Rapid recovery of function after partial denervation of the rat pineal gland suggests a novel mechanism for neural plasticity

(norepinephrine uptake/serotonin N-acetyltransferase/superior cervical ganglion/sympathetic nervous system)

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ABSTRACT The activity of serotonin N-acetyltransferase (NATase) in the rat pineal gland exhibits a large (approximately 100-fold) circadian variation, with peak activity occurring in the dark part of the light/dark cycle. Surgical removal of both superior cervical ganglia abolishes this rhythm in enzyme activity. Unilateral ganglionectionomy caused a 75% decrease in NATase activity during the dark period immediately following the operation; however, by the subsequent dark period (32 hr after operation) the rhythm in NATase activity had returned to normal. Similar results were found after the internal carotid nerve was cut, and data are presented indicating that this is the postganglionic trunk by which sympathetic neurons reach the pineal gland. Denervation of one superior cervical ganglion (unilateral "decentralization") also produced a 75% decrease in NATase activity during the dark period immediately following the operation; however, after decentralization, enzyme activity did not return to normal in subsequent cycles. It is hypothesized that this recovery is due to loss of norepinephrine uptake sites in the degenerating sympathetic nerve terminals. As a result of decreased norepinephrine uptake, the effectiveness of the norepinephrine released by surviving neurons may be enhanced. This hypothesis is supported by experiments in which pharmacological blockade of norepinephrine uptake in unilaterally decentralized animals increased NATase activity to control levels. We propose that neural systems which use transmitter uptake as the mechanism of transmitter inactivation have a built-in "reserve stimulatory capacity."

Although a great deal of interest has been focused in recent years on the ability of the nervous system to recover after subtotal neural damage, there are only a few systems available in which functional recovery has been studied quantitatively on a cellular level. A useful system for such an investigation should have two characteristics: (i) permit the making of a lesion of a reproducible size in a specific part of the nervous system, and (ii) permit quantitative measurement of the degree of functional recovery after the lesion is made. The innervation of the rat pineal gland offers such a model system.

The pineal gland is a midline structure innervated by adrenergic sympathetic neurons whose cell bodies are located in the right and left superior cervical ganglia (SCGs) (1–4). In the rat these ganglia are the primary source of neurons innervating this tissue (3–5). Ganglionic neurons are important in regulating a number of aspects of the biochemistry of the pineal parenchymal cells, particularly the synthesis of the hormone melatonin. Pinealocytes synthesize melatonin from serotonin in two steps catalyzed by the enzymes serotonin N-acetyltransferase (arylamine N-acetyltransferase, EC 2.3.1.5) (NATase) and hydroxyindole O-methyltransferase (acetylserotonin methyltransferase, EC 2.1.1.4).

The sympathetic neurons innervating the pineal gland appear to constitute the final pathway by which changes in environmental lighting alter the activity of these two enzymes (1, 2, 6, 7). The influence of these neurons on pineal biochemistry is most evident in the regulation of the large circadian rhythm in NATase activity (7). This rhythm is almost completely abolished by removal of both SCGs or by cutting the axons of the pre-ganglionic neurons that innervate these ganglia ("decentralization") (7). The residual rhythm in NATase activity (having an amplitude of less than 2-fold) is thought to depend on a rhythm in circulating catecholamines (7, 8).

Further evidence for the involvement of sympathetic neurons in the regulation of NATase activity are the findings that (i) isoproterenol, a β-adrenergic agonist, produces a dose-dependent increase in NATase activity if injected during the light part of the cycle when enzyme activity is normally low, and (ii) propranolol, a β-adrenergic antagonist, decreases NATase activity if injected during the dark part of the cycle when enzyme activity is normally high (9, 10). In addition, it has been shown recently that a change in NATase activity comparable to the normal night-time increase can be produced by bilateral electrical stimulation of the cervical sympathetic trunks at 5 Hz (11).

These results together with measurement of norepinephrine turnover (12) support the hypothesis that the sympathetic nerves innervating the pineal gland fire more rapidly at night than during the day and that the consequent increased release of norepinephrine leads to an increase in NATase activity. Based on these results, we have used NATase activity as an index of synaptic stimulation of the pineal gland after partial denervation. We report here that 8 hr after unilateral denervation the amplitude of the pineal NATase rhythm is decreased by more than 50% but that 32 hr after operation there is a complete recovery of the normal NATase rhythm. In the course of these studies we found evidence for a novel mechanism that may account for recovery of function after partial denervation in a number of neural systems and that also may operate in intact systems to modulate synaptic efficacy.

MATERIALS AND METHODS

Sprague–Dawley rats (100–125 g) were housed in individual cages, kept under a reversed light/dark cycle (lights on at 2200; lights off at 1000), and given food and water ad lib for 10 days after their arrival from the supplier (Charles River Breeding Laboratories). All operations were performed during the 3 hr preceding the onset of the dark period. Animals were anesthetized with chloral hydrate (0.4 g/kg, intraperitoneally). Four types of surgical operations were performed: (i) removal of the SCG; (ii) cutting of the internal carotid nerves (ICNs); (iii) cut-

Abbreviations: NATase, serotonin N-acetyltransferase; SCG, superior cervical ganglion; ICN, internal carotid nerve.
ting of the external carotid nerves; and (c) cutting of the cervical sympathetic trunks ("decentralization"). These operations were performed either unilaterally or bilaterally. Except where noted, all unilateral operations were performed on the right side.

In all but one experiment, animals were killed by decapitation under dim red light (Kodak Safelight filter 2, 15-W bulb) between 5 and 8 hr into the dark period during which NATase activity in intact (not operated on) animals has been found to be maximal in our laboratory (11). Within 1 min after decapitation, the pineals were removed, freed of connective tissue, and frozen (−80°C). NATase activity was measured according to the method of Deguchi and Axelrod (13) as modified by Parfitt et al. (14). Details of the assay conditions have been reported (11). Tubes with no tissue homogenate served as blanks and yielded values equivalent to about 170 pmol of product in 20 min. Pineals from certain groups of animals yielded values less than twice these blank values (i.e., bilaterally decentralized animals, bilaterally denervated animals killed 8 hr after denervation, and all animals killed during the light period). Protein was measured by the method of Lowry et al. (15) with bovine serum albumin as the standard. The protein content per pineal was approximately 130 μg. All data are expressed as mean ± SEM. The NATase values of different groups were compared by using Student's two-tailed t test for two means.

RESULTS AND DISCUSSION

In the first experiment, four groups were studied: animals not operated on, sham-operated animals, and animals in which the SCGs had been removed either unilaterally or bilaterally. Pineal NATase activity was assayed at 5 hr into the dark cycle, approximately 8 hr after the operations were performed. The NATase activities of sham-operated and intact animals were not significantly different (Fig. 1A). Animals that had undergone bilateral ganglionectionomy had extremely low NATase activity, amounting to less than 1% of that in sham-operated controls. Unilaterally ganglionectionomized animals had an intermediate level of NATase activity which was significantly higher than that of the bilaterally ganglionectionomized animals (P < 0.001) and significantly lower than that of the sham-operated animals (P < 0.001). The NATase activity of unilaterally ganglionectionomized animals was also significantly lower than one-half of the values in sham-operated animals (P < 0.005).

When the animals were sacrificed at 32 hr after the same operations (during the second dark period post-operatively), quite different results were found (Fig. 1B). In this case, NATase activity in the unilaterally ganglionectionomized animals was not significantly different from that in the sham-operated or intact animals. In addition, although the NATase activity of the bilaterally ganglionectionomized animals was less than 3% of that in sham-operated controls, it was significantly higher than in the same group in the first experiment (P < 0.01).

Because removal of the SCG results in the denervation of many tissues, we thought that it would be preferable to make a more specific lesion. We could find no evidence in the literature as to the trunk by which the sympathetic nerves innervating the pineal gland reach their target, so we studied the effects of bilateral section of the ICN or the external carotid nerves, the two major postganglionic trunks of the SCG. Bilateral section of the external carotid nerves did not affect NATase activity in the pineal gland; bilateral section of the ICN produced low NATase activity comparable to that seen in bilaterally ganglionectionomized animals (Table 1). These results suggest that all the neurons innervating the rat pineal gland exit from the SCG via the ICN.

![Fig. 1. Effect of removal of the SCG on NATase activity in the pineal gland. Rats were intact (a), sham operated (b), unilaterally ganglionectionomized (c), or bilaterally ganglionectionomized (d) and were killed on the same day (A) or on the day after the operation (B). The number of animals in each group is shown in parentheses. (Inset) Normal circadian variation in NAT activity over several 24-hr light/dark cycles. The dark periods are represented by horizontal lines. The time of the operations is indicated by an arrow; letters indicate cycles studied.](image1)

![Fig. 2. Effect of cutting the ICN on NATase activity in the pineal gland. Rats were either sham operated (b) or the ICN was cut unilaterally (c) or bilaterally (d). The animals were killed on the same day (A), on the following day (B), or 3 days after the operation (C). (Inset) As in Fig. 1.](image2)

### Table 1. Effect of bilateral section of internal (ICN) or external (ECN) carotid nerves on NATase activity in pineal gland

<table>
<thead>
<tr>
<th>Treatment</th>
<th>n</th>
<th>pmol/20 min/μg protein</th>
<th>pmol/20 min/pineal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intact</td>
<td>7</td>
<td>78.2 ± 6.1</td>
<td>10.4 ± 1.2</td>
</tr>
<tr>
<td>Sham operated</td>
<td>9</td>
<td>84.6 ± 9.5</td>
<td>10.3 ± 1.3</td>
</tr>
<tr>
<td>Bilateral ECNX</td>
<td>8</td>
<td>74.5 ± 6.6</td>
<td>8.0 ± 0.8</td>
</tr>
<tr>
<td>Bilateral ICNX</td>
<td>8</td>
<td>1.9 ± 0.4</td>
<td>0.2 ± 0.1</td>
</tr>
</tbody>
</table>

All operations were performed at the end of the light cycle, and the animals were killed 32 hr later (during the dark cycle).
These (see above), operations were approximately 25% of the pineal gland. Eight procedures produced the effect tralization it sham-operated control values whereas after the amined (Fig. 3). Therefore, the cervical sympathetic trunks, which contain the preganglionic input to the SCG (16) (Fig. 3). Therefore, the cervical sympathetic trunks were cut either unilaterally or bilaterally and NATase activity was examined 8, 32, or 80 hr later. At all times studied after unilateral decentralization, the NATase activity was about 25% of the sham-operated control values whereas after bilateral decentralization it was less than 1% of controls (Fig. 4, Table 2).

Thus, the effect of unilateral decentralization was similar to the effect seen 8 hr after section of one ICN. These unilateral procedures produced NATase levels that were 20–30% of control values. Because in all these cases the operations were performed on the right side of the animal, an experiment was designed to ensure that there was no right–left asymmetry in the innervation of the pineal gland. Eight hours after unilateral denervation or decentralization, NATase levels were approximately 25% of control values regardless of the side on which the operation was performed (Table 2). As after unilateral gangliectomy (see above), these values were significantly lower than one-half the values for sham-operated controls ($P < 0.05$). These results indicate that activity in neurons located in both

### Table 2. Effects of unilateral denervation (ICNX) and decentralization (CSTX) on NATase activity in pineal gland

<table>
<thead>
<tr>
<th>Treatment</th>
<th>$n$</th>
<th>NAT activity, pmol/µg protein/20 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham operated</td>
<td>8</td>
<td>80.7 ± 10.4</td>
</tr>
<tr>
<td>ICNX:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Right</td>
<td>8</td>
<td>20.8 ± 6.7</td>
</tr>
<tr>
<td>Left</td>
<td>9</td>
<td>18.3 ± 5.4</td>
</tr>
<tr>
<td>CSTX:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Right</td>
<td>8</td>
<td>22.0 ± 4.9</td>
</tr>
<tr>
<td>Left</td>
<td>8</td>
<td>20.1 ± 6.2</td>
</tr>
</tbody>
</table>

All operations were performed at the end of the light cycle and the animals were killed approximately 8 hr later (during the dark cycle). The ICN or the cervical sympathetic trunk (CST) was cut on either the right or left side (see Fig. 3).

SCG is necessary for the maintenance of the normal rhythm in pineal NATase and that the effects of neurons in the two ganglia are not simply additive. Rather, the data suggest some interaction of the two populations of neurons in their regulation of NATase activity.

All the experiments referred to thus far involved measurements of NATase activity at a single time point during the light/dark cycle. In order to determine whether the NATase activity in unilaterally denervated animals is similar to that in sham-operated animals throughout the cycle and whether the NATase activity in unilaterally decentralized animals is decreased throughout the cycle, enzyme activity was measured at four time points. The earliest measurements were made 48 hr after the operations. The NATase activity in the unilaterally denervated animals was not significantly different from that of sham-operated animals at any time (Fig. 5). However, the values for unilaterally decentralized animals were significantly lower than for sham-operated animals at both 1500 and 2000.

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**Fig. 3.** Diagram of the sympathetic innervation of the pineal (Left) and of the location of the lesions made to unilaterally denervate (Center) and unilaterally "decentralize" (Right) the gland. CST, cervical sympathetic trunk; broken lines, anterograde degeneration resulting from the lesions.

**Fig. 4.** Effect of decentralization on NATase activity in the pineal gland. Rats were sham operated (b) or the cervical sympathetic trunk was cut unilaterally (c) or bilaterally (d). Animals were killed on the following day (A) or 3 days after the operation (B). (Inset) As in Fig. 1.

**Fig. 5.** Effect of unilateral denervation (section of ICN on one side) and unilateral decentralization (section of cervical sympathetic trunk on one side) on the rhythm of NATase activity in the pineal gland. Animals were operated on between 0700 and 1000. Beginning 48 hr later, groups of six animals were killed at 0700, 1500, 2000, and 0100. □, Sham operated; ○, unilaterally denervated; △, unilaterally decentralized.
These results support the conclusion that a normal NATase rhythm rapidly reappears after unilateral denervation of the pineal gland but that a NATase rhythm with a reduced amplitude persists after unilateral decentralization. At least four possible mechanisms might account for the recovery of normal pineal function after unilateral denervation: (a) reinnervation of the pineal, (b) increased sympathetic nerve activity on the intact side, (c) development of postjunctional supersensitivity in the pinealocytes, and (d) loss of uptake sites due to degeneration of the severed axons. Based on the arguments and the preliminary data presented below, we believe that the last hypothesis is the most likely explanation for our findings.

The possibility that the recovery of the NATase rhythm depends on regenerative or collateral sprouting seems remote because in situations in which these processes have been shown to take place in the cervical sympathetic system, they required weeks to months to occur (4, 17-19). The second hypothesized mechanism, an increase in sympathetic nerve activity on the intact side, might compensate for the lack of transmitter release from the sectioned nerves. Although no evidence exists for such a change in nerve firing, an increase in neural activity might be triggered by the decrease in various sympathetic functions that occurs after unilateral ganglionectomy or unilateral cutting of the ICN (e.g., the decrease in melatonin secretion) (20). However, it is difficult to imagine why, if such a reflex existed, it would not also be activated by unilateral decentralization of the SCG because this procedure would be expected to lead to functional losses similar to those after unilateral ganglionectomy (Fig. 3).

Postjunctional supersensitivity to exogenously administered β-adrenergic agonists has been shown to occur in the pineal gland after bilateral ganglionectomy and after bilateral decentralization (21); however, for this mechanism to explain the recovery we have observed, postjunctional supersensitivity would have to develop more rapidly or to a greater extent after denervation than after decentralization. Preliminary experiments in our laboratory indicate that neither of these possibilities occurs. A submaximal dose of isoproterenol (0.5 mg/kg) was injected into animals that had been bilaterally denervated, bilaterally decentralized, or sham operated 32 hr previously. The increase in NATase seen in the denervated and decentralized animals was 2.5-3 times greater than the increase seen in the controls, indicating that these two procedures result in the same level of supersensitivity. Although comparable studies have not been reported after unilateral operations, these results suggest that changes in postjunctional sensitivity are unlikely to account for the recovery of pineal function that occurs after unilateral denervation.

One change that almost certainly occurs after denervation but not after decentralization of the pineal gland is a loss of norepinephrine uptake resulting from the degeneration of sympathetic nerve terminals (Fig. 3). In various tissues, total sympathetic denervation has been associated with a form of supersensitivity termed "prejunctional supersensitivity" because it is attributed to the increased accumulation of injected agonist in the vicinity of postjunctional adrenergic receptors due to the loss of neuronal uptake sites (22). Although the time course of the disappearance of neuronal uptake of norepinephrine has not been reported after denervation of the pineal, ultrastructural signs of neuronal degeneration occur by 24 hr, at which time endogenous norepinephrine is no longer detectable in the gland (23). Furthermore, the capacity to take up norepinephrine is lost from the iris, another tissue innervated by the ICN, 12-24 hr after superior cervical ganglionectomy, just before the disappearance of norepinephrine fluorescence from this tissue (24). Therefore, it seems likely that the capacity to take up norepinephrine is lost from the pineal within 32 hr after denervation.

The loss of uptake sites after unilateral denervation might make the pineal gland more sensitive both to circulating catecholamines and to norepinephrine released by the remaining neurons innervating the gland; however, experiments with bilaterally denervated animals suggest that the former can only play a minor role in the recovery process. Thus, although the small increase in NATase activity that occurs 32 and 80 hr after bilateral denervation (Figs. 1 and 2), but not after bilateral decentralization (Fig. 4), probably results from an increased response to circulating catecholamines due to decreased norepinephrine uptake, NATase levels in these animals are still less than 3% of that in normal rats. Therefore, we believe that the predominant stimulus to the pineal gland in unilaterally denervated animals is the intact ICN.

By hypothesizing that the recovery of pineal function after unilateral denervation is due to the loss of presynaptic uptake sites, we are suggesting that the endings of neurons from the two SCGs are situated in the pineal gland such that nerve endings of cell bodies in the right SCG can take up norepinephrine released by neurons whose cell bodies are in the left SCG. The observation that the two nerves innervating the pineal gland interact in their control of pineal NATase activity (i.e., unilateral decentralization results in NATase activity that is less than half that seen in intact animals) is consistent with a close anatomical arrangement of the nerve terminals from the two ganglia. According to this view, after unilateral decentralization and during the first few hours after unilateral denervation, the uptake capacity of the neurons on the inactive side would be intact and would thus limit the effectiveness of the active neurons in stimulating NATase activity. However, between 8 and 32 hr after unilateral denervation, the uptake capacity of the degenerating neurons would be lost, thus enhancing the stimulatory capacity of the remaining neurons. If it is this disappearance of norepinephrine uptake sites that accounts for the recovery of the NATase rhythm, it should be possible to produce normal NATase activity in unilaterally decentralized animals by pharmacologically blocking the norepinephrine uptake sites. Injection of desmethylimipramine into unilaterally decentralized animals does in fact restore NATase activity to control values (Fig. 6).

These results raise the general possibility that a molecule of norepinephrine can be taken up not only by the neuron that releases it, as is normally assumed to occur, but also by neighboring neurons. In this way, the effectiveness of the norepinephrine released by adrenergic nerve terminal in stimulating postjunctional receptors would be limited by the norepinephrine uptake system of nearby terminals. Such a reserve stimulatory capacity could serve as a built-in mechanism for rapid recovery of function after partial denervation in various peripheral and central nervous structures in which the inactivation of the neurally released transmitter is by neural uptake. The pineal gland may simply offer a convenient system in which to detect this mechanism, both because of the ease and reproducibility with which partial deafferentation can be performed and because of the possibility of monitoring pineal function after this procedure. However, a similar mechanism might also explain why in certain regions of the central nervous system it has been found that the vast majority of neurons must be destroyed before any long-term deficits are seen (e.g., ref. 26). Finally, our results lead also to the speculation that, in normally func-

* An increased response to circulating catecholamines has been proposed to explain the small increase in daytime NAtase activity seen in intact rats given the norepinephrine uptake blocker desmethylimipramine.
Fig. 6. Effect of desmethylimipramine on NATase activity in normal and decentralized animals. Rats were sham-operated (a, b), unilaterally decentralized (c), or bilaterally decentralized (d). The next day they were injected with saline (a) or desmethylimipramine (b, c, and d) 30 min before the onset of darkness (20 mg/kg) and again 5 hr after the onset of darkness (10 mg/kg). Two hours later they were killed and NATase activity was measured.

tioning neural systems, alteration in the uptake capacity of certain neurons could modulate the synaptic efficacy of other neurons.

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