing pads. The development of [3 H]ACh synthesis in intact footpads paralleled that observed for CAT activity in footpad homogenates (Fig. 2, inset).

Footpads are complex pieces of tissue that contain sensory as well as sympathetic nerve fibers. Also, muscle fibers with their accompanying cholinergic innervation may contaminate footpad samples obtained from younger animals. Hence, we wished to determine whether CAT activity observed in developing rat footpads does in fact arise from the sympathetic innervation of the pads. Since it has been shown that neonatal 6-OHDA treatment destroys the developing sympathetic innervation of the sweat glands (Yodkowski et al., 1984), we examined the effect of neonatal 6-OHDA treatment on the development of CAT activity in the footpads.

Footpads of rats treated neonatally with either 6-OHDA or vehicle were assayed for acetylating activity on postnatal day 4, 9, 14, or 19. Irides of the 6-OHDA-treated animals were devoid of catecholamine fluorescence after glyoxylic acid fixation, suggesting that the treatment protocol was effective in destroying sympathetic terminals. Mean body weights of the 6-OHDA-treated animals were reduced 10–18% compared with control animals, depending on the age at which they were examined. 6-OHDA treatment also reduced the protein content of 14 and 19 d footpads by 30 and 15%, respectively. Protein content of 4 and 9 d footpads was not measured, because at these ages it is not possible to separate the gland-rich portion of the pads from the overlying dermis. Acetylating activity in footpads of these ages is therefore expressed in terms of activity per pad/gm body weight.

The 6-OHDA treatment decreased the specific acetylating activity of both 14 and 19 d footpads by approximately 75% (Fig. 3). In a separate experiment (Table 2), we examined the effect of PVN on acetylating activity in footpad homogenates of control and 6-OHDA-treated 19 d rats. PVN still caused some reduction in the activity of footpads of 6-OHDA-treated rats. However, it can also be seen that 6-OHDA treatment eliminated 89% of the PVN-sensitive acetylating activity of 19 d footpads. These results suggest that virtually all of the CAT activity present in rat footpads arises from the sympathetic innervation of the pads.

Table 1. Acetylating activity and [3 H]ACh synthesis in adult rat footpads

A. Acetylating activity	pmol/pad/hr
Control	389.4 $\pm$ 1.7
+ 0.3 mM PVN	36.9 $\pm$ 6.4
- choline	25.9 $\pm$ 9.9
B. [3 H]ACh synthesis	fmol/pad/hr
Control	36.2 $\pm$ 3.3
5 μ M Hemicholinium	18.1 $\pm$ 0.3

A. Aliquots of a homogenate containing two adult rat footpads were assayed in triplicate under the conditions specified. Each figure is the mean $\pm$ SEM of these triplicate determinations. B. Intact footpads were incubated in the presence of 0.5 μ M [3 H]choline for 1 hr. Each figure is the mean $\pm$ SEM of determinations in three separate footpads.

Figure 3. Effect of neonatal 6-OHDA treatment on acetylating activity in rat footpads. Animals were injected with 6-OHDA (100 mg/kg) or vehicle on postnatal days 1, 3, 5, 8, and 13, and killed at the ages indicated. Each bar represents the mean $\pm$ SEM of activity determinations in the number of homogenates indicated on the bar. Each homogenate contained footpads pooled from one rat. Note that acetylating activity is expressed in different units on the left and right sides of the figure. *, Differs from control group at $p < 0.005$, ** $p < 0.025$ (one-tailed Mann-Whitney U test).

6-OHDA treatment produced small (15%) but statistically insignificant decreases in the acetylating activity of 4 and 9 d footpads. 6-OHDA treatment caused similar decreases in the body weights of these animals; acetylating activity per gram body weight was thus not affected by 6-OHDA treatment in 4 and 9 d animals (Fig. 3). Moreover, PVN inhibited acetylating activity to the same extent in the footpads of 9 d 6-OHDA-treated rats as it did in control rats of the same age (not shown). These results suggest that the low level of PVN-sensitive activity observed prior to postnatal day 11 reflects either (1) CAT present in muscle innervation in the pads or (2) a nonspecific inhibitory effect of PVN. The finding that 6-OHDA treatment greatly reduced CAT activity in 14 and 19 d footpads, but did not affect acetylating activity in 4 or 9 d footpads, indicates that the sympathetic fibers in the footpads do not contain detectable CAT until after postnatal day 9.

Discussion

The present results support and extend previous evidence that the sympathetic innervation of adult rat sweat glands is cholinergic. CAT immunoreactivity was observed in the nerve fibers surrounding the sweat glands, and CAT enzyme activity was present in footpad homogenates. Synthesis and storage of [3 H]ACh was also observed in intact, gland-rich footpad chunks. Finally, we obtained indirect evidence that a high-affinity choline uptake system, another characteristic feature of cholinergic nerve terminals, is present in the footpads.

In most classes of cholinergic neurons, cholinergic traits appear very early in development. For instance, the initial expression of CAT activity usually precedes the establishment of connections with target structures (Bader et al., 1978; Burt, 1968; Smith et al., 1979). Thus, CAT can be detected in the developing chick limb bud immediately on the arrival of skeletal motor fibers (Burt, 1968; Giacobini, 1972) and in the iris as soon as it becomes innervated by the parasympathetic fibers of the ciliary ganglion (Chiappinelli et al., 1976). In contrast, we were unable to detect either CAT activity or [3 H]ACh synthesis in the sympathetic fibers that innervate rat sweat glands until a full week after their arrival at the glands. Thus, the sweat gland neurons, unlike other cholinergic neurons, do not detectably express the cholinergic phenotype when they first contact their target structure.

The postnatal development of the sympathetic innervation

Table 2. Effect of PVN on acetylating activity in footpads of 19-d-old control and 6-OHDA-treated rats

	Total activity (pmol/pad/hr)	Control - PVN (pmol/pad/hr)
Control + PVN	102.1 $\pm$ 7.0	
	32.1 $\pm$ 7.6	70.1 $\pm$ 0.5
6-OHDA + PVN	23.4 $\pm$ 1.1	
	16.0 $\pm$ 2.5	7.4 $\pm$ 1.4

Rats received injections of 6-OHDA (100 mg/kg) or vehicle on postnatal days 1–7 and 12, and were killed on Day 19. The footpads of each rat were pooled and homogenized, and aliquots of the homogenate were assayed duplicate or triplicate in the presence or absence of 0.5 mM PVN. Each figure is the mean $\pm$ range of activity determinations in two rats.

of the sweat glands appears to involve not only the acquisition of cholinergic traits, but also the gradual reduction or loss of certain previously expressed noradrenergic properties (Landis and Keefe, 1983; Siegel et al., 1982). The disappearance of noradrenergic properties from the sweat gland innervation occurs with a time course similar to that described here for the appearance of CAT activity in the innervation. For instance, CAT activity was first detected in the sweat gland fibers around postnatal day 11; the intensity of catecholamine fluorescence in the fibers begins to diminish around day 14. These findings are consistent with the idea that the sweat gland innervation changes from noradrenergic to cholinergic as it matures. An alternate explanation is that the delayed appearance of CAT activity in the footpads reflects a late ingrowth of cholinergic fibers. However, neither histochemical nor ultrastructural examinations of the developing footpads provide evidence for the arrival of a second population of fibers. Moreover, we have shown here that the appearance of CAT activity in the pads is prevented by neonatal 6-OHDA treatment, indicating that CAT arises in nerve fibers possessing a catecholamine uptake system.

The apparent shift in the neurotransmitter phenotype of the sweat gland neurons could be a quantitative one, in which the neurons initially express a low level of both adrenergic and cholinergic traits, and ensuing developmental events then favor the further expression of only cholinergic traits. However, we did not detect CAT activity in the sweat gland fibers when they first innervate the glands, and AChE is not detectable in most fibers by histochemical methods until postnatal day 10. Moreover, the sweat gland fibers initially contain the same proportion of small granular vesicles, as do nearby fibers that innervate blood vessels and that never become cholinergic (Landis and Keefe, 1983). While each assay for the cholinergic phenotype has a finite sensitivity, the present evidence is consistent with the idea that the sweat gland fibers are simply adrenergic at the outset.

The finding that the sweat gland innervation loses its adrenergic properties and acquires cholinergic ones only after it has been in contact with the target for several days is consistent with the hypothesis that the target is responsible for mediating these changes. The idea that nonneuronal cell-derived factors can alter the neurotransmitter phenotype of developing sympathetic neurons gains support from studies of the differentiation of neonatal rat superior cervical ganglion (SCG) neurons *in vitro*. When these neurons are cultured in the absence of nonneuronal cells, they retain their noradrenergic phenotype, as assessed by the ability to synthesize and store NE and the presence of numerous small granular vesicles (Landis, 1980; Patterson and Chun, 1977b; Walicke et al., 1977; Wolinsky et al., 1985). However, when the neurons are cultured in the presence of nonneuronal cells or medium conditioned by them, they acquire cholinergic properties such as CAT activity, synthesis and storage of ACh, formation of cholinergic synapses, and a

predominance of small clear vesicles (Furshpan et al., 1976; Landis, 1980; O'Lague et al., 1978; Patterson and Chun, 1974, 1977a). At high concentrations of conditioned medium (CM), the acquisition of cholinergic properties is accompanied by suppression of adrenergic ones, as evidenced by reduced TH, dopa decarboxylase, and DBH activities and NE synthesis (Patterson and Chun, 1977a; Swerts et al., 1983; Wolinsky and Patterson, 1983). SCG neurons grown in the presence of human placental serum and chick embryo extract also differentiate cholinergically (Johnson et al., 1976; Wakshull et al., 1978). However, under these culture conditions, the activities of NE-synthesizing enzymes rise in parallel with CAT (Iacovitti et al., 1981; Johnson et al., 1980). It is not clear whether the active factor in the two different culture systems is identical. Nevertheless, both sets of results indicate that diffusible, nonneuronal cell-derived factors can elicit changes in cultured sympathetic neurons that are similar to those observed in the sweat gland innervation *in vivo*. It remains to be determined if such a factor does, in fact, mediate the changes seen *in vivo* and, if so, what the source of this factor might be.

A second factor that may contribute to the regulation of CAT activity in sweat gland neurons is NGF. Treatment of neonatal rats with anti-NGF destroys the developing sympathetic innervation of the sweat glands (Landis et al., in press), just as it does the adrenergic sympathetic innervation of targets such as heart and iris (Gorin and Johnson, 1980; Levi-Montalcini and Booker, 1960). Thus, as has been proposed for the latter systems, it appears that the developing sweat gland neurons are normally exposed to NGF or an antigenically related molecule and that their survival depends on this factor. In cultured sympathetic neurons that have been induced to differentiate cholinergically by the presence of CM, NGF not only enhances neuronal survival, but also increases levels of ACh synthesis per neuron, suggesting that NGF has a trophic effect on CAT activity (Chun and Patterson, 1977b). NGF administration also enhances CAT activity in parasympathetic ciliary ganglion neurons *in vivo* (Kessler, 1985) and in cholinergic forebrain neurons, both *in vivo* and *in vitro* (Gnahn et al., 1983; Hefti et al., 1985; Honegger and Lenoir, 1982). However, while NGF may be important in maintaining CAT enzyme levels in sweat gland neurons, it seems unlikely for several reasons that NGF is responsible for inducing these neurons to change from adrenergic to cholinergic. First, NGF enhances catecholamine synthesis in neurons cultured in the absence of CM to the same extent that it enhances ACh synthesis in neurons grown in the presence of CM (Chun and Patterson, 1977a, b). Similarly, NGF enhances TH and DBH activity *in vivo* to an equal or greater extent than it does CAT activity (Kessler, 1985; Thoenen et al., 1971). Second, NGF appears to be present in most sympathetic target structures (Korsching and Thoenen, 1983; Shelton and Reichardt, 1984), but only the innervation of the sweat glands changes from adrenergic to cholinergic.

The time course of appearance of CAT activity in the innervation of rat sweat glands resembles that previously reported for AChE and VIP. Hence, it is possible that a single factor regulates the expression of all three of these proteins in the sweat gland neurons. However, coordinate expression of CAT and AChE, at least, does not appear to be a general rule for sympathetic neurons. For instance, while CM promotes the expression of CAT in cultured sympathetic neurons, it has been found to decrease levels of AChE activity in these neurons (Swerts et al., 1984). Furthermore, the adrenergic sympathetic innervation of the kidney and pineal contain AChE, but not CAT (Barajas and Wang, 1975; Eranko et al., 1970). Whether CAT and VIP are always expressed together in sympathetic neurons remains to be determined. In sympathetic ganglia of the cat, VIP does tend to be localized in those sympathetic neurons that are presumed to be cholinergic on the basis of their high cholinesterase

content (Lundberg et al., 1979). It is not known what tissues other than the sweat glands are innervated by cholinergic sympathetics in the rat, although there is evidence for a cholinergic sympathetic innervation of skeletal muscle vasculature in other species (Uvnäs, 1970). It will be interesting to determine whether all cholinergic sympathetic neurons undergo the same apparent change from adrenergic to cholinergic phenotype that has been observed in the innervation of the rat sweat glands.

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