IMPAIRED MATURATION OF PRE-SYNAPTIC CHOLINERGIC
NERVE TERMINALS IN THE SUPERIOR CERVICAL
GANGLIA AFTER ADMINISTRATION OF GUANETHIDINE
AND DEXAMETHASONE

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Abstract - The role of post-synaptic cells in the development of pre-synaptic cholinergic
nerve terminals has been investigated in immature rat superior cervical ganglia (SCG)
and adrenals employing chemical agents which prevent the normal maturation of post-
synaptic cells. A marked atrophy of ganglion adrenergic neurons after guanethidine
administration was accompanied by the complete failure of normal maturation of
choline acetyltransferase (ChAc) activity in the presynaptic endings. However, the
same treatment failed to alter the levels of ChAc in the mature ganglia despite the
marked atrophy of adrenergic neurons. Administration of dexamethasone resulted in a
growth retardation of ganglion neurons as well as adrenal chromaffin cells reflected by
the lower levels of tyrosine hydroxylase and dopamine-β-hydroxylase than those in
untreated tissues. The levels of ChAc were significantly lower in the ganglia, but not
in the adrenals when treatment was started immediately after birth. These results
support the view that the normal synapse formation in the SCG depends on the normal
maturation of adrenergic neurons, and suggest that this dependence is detectable only
during a limited period of life.

It has been suggested that the normal development of the post-synaptic cells is critical
for the normal maturation of the presynaptic cholinergic nerve terminals in the peripheral
sympathetic nervous system. Thus the surgical, immunological or pharmacological mani-
pulations (1, 2) for destruction of sympathetic ganglion cells block the normal development
of choline acetyltransferase (ChAc), the enzyme restricted to the cholinergic nerve terminals
(3).

In this study, guanethidine and dexamethasone were employed to investigate further
the role of the post-synaptic cells in the maturation of pre-synaptic nerve terminals.
Prolonged administration of guanethidine to newborn or adult rats produces irreversible
destruction of peripheral adrenergic cell bodies (4, 5). Dexamethasone retards the normal
growth of these neurons (6) with possible concomitant proliferation of adrenaline-containing
neurons (7, 8). This drug was found herein to have an additional growth retarding effect
on the adrenal chromaffin cells, a property not shared with guanethidine (9). This agent
may, therefore, provide a tool for studying the possible retrograde trans-synaptic control
mechanism in chromaffin cells.

Part of this work was presented in Sendai, Japan, June 1976 at the annual meeting of the
Physiological Society of Japan.
MATERIALS AND METHODS

Guanethidine dissolved in physiological saline was injected daily s.c. at a dose of 15-50 \( \mu g/g \) of body weight into newborn Wistar rats of both sexes and 25 mg/kg to adult male rats. Dexamethasone was injected daily s.c. at a dose of 0.1 \( \mu g/g \) into newborn rats and 0.2 \( \mu g/g \) to rats of various ages. The animals were sacrificed by exposure to ether. Superior cervical ganglion (SCG) pairs and duodenum (approx. 1 cm length) were homogenized in 0.2-0.4 ml of 10 mM tris buffer, pH 7.4 containing 0.2\% triton X-100 using a small glass-to-glass homogenizer. Adrenal pairs were homogenized in 0.2-2.0 ml of the same solution using a teflon pestle glass homogenizer and the supernatant was used for the assay of enzymes after centrifugation at 10,000 g for 10 min (conventional refrigerated centrifuge).

The activity of tyrosine hydroxylase (TH) was determined by the \(^{14}\)CO\(_2\) trapping method developed by Waymire et al (10) with a 73 \( \mu M \) tyrosine and a 2 mM 5,6-dimethyltetrahydropterine concentration. The activity of dopamine-\(\beta\)-hydroxylase (DBH) was assayed by the phenylethanolamine N-methyltransferase (PNMT) coupled method of Molinoff et al (11) employing an optimal copper concentration. The ChAc and the PNMT activities were measured by the methods of Fonnum (12) and Saavedra et al (13) with slight modifications (14), respectively. The protein was assayed by the method of Lowry et al (15) employing bovine serum albumin as a standard. Reagent blanks included the same concentrations of triton X-100 as those in samples.

RESULTS

Effects of guanethidine on the activities of some enzymes in the SCG and adrenals

Daily injection of guanethidine to newborn rats for 13 days impaired little the normal developmental increase of body weight. However, the treated ganglia failed to develop normally as was reflected by the total protein contents which were less than the levels found in ganglia of animals 6 days of age. This treatment caused not only a failure of the normal developmental increase, but also a marked decrease of the TH and DBH activities in the SCG (Table 1). These results are consistent with the morphological (4) and the biochemical (5) evidence for the destruction of ganglion cells after this drug. The activity of ChAc also failed to develop normally and remained at the levels of 6 days of age (Table 1). On the other hand, the same treatment failed to reduce the ChAc activity in the adult ganglia, despite the marked decrease of the TH activity (Table 2). Enzyme activities and total protein content in the adrenals were not affected by treatment with this drug.

Effects of dexamethasone on the activities of some enzymes in the SCG and adrenals

Daily administration of dexamethasone to newborn rats for 5-7 days resulted in a marked increase of the PNMT activity in the SCG, this result verifying the efficacy of this treatment (7). On the other hand, less PNMT activity was found in the dexamethasone treated adrenals. Concomitant with the growth failure (decrease of body weight) observed, however, a marked retardation of normal developmental increases of the activities of TH and DBH was noted in the SCG as well as in the adrenals (Table 3).
### Table 1. Effect of guanethidine on the development of enzyme activities and total protein contents in the SCG and the adrenals of newborn rats

| Tissue | duration (days) | control | treated | control | treated | control | treated | Protein (μg) | treated |
|--------|----------------|---------|---------|---------|---------|---------|---------|-------------|---------|
| SCG    | 5              | -       | -       | 12.8±0.7(4) | 6.7±1.4(3)* | 5.0±0.2(4) | 4.7±0.3(3) | 188±4(6) | 159±8(3)** |
|        | 8              | -       | -       | 13.2±1.3(6) | 3.1±0.2(10)* | 7.0±0.4(6) | 5.1±0.3(6)* | 221±3(6) | 164±19(8)* |
|        | 13             | 1.50±0.20(3) | 0.08±0.04(3)* | 16.7±1.1(6) | 0.9±0.4(6)* | 25.3±2.8(5) | 4.6±0.7(6)* | 275±22(8) | 159±12(10)* |
|        | 17             | 2.8(2) | 0.12(2)* | 40.7(2) | 0.8(2)* | 37.7(2) | 6.6(2)* | 353(2) | 119(2)* |
| Adrenal| 8              | -       | -       | 18.4±0.6(6) | 17.8±0.6(6) | 3.3±0.1(4) | 3.2±0.2(4) | 485±30(7) | 503±24(8) |
|        | 13             | 7.9±0.5(3) | 8.7±0.5(3) | 33.6±1.8(3) | 35.3±1.2(3) | 12.7±0.8(3) | 13.3±1.4(3) | 570±33(3) | 496±43(3) |

Newborn rats were given guanethidine (50 μg/g/day, s.c.) at various times.*
Enzyme activities are expressed as nmole products formed/hr/pair. Numbers of animals as indicated in parentheses.
* significantly different from control groups, p<0.01. **p<0.05

### Table 2. Effect of guanethidine on TH and ChAc activities in the SCG of adult rats

| Duration | TH          | ChAc          |
|----------|-------------|---------------|
|          | Control     | Treated       | Control     | Treated     |
| 11 days  | 3.98±0.20(4) | 1.81±0.25(4)* | —           | —           |
| 18 days  | 3.55±0.30(8) | 1.19±0.21(9)* | 30.5±2.5(8) | 29.8±2.3(9) |
| 30 days  | —           | —             | 36.0±2.7(4) | 33.3±2.2(4) |

Adult male rats were given guanethidine (25 mg/kg/day, s.c.) at various times.* Enzyme activities are expressed as nmole products formed/hr/pair. *significantly different from control groups, p<0.01
### Table 3. Effect of dexamethasone on enzyme activities and total protein contents in the SCG and adrenals of newborn and young rats

| Tissue | age | Duration (days)* | TH (U/mg protein) | DBH (U/mg protein) | % of mean of control PNMT | ChAc (U/mg protein) | Protein (mg) |
|--------|-----|-----------------|-------------------|-------------------|--------------------------|---------------------|--------------|
| SCG    | 1   | 5               | 47.7 ± 2.5(12)*   | 52.0 ± 3.0(4)*    | 1119 ± 149(13)*          | 73.2 ± 4.1(11)*    | 64.4 ± 3.1(11)* |
|        | 6   | 6               | 46.7 ± 5.8(6)*    | 31.9 ± 2.1(9)*    | 1715 ± 271(8)*           | 70.2 ± 5.7(5)*     | 68.8 ± 3.5(13)* |
|        | 7   | 7               | 52.4 ± 4.6(5)*    | 32.4 ± 4.6(5)*    | —                        | 72.5 ± 3.7(11)*    | 61.0 ± 2.6(10)* |
| Adrenal| 1   | 10              | 60.9 ± 3.3(10)*   | 51.6 ± 3.7(5)*    | —                        | 85.3 ± 3.8(7)**    | 71.9 ± 1.5(10)* |
|        | 15  | 10              | 90.5 ± 5.8(4)     | —                 | —                        | 94.3 ± 4.0(4)      | —            |
|        | 1   | 5               | 53.8 ± 2.2(11)*   | 37.3 ± 2.5(4)*    | 77.5 ± 3.4(11)*          | 91.6 ± 4.4(7)      | 48.0 ± 1.0(4)*  |
|        | 6   | 6               | 50.5 ± 2.7(6)*    | 46.5 ± 4.8(6)*    | 76.1 ± 2.0(16)*          | 100.0 ± 14.8(3)    | 49.3 ± 2.9(6)*  |
| Adrenal| 10  | 7               | 66.0 ± 4.4(10)*   | 80.0 ± 6.4(5)**   | 96.6 ± 5.7(10)           | 47.8 ± 4.0(5)**    | —            |

Dexamethasone (0.1–0.2 μg/g/day) was injected into newborn or young rats. Treatment continued for several days*. Enzyme activities and protein contents of treated ganglia and adrenals are normalized as % of controls in each experiment. These values are combined and expressed as % ± S.E.M. Control values are slightly different from group to group, but the variations in the same group are minute. Numbers of animals as indicated in parentheses. Control group included 4–11 animals. *significantly different from control group, p < 0.01. **p < 0.02. ★ denotes no comparison because of the undetectably low activity of PNMT in the treated and control ganglia.

### Table 4. Effect of guanethidine and dexamethasone on enzyme activities and protein contents in the SCG and duodenum of newborn rats

| Treatment          | Duration (days)* | Tissues | ChAc (U/mg protein) | TH (U/mg protein) | Protein (mg) |
|--------------------|------------------|---------|---------------------|-------------------|--------------|
|                    |                  | Control | Treated             | Control           | Treated      | Control      | Treated      |
| Dexamethasone      | 7                | SCG     | 8.8 ± 0.8(8)        | 5.5 ± 0.5(9)*     | 21 ± 13(8)   | 116 ± 5(9)*  |
|                    |                  | duod.   | 3.4 ± 0.2(8)        | 3.7 ± 0.2(9)      | —            | —            |
| Guanethidine       | 13               | SCG     | 25.2 ± 0.9(4)       | 6.0 ± 0.5(6)*     | 3.6 ± 0.4(6) | 280 ± 17(4)  | 118 ± 8(6)*  |
|                    |                  | duod.   | 4.2 ± 0.5(4)        | 4.2 ± 0.4(4)      | —            | —            |

Newborn rats were treated with dexamethasone (0.1 μg/g/day) or guanethidine (15 μg/g/day) at various times. Enzyme activities are expressed as nmole products/hr/pair for SCG and nmole products/hr/mg protein for duodenum. No. of animals as indicated in parentheses. *significantly different from control groups, p < 0.01.
The same treatment also resulted in a significant delay in the development of ChAc activity in the SCG. In sharp contrast to the ganglia, no difference of the ChAc activity was found between dexamethasone treated and untreated adrenals. Marked delay in the increases of TH and DBH activities was still observed in the SCG and adrenals of 10-day old rats (Table 3), however, the reduction of ChAc activity was less marked in the SCG.

**Effects of guanethidine and dexamethasone on ChAc activity in the duodenum**

To determine whether or not guanethidine and dexamethasone directly inhibit the development of cholinergic fibers in the immature rats, ChAc activity was measured in the duodenum and a reduced activity of ChAc in the duodenum was not apparent after treatment with these drugs (Table 4).

**DISCUSSION**

Guanethidine treatment resulted in a lack of formation of the cholinergic synapse in the immature ganglia as was demonstrated by the absence of normal developmental increase of ChAc activity. These results suggest that this failure is not due to a direct effect on the cholinergic terminals, but rather to the indirect effect of destruction of the adrenergic neurons by the agent. There was a marked decrease of the activities of TH and DBH in the immature ganglia, while the ChAc activity remained at fixed levels (Table 1). There was no apparent alteration of ChAc activity in the mature ganglia despite the marked reduction of the activities of TH and DBH (Table 2). These data also suggest that there is a critical period during which the retrograde control mechanism (i.e. influence of the post-synaptic neurons on the pre-synaptic terminals) exists. In addition, no alteration in ChAc activity was found in the duodenum, where cholinergic fibers do not terminate at adrenergic neurons. This conclusion is in good agreement with that drawn in the earlier studies (1, 2).

Administration of dexamethasone retarded the increases of activities of TH and DBH in the immature ganglia and adrenals. These changes appear to be related to the growth retardation of some tissues reflected by the lower levels of DNA, protein and other cellular constituents as compared to the controls (16). ChAc activity was lower in ganglia only when the drug was given at the early stage of development (Table 3).

Ciaranello and Axelrod (6) also found the reduced activities of TH and ChAc in ganglia after dexamethasone. They suggested that this drug primarily impaired the preganglionic neurons and thus prevented the normal growth of the post-synaptic neurons according to the well demonstrated orthograde trans-synaptic control mechanism (17, 18). However, it should be kept in mind that this concept was based on the rather drastic experiments in which preganglionic fibers were surgically transected. Even when the ChAc activity fell to less than 20% of control after operation, the TH activity was maintained at the levels of 50–60% of control (17). If the same mechanism operates after dexamethasone, the extent of reduction of the TH and DBH activities may have been much smaller than those observed, since the ChAc activity remained at the level of approx. 70% of control. Furthermore, the reduced activities of TH and DBH were still marked, but that of ChAc was less marked when administration was initiated at 10 days of age. These data together with the fact
that there was no alteration in ChAc in the duodenum after treatment with dexamethasone suggest that this drug inhibits the maturation of the adrenergic neurons thereby impairing development of the preganglionic nerve terminals.

The reduced activities of TH, DBH and PNMT were also found in the adrenal chromaffin cells after dexamethasone (Table 3). PNMT levels were the least reduced. This difference may be related to the findings that the reduced PNMT activity after hypophysectomy is replaced by systemic administration of dexamethasone in adult rats (19), but not the activities of TH and DBH (20, 21). On the other hand, there was no significant reduction in ChAc activity after dexamethasone. This observation taken together with the results in which the reduced ChAc activity was found in the fetal adrenals when dexamethasone was given to the mother during different stages of gestation (6) suggests that the retrograde control mechanism exists in the adrenals in an early limited time in fetal life, but disappears after birth.

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