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EFFECTS OF POSTNATAL RESERPINE ADMINISTRATION ON SYMPATHO-ADRENAL DEVELOPMENT IN THE RAT

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Abstract—Reserpine (2.5 or 5 mg/kg, s.c.) was administered to neonatal rats and the adrenals were analyzed for catecholamines (CA), tyrosine hydroxylase (TH) and dopamine β-hydroxylase (DBH) activities and for the ability of isolated storage vesicles to incorporate [^{3}H]epinephrine. Four hr after reserpine administration, an acute depletion of CA was observed, and by 24 hr CA fell to 10–20 per cent of controls; recovery required 2 weeks. Pretreatment of neonates with chlorisondamine (10 mg/kg, s.c.) did not prevent acute (4 hr) CA depletion by reserpine, but did block the decline observed in adult rats, indicating that the acute depletion in neonates is not due to neurogenic stimulation via the splanchnic nerve. At no time after the administration of reserpine to neonates was a change in TH observed, and only a small increment in DBH activity was obtained. In contrast, adult rats given reserpine showed marked increases in both TH and DBH, and depletion of CA was less marked and of shorter duration. Administration of reserpine to rats of different ages demonstrated that an "adult" pattern of response (TH induction) was obtained only after 8 days of age. On the other hand, nicotine (10 mg/kg, s.c.) given to neonates did evoke TH induction, indicating that at birth the tissue is capable of induction if stimulated directly. Inhibition of [^{3}H]epinephrine uptake by reserpine *in vitro* (10^{-7} M) was lower in vesicles from neonates than that observed in vesicles from adults, but drug administration *in vivo* produced marked inhibition in both adults and neonates by 4 hr, with recovery occurring by 4 days post-injection. These data show that the administration of reserpine to neonatal rats produces an acute depletion of CA which is not dependent on either blockade of vesicular uptake or on reflex actions of the drug, and which is not accompanied by TH induction. These differences from the adult may account for the more intense and longer-lasting effects of the drug in neonates. The lack of functional connections between the central nervous system and the neonatal adrenal medulla may be responsible for the immature response pattern.

Administration of reserpine to adult rats produces depletion of catecholamines by two mechanisms: blockade of amine uptake into storage vesicles [1–3], and stimulation-induced secretion of catecholamines [4]. As a result of stimulation, administration of reserpine produces increases in tyrosine hydroxylase (TH) and dopamine β-hydroxylase (DBH) activities in both adrenergic neurons and in the adrenal medulla ("trans-synaptic induction") [5–7]; additionally, there is an increase in the formation of storage vesicles [8, 9]. These changes aid in the rapid replenishment of catecholamine stores [9, 10].

Studies conducted in developing rats have shown that the administration of reserpine or tetrabenazine to neonates produces a depletion of brain catecholamines which is more profound and longer-lasting than that observed in adult rats [11]. Additionally, the metabolism of reserpine in neonates appears to be impaired compared to that in adults [12]. However, few studies have been conducted on the effects of reserpine in developing peripheral tissue, nor have the mechanisms for the different effects of reserpine in neonates been identified.

During the maturation of the rat adrenal medulla, catecholamine biosynthetic enzymes and storage vesicles undergo a series of changes which are in part dependent upon the levels of neuronal input to the gland [13–16]. In the neonatal rat and calf, innervation of the adrenal medulla appears to be non-functional in that electrical or pharmacological stimulation of the splanchnic nerve fails to cause secretion of adrenal catecholamines [14, 17]. Additionally, morphine, which elicits trans-synaptic induction in adult rats, fails to do so in perinatally addicted rats [18].

Because the actions of reserpine on the adrenal medulla depend to a large measure on the integrity of the nerve supply to the tissue, it seemed likely that the effects of the drug in developing rats would be markedly different from those in mature rats. In the present study, the effects of reserpine on the synthesis, uptake and storage of catecholamines in developing rat adrenal medulla have been examined, along with the mechanisms underlying the different actions of the drug in neonates.

METHODS

Treatment of rats. Timed pregnant Sprague–Dawley rats (Zivic–Miller) were housed in individual breeding cages and maintained at 22° with 12-hr alternating light–dark periods. Water and food were provided *ad lib.* Pups received a single injection of reserpine (2.5 or 5.0 mg/kg, s.c.) at birth, while control pups received saline. The rats were killed by decapitation at various time intervals from 4 hr to 29 days after drug administration. In other experiments, reser-

pine was administered at different ages, with a single dose (2.5 mg/kg, s.c.) given at age 0, 4, 8, 11 or 17 days; assays were performed 3 days after each injection.

For short-term studies, rats were pretreated with saline or chlorisondamine (10 mg/kg, s.c.) followed 30 min later by saline or reserpine and were killed 4 hr after the second injection; other groups received saline or nicotine (10 mg/kg, s.c.) and were killed 24 hr later, or α-methyl-*p*-tyrosine methyl ester (300 mg/kg, i.p.) and were killed 4 hr later.

Assays. Adrenals were excised and homogenized (glass-to-glass) in 2.5 ml of ice-cold 300 mM sucrose containing 25 mM Tris (pH 7.4) and 0.01 mM iproniazid (irreversible monoamine oxidase inhibitor). At early stages of development, adrenals from several pups were pooled to obtain sufficient material. One-tenth ml of the homogenates was removed and deproteinized with 1.9 ml of 3.5% perchloric acid (PCA), and centrifuged at 26,000 *g* for 10 min. The supernatants were analyzed for catecholamines by the trihydroxyindole method, using an autoanalyzer [19]. One-half ml of the remaining homogenate was added to an equal volume of water containing 2000 units/ml of beef catalase, and used for duplicate assays of dopamine β-hydroxylase (DBH) activity by the method of Friedman and Kaufman [20], using 10 μM tyramine[G-^{3}H] as a substrate. Parahydroxymercuribenzoate (optimal concentration, 0.5 mM) was used to inactivate endogenous inhibitors [21].

An aliquot of homogenate was centrifuged at 26,000 *g* for 10 min to sediment the catecholamine-containing storage vesicles, and duplicate 0.1-ml portions of the supernatant were assayed for tyrosine hydroxylase activity by the method of Waymire *et al.* [22], using L-tyrosine[1-^{14}C] (100 μM) as substrate.

The remainder of the homogenate was centrifuged at 800 *g* for 10 min, and the supernatant used for the determination of [^{3}H]epinephrine uptake into storage vesicles by standard techniques described previously [13]. Duplicate tubes were prepared containing 0.5 ml of the vesicle-containing 800 *g* supernatant, 5 μmoles ATP and Mg^{2+}, 5 μCi of [^{3}H]epinephrine, 0.1 μmole of unlabeled epinephrine (to obviate any differences in extravesicular catecholamine concentration among the samples), and sucrose Tris in a final volume of 1 ml. Samples were incubated at 30°, while duplicates were kept on ice to serve as blanks. Uptake was stopped by the addition of 2 ml of ice-cold sucrose–Tris and labeled vesicles were sedimented at 26,000 *g* for 10 min. The supernatant was deproteinized with an equal volume of 7% PCA, centrifuged, and analyzed for catecholamines and radioactivity by liquid scintillation spectrometry; this enabled determination of the specific activity of the labeling medium. The vesicular pellet was washed with fresh sucrose Tris and recentrifuged twice, and then the final pellet was resuspended in 3 ml of 3.5% PCA, centrifuged, and the supernatant analyzed for catecholamines and radioactivity. The temperature-dependent component of the uptake in each sample was calculated as described previously [23], and expressed as uptake per gland (a composite measure reflecting the number of functional vesicles per gland as well as the uptake capability of each vesicle), or as uptake per unit of catecholamines (a measure of the ability of individual vesicles to incorporate [^{3}H]epinephrine relative to endogenous content, independently of the number of vesicles present) [23, 24]. Although contaminating particles are present in this preparation, under these conditions labeling occurs solely in the storage vesicles [13, 24].

Studies of the effects of reserpine *in vitro* on [^{3}H]epinephrine uptake were conducted in a similar fashion, except that the animals received no drug treatment; instead, reserpine (10^{-7} M) was added directly to the incubation medium.

Statistics. Results are expressed as means ± standard errors, and levels of significance calculated by Student's *t*-test [25].

Materials. Tyramine[G-^{3}H] (10 Ci/m-mole), *dl*-epinephrine[7-^{3}H] (10 Ci/m-mole) and L-tyrosine [1-^{14}C] (10 mCi/m-mole) were obtained from New England Nuclear Corp. Reserpine phosphate and chlorisondamine chloride were obtained from Ciba Pharmaceutical Co. Epinephrine bitartrate was obtained from Winthrop Laboratories, and iproniazid phosphate, nicotine, parahydroxymercuribenzoate, beef liver catalse and DL-α-methyl-*p*-tyrosine methyl ester hydrochloride from Sigma Chemical Corp.

RESULTS

Effects of reserpine administered at birth. During the course of development, control rats increased in weight from 7 g at birth to 83 g at 29 days of age. In neonates injected with reserpine (2.5 mg/kg), body weight was approximately 10–15 per cent below controls through the first 2 weeks (Fig. 1). Neonates given the higher dose of reserpine (5 mg/kg) showed a greater retardation in weight gain in week 1, but recovered to approximately normal body weight by week 2.

Over the same period of development, adrenal catecholamines in control rats increased from 0.2 μg/gland at birth to 5 μg/gland at 29 days (Fig. 2). Four hr after the injection of the low dose of reserpine to neonates, a marked decrease was observed in adrenal catecholamine content. One day later, catecholamine levels had declined to 20 per cent of control. With the higher dose, depletion was nearly com-

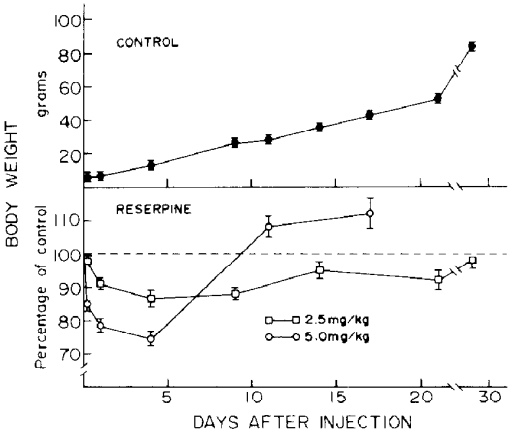

Fig. 1. Body weights of developing rats after administration of a single dose of reserpine given at birth. Each point represents mean ± standard error of six determinations.

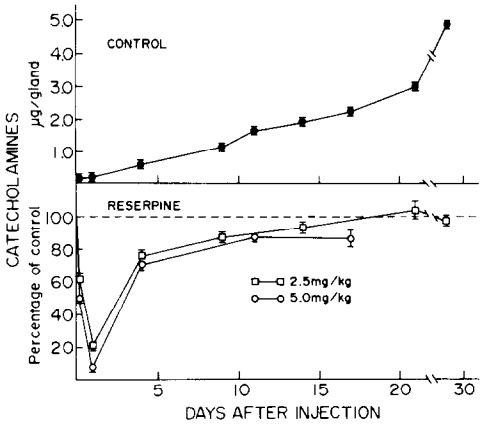

Fig. 2. Adrenal catecholamines in developing rats after administration of a single dose of reserpine given at birth. Each point represents mean ± standard error of six determinations.

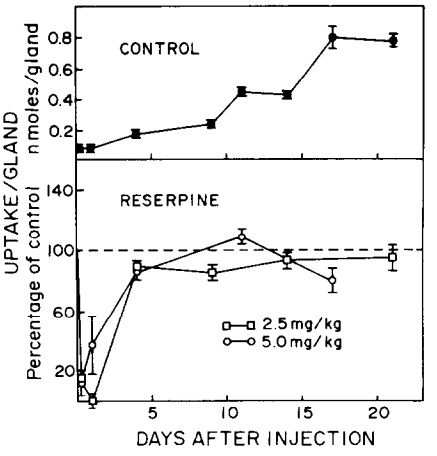

Fig. 4. [^{3}H]epinephrine uptake per gland in isolated adrenal storage vesicles of developing rats after administration of a single dose of reserpine given at birth. Each point represents mean ± standard error of six determinations.

plete within 24 hr. At both doses, the catecholamine depletion observed 24 hr after drug administration was followed by a rapid recovery to 80 per cent of normal 4 days after drug administration. After that point, recovery slowed such that normal levels for developing adrenals were not obtained until about 2 weeks of age.

The effects of neonatal reserpine treatment on tyrosine hydroxylase (TH), and dopamine β-hydroxylase (DBH) activities in developing adrenal medullae are shown in Fig. 3. Control activities increased from 1.2 nmoles/hr/gland at birth to 7.4 units at 29 days for TH, and from 0.04 nmole/hr/gland at birth to 0.47 unit at 29 days for DBH. At no time after neonatal administration of either dose of reserpine was there an alteration in the development of TH activity. DBH activity similarly displayed only minor differences from controls, with small increases at 4 and 21 days after the low dose, and an increase at 4 and decrease at 17 days after the high dose.

The abilities of isolated storage vesicles to incorporate [^{3}H]epinephrine, expressed per gland, increased approximately 10-fold over the first 3 weeks of development in control animals (Fig. 4), while uptake per unit of catecholamines (CA) ranged from 45 nmoles/100 μg of CA at birth to 25 nmoles/100 μg of CA at 3 weeks of age. These data indicate that in normal development the number of functional vesicles increases and that the uptake capabilities of the vesicles relative to CA content change [13, 15]. Four hr after administration of either dose of reserpine to neonates, there was nearly complete blockade of [^{3}H]epinephrine uptake expressed either per gland or per unit of CA. Recovery of uptake per gland was nearly complete by 4 days of age (Fig. 4). Uptake

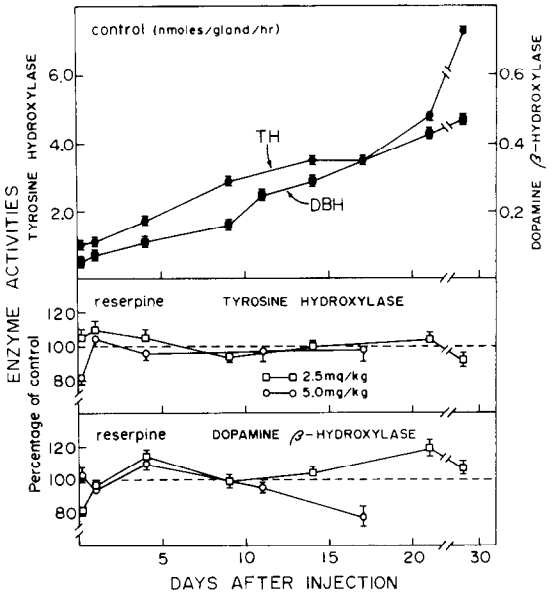

Fig. 3. Adrenal tyrosine hydroxylase and dopamine β-hydroxylase activities in developing rats after administration of a single dose of reserpine given at birth. Each point represents mean ± standard error of six determinations.

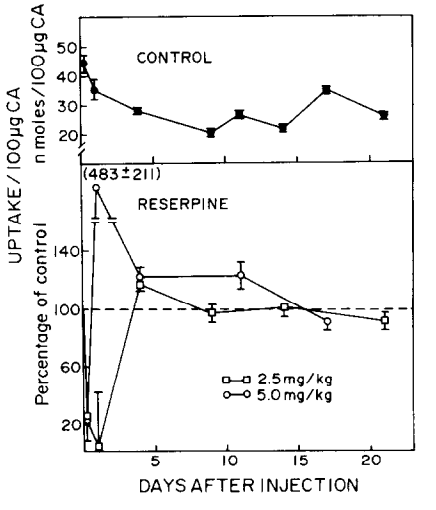

Fig. 5. [^{3}H]epinephrine uptake per 100 μg of endogenous catecholamines in isolated adrenal storage vesicles of developing rats after administration of a single dose of reserpine given at birth. Each point represents mean ± standard error of six determinations.

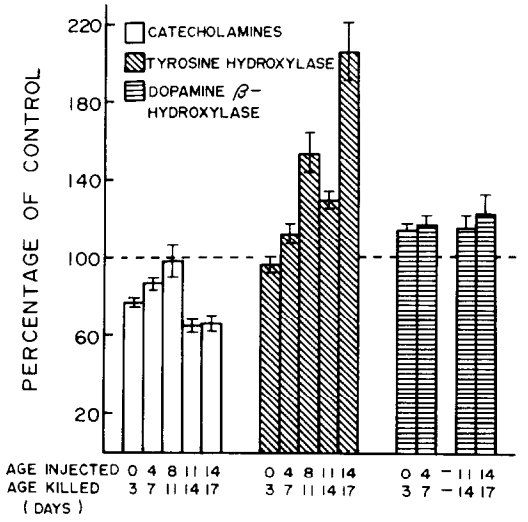

Fig. 6. Effects of reserpine administered to rats of different ages on the adrenal catecholamine content and tyrosine hydroxylase and dopamine β-hydroxylase activities. Rats received a single dose of reserpine (2.5 mg/kg, s.c.) at the ages indicated and were killed 3 days later. Bars represent means ± standard errors of four to six determinations.

per unit of CA demonstrated a completely different pattern; after treatment with 2.5 mg/kg, there was a rebound from complete inhibition at 1 day of age to supranormal levels ($P < 0.02$) by 4 days of age and normal uptake per unit of CA by 9 days of age (Fig. 5). The rebound occurred sooner and was much more pronounced and prolonged after the high dose, indicating that the remaining vesicles have been markedly depleted of endogenous CA.

Effects of reserpine administered at different ages. Three days after drug administration, catecholamine depletion was evident in all age groups except one (Fig. 6). There was no significant change in TH activity in neonates or 4-day-old pups after reserpine treatment. However, the administration of the drug at 8, 11 or 14 days of age produced TH increases ranging from 30 to 100 per cent above controls. Three days after reserpine administration, DBH activity was 15–20 per cent above control values ($P < 0.05$) regardless of age at the time of injection.

Effects of chlorisondamine on acute catecholamine depletion after reserpine administration. Reserpine alone at either 2.5 or 5 mg/kg caused an acute (4 hr) depletion of neonatal adrenal catecholamines (Table 1). In adult rats, 2.5 mg/kg failed to cause acute depletion, but 5 mg/kg reduced catecholamines to 57 per cent of controls. To determine the possible role of splanchnic stimulation in the reserpine-induced depletion of neonatal catecholamines, chlorisondamine (a long-acting ganglionic blocker) was administered 30 min prior to reserpine. Chlorisondamine did not prevent depletion in neonates, but the same dose of chlorisondamine completely blocked the loss of catecholamines in adult rats. Chlorisondamine itself had no effect on adrenal catecholamine content in either neonates or adult rats.

Effects of reserpine in vitro on [^3H]epinephrine uptake. In order to find out whether the enhancement of reserpine-induced depletion in neonates could be due to a greater effect of reserpine on the vesicular uptake mechanism, the drug was added directly to vesicle preparations from untreated animals of various ages. While reserpine at 10^{-7} M inhibited uptake 70–75 per cent in vesicles from 15-, 30- or 50-day-old rats (Table 2), it was significantly less effective in vesicles from neonates, indicating that the actions on uptake cannot account for enhanced depletion in neonates.

Effects of DL-α-methyl-p-tyrosine. To determine whether the greater CA depletion in neonates could result from an inherently higher rate of turnover, α-methyl-*p*-tyrosine was administered to block CA synthesis and CA was determined 4 hr later (Table 3). While a drug-induced decrease was observed in adult CA, neonatal CA levels did not differ from con-

Table 1. Effects of reserpine and chlorisondamine on neonatal and adult adrenal catecholamines*

Treatment		Catecholamines (4 hr after second injection) (Percentage of control)	
First injection	Second injection (30 min later) (mg/kg)	Neonates	Adults
Saline	Saline	100 ± 6 (27)	100 ± 5 (27)
Chlorisondamine	Saline	103 ± 13 (9)	110 ± 8 (6)
Saline	Reserpine (2.5)	76 ± 3† (11)	96 ± 6 (6)
Chlorisondamine	Reserpine (2.5)	77 ± 4‡ (10)	105 ± 3 (6)
Saline	Reserpine (5)	71 ± 3§ (6)	57 ± 5§ (6)
Chlorisondamine	Reserpine (5)	73 ± 5† (6)	
Saline	Reserpine (10)		65 ± 3§ (6)
Chlorisondamine	Reserpine (10)		90 ± 9 (6)

* Control values were 0.224 ± 0.014 (26) and 16.8 ± 0.9 (27) μg/gland, for neonates (1 day old) and adult rats (50 days old) respectively. All drugs were administered subcutaneously; the dose of chlorisondamine was 10 mg/kg. Data represent means ± standard errors. Numbers in parentheses denote number of determinations.

‡ $P < 0.005$ vs saline saline.

† $P < 0.002$.

§ $P < 0.001$.

Table 2. Effects of reserpine *in vitro* on [^{3}H]epinephrine uptake into adrenal storage vesicles of developing rats*

Age (days)	Epinephrine uptake (nmoles/100 μg catecholamines) Control	Reserpine (10^{-7} M)	Percentage inhibition
1	27.9 ± 2.5	13.9 ± 1.6	50 ± 6†
15	22.8 ± 0.3	4.7 ± 0.4	79 ± 2
30	21.7 ± 0.5	5.6 ± 0.3	74 ± 1
50	19.3 ± 0.4	5.1 ± 0.2	74 ± 1

* Animals received no drug treatment; reserpine was added directly to the uptake incubation medium. Data represent means ± standard errors of four determinations.
† $P < 0.01$ vs adult (50-day).

trol values, indicating that the enhanced depletion after reserpine in neonates cannot be accounted for solely on the basis of alterations in turnover.

Effects of nicotine on adrenal catecholamines and tyrosine hydroxylase activity. To determine whether the lack of TH induction in reserpinized neonates resulted from an inability of the adrenal to increase enzyme levels, nicotine was administered to evoke direct stimulation. Twenty-four hr after administration of 10 mg/kg of nicotine to 4- and 35-day-old rats, both groups showed significant induction of TH but normal CA levels (Table 4).

Effects of reserpine administered to adult rats. In order to compare the actions of reserpine on the immature and mature adrenal, the effects of reserpine on catecholamine content, tyrosine hydroxylase and dopamine β-hydroxylase activities were determined in adult rats given 2.5 or 5 mg/kg. Four hr after 2.5 mg/kg, there was no significant CA depletion in adult adrenals, but at the higher dose, depletion was marked (Fig. 7). After 24 hr, depletion of catecholamines was obtained with both doses, but not to as great an extent as in neonates (Fig. 2). Within 3–6 days, catecholamine content returned to normal adult levels.

Twenty-four hr after the low dose of reserpine, TH activity was increased and reached an elevation of 60 per cent above control by 3 days. Maximal TH activity with the higher dose (80 per cent above control) was reached 24 hr after injection, and remained elevated at 7 days. DBH activity was elevated by 1 or 3 days post-reserpine at high and low doses, respectively, and reached values 25–65 per cent above controls.

The effects of reserpine on [^{3}H]epinephrine uptake into vesicles from adult adrenals were similar to those seen in reserpinized neonates (Fig. 8). Uptake per gland was nearly completely blocked 4 hr after either dose and returned to normal by 3–7 days. Uptake per unit of CA was low at 4 hr post-reserpine and showed rebound elevations above control levels at 1 (high dose) and 3 (low dose) days after administration; previous studies have shown that the elevations indicate that the intact vesicles remaining in the tissue after the initial secretory response are partially depleted of endogenous CA, and that resynthesis of new vesicles (which have an initially low CA content) has begun [4, 8, 9, 23, 24, 26].

DISCUSSION

Catecholamine (CA) depletion observed in adult rats after reserpine administration is biphasic: initially (first 4 hr) there is a reflex secretion of adrenomedullary CA (higher dose only), which is followed by a slower depletion (24–48 hr, either dose) resulting from reserpine-induced blockade of the vesicular CA uptake mechanism [1–4]. While 2.5 mg/kg of reserpine produced no acute CA depletion in adults, the same dose given to neonates resulted in marked depletion within 4 hr. A number of hypotheses can be advanced to explain this phenomenon. First, reserpine could cause a greater degree of splanchnic stimulation in neonates. This was ruled out by experiments showing that pretreatment with chlorisondamine, which blocks neuronal input to the adrenal [26], did not prevent the reserpine-induced acute depletion in neonates. The same dose of chlorisondamine prevented the stimulatory effect of higher doses of reserpine in the adult rat. Second, reserpine might be a more potent blocker of vesicular uptake in neonates. This was ruled out by experiments *in vitro* showing that the degree of blockade by reserpine is less in neonates than in adults; studies after administration *in vivo* also confirmed that the time course of blockade of vesicular uptake was similar in adults and neonates. Third, catecholamine turnover may be normally high in neonates, thus enhancing the depleting effect of reserpine. This was ruled out by studies with α-methyl-*p*-tyrosine, which inhibits CA synthesis. If the enhanced CA depletion were due solely to higher turnover, α-methyl-tyrosine should produce a loss of CA equivalent to that seen after reserpine; instead, no depletion was seen. Fourth, reserpine could cause secretion of adrenal CA by a mechanism not involving neurogenic stimulation or nicotinic receptors. This hypothesis is the most likely, since the acute depletion occurs too quickly to be accounted for by direct effects of reserpine on vesicles or by altered turnover; furthermore, direct electrical stimulation of the splanchnic nerve or administration of agents which evoke increased splanchnic activity does not cause CA secretion in neonatal rats and calves [14, 17], while certain stresses and drugs do elicit secretion [14, 17, 18]. In the present study, the acute loss of CA after reserpine could not be prevented by nicotinic receptor blockade (30 min pretreatment with 10 mg/kg of chlorisondamine), indicating the non-neurogenic nature of the depletion.

Table 3. Effects of DL-α-methyl-*p*-tyrosine methyl ester HCl (αMPT) on catecholamine content of neonatal and adult rat adrenals*

Treatment	Catecholamines (μg/gland) Neonates	Adults
Control	0.218 ± 0.005 (5)	15.2 ± 0.4 (6)
αMPT	0.228 ± 0.008 (7)	13.4 ± 0.4† (6)

* Rats were killed 4 hr after receiving 300 mg/kg of αMPT, i.p. Numbers in parentheses denote number of determinations.
† $P < 0.02$ vs control.

Table 4. Effects of nicotine on adrenal catecholamines and tyrosine hydroxylase activity*

Treatment	Catecholamines (μg gland) (4 days old)	Catecholamines (μg gland) (35 days old)	Tyrosine hydroxylase (nmoles gland hr) (4 days old)	Tyrosine hydroxylase (nmoles gland hr) (35 days old)
Control	0.541 ± 0.000 (4)	8.31 ± 0.45 (6)	1.20 ± 0.04 (4)	10.5 ± 1.0 (6)
Nicotine	0.561 ± 0.028 (5)	8.27 ± 1.03 (6)	1.79 ± 0.08† (5)	19.7 ± 3.0‡ (6)

* Rats were killed 24 hr after receiving 10 mg/kg of nicotine, s.c. Numbers in parentheses denote number of determinations.
† $P < 0.001$ vs control.
‡ $P < 0.02$.

Another factor which may operate in the enhanced reserpine-induced loss of CA in neonates is the lower rate of drug metabolism at that age [12]. However, the effect of reserpine administration on amine uptake mechanisms was equivalent in both degree and duration in adults and neonates, indicating little or no difference in the intensity or persistence of direct effects of the drug. This is in keeping with the finding of Mueller and Shideman [12] that, although reserpine metabolism is slower in the neonatal rat, the whole-body half-life for the drug is less than 2 hr. In any case, the effects of higher doses in adults still were not equivalent to the effects seen in neonates, since the mechanism of acute CA depletion was entirely different.

In adult rats, one of the key factors in re-establishment of CA stores after reserpine-induced depletion is the trans-synaptic induction of the catecholamine biosynthetic enzymes, tyrosine hydroxylase and dopamine β-hydroxylase [10]. Since induction requires neural input, and since neural connections between the CNS and the adrenal medulla appear to be absent at birth [14, 17], it was important to determine whether TH and DBH activities increased in reserpinized neonates and whether the rate of recovery of CA stores was affected. At no time after neonatal reserpine treatment was an increase in TH noted, and a consistent but small increment in DBH activity was seen at 3–4 days of age (Figs. 3 and 6); recovery of adrenal CA required 2 weeks (Fig. 2). In contrast, reserpine in adult rats caused a marked increase in both TH and DBH, and CA recovered to within 10 per cent of normal within 3 days. These data demonstrate that reserpine exerts a more intense and prolonged effect on CA in neonates as compared to adults, and that the difference results in part from a failure of the neonatal adrenal to compensate for depletion by enzyme induction. Reserpine administered early in development thus delayed the normal maturational increase in CA stores for up to 2 weeks.

The lack of TH induction in reserpinized neonates raised two important questions: is the neonatal

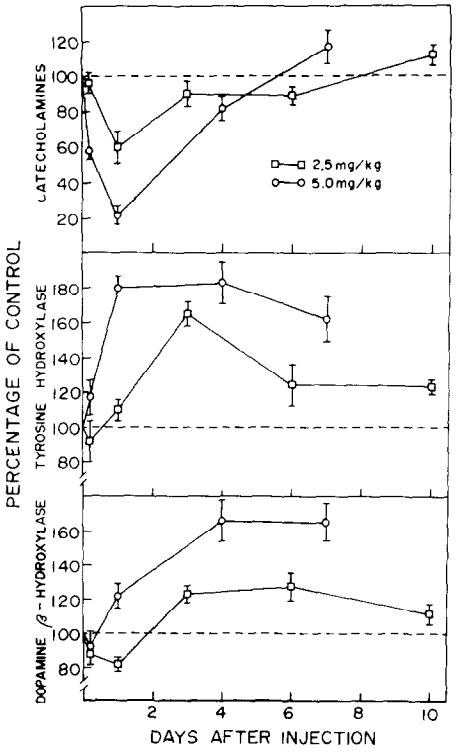

Fig. 7. Effects of reserpine on adrenal catecholamine content and tyrosine hydroxylase and dopamine β-hydroxylase activities of adult rats (50 days old). Each point represents mean ± standard error of five to six determinations.

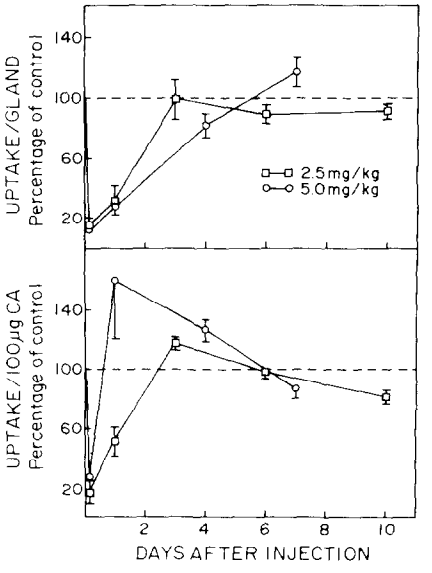

Fig. 8. Effects of reserpine on [^{3}H]epinephrine uptake per gland and per 100 μg of endogenous catecholamines in isolated adrenal storage vesicles of adult rats (50 days old). Each point represents mean ± standard error of five to six determinations.

adrenal inherently incapable of induction, and when does reserpine-induced induction first become possible? To test the ability of the tissue to induce TH, direct stimulation was evoked by nicotine administration; there was a prompt (24 hr) and marked increment in TH activity, indicating that the neonatal adrenal is indeed capable of induction and, further, that functional nicotinic receptors are present. However, not until 8 days of age did reserpine administration evoke TH induction; this corresponds to the age at which innervation of the adrenal medulla becomes functional [14]. Since in adults reserpine causes induction via increased neuronal activity, these data suggest that the inability of reserpine to increase TH activity in neonates results from lack of functional connections and not from a deficiency inherent in the adrenal itself.

The hypothesis that neuronal connections are required for an adult response pattern to reserpine is supported by studies with adult rats in which one adrenal was denervated [10]. Reserpine produced a long-lasting CA depletion and no TH induction in the denervated gland, but did evoke DBH induction. This pattern is qualitatively similar to that found in reserpinized neonates. The ability of drugs to induce DBH in the absence of neuronal input in denervated adult adrenals or in neonates may result from an additional, nontrans-synaptic regulatory mechanism [27].

In conclusion, these studies in developing rats indicate effects of reserpine completely different from those seen in adults; there is acute non-neural depletion without compensatory induction of catecholamine biosynthetic enzymes, resulting in part from a lack of functional connections between the neonatal adrenal and the central nervous system. These differences from the adult produce a marked delay in maturational increases in CA stores in reserpine-treated neonates.

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