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MATURATION OF THE ADRENAL MEDULLA—II

CONTENT AND PROPERTIES OF CATECHOLAMINE STORAGE VESICLES OF THE RAT*

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Abstract—Albino Wistar rats were sacrificed at 10-day intervals from birth to 50 days of age. The adrenals were analyzed for catecholamines (CA) and dopamine β-hydroxylase (DBO), and purified storage vesicles were analyzed for CA and ATP. At birth, all three parameters were 3–5 per cent of 50-day levels, about 10 per cent at 10 days, 20–30 per cent at 20 days, 40–55 per cent at 30 days and 70–75 per cent at 40 days. Although all three increased approximately in parallel, CA/DBO was below normal from birth to 20 days and CA/ATP was above normal at 10 days. At birth, the vesicles were more fragile, but discontinuous density gradient studies indicated fewer "light" vesicles (vesicles deficient in CA and ATP which do not penetrate 1·6 M sucrose) compared to adults. At 10 and 20 days, however, more light vesicles were present and vesicle fragility was the same as in adults. Neonates did not secrete adrenal CA in response to insulin-induced hypoglycemia, but secretion was observed at all other ages. Isolated neonatal adrenals as well as adult adrenals exhibited Ca^{2+}-dependent CA secretion when exposed to high K^+ concentrations. These data suggest that there are age-dependent changes in the content and properties of adrenal CA storage vesicles which affect the uptake and storage of amines in the vesicles. The maturation of vesicles is probably dependent on the relative rates of synthesis of vesicle components, which in turn are affected by the degree of neural input to the gland.

THE SYMPATHETIC neuron and its endocrine counterpart, the adrenal medulla, undergo profound developmental changes both pre- and postnatally. These include marked increases in catecholamine levels and in catecholamine-synthesizing enzymes as well as alterations in the ability of the adrenal medulla to secrete amines upon stimulation of the splanchnic nerve.[1–6] In addition, there is evidence that the adrenal medullary storage vesicles themselves undergo maturation; Elfvin[7] and Daikoku *et al.*[8] have presented ultrastructural evidence for developmental changes in the vesicles, and recent work from our laboratory has demonstrated age-dependent alterations in the uptake and storage of amines and in the buoyant densities of isotopically labeled vesicles.[9] It was suggested that neural input could play a determining role in the maturation of the vesicles.[9] In the present study, the content and properties of the vesicles have been determined in order to elucidate the nature of the developmental process in the adrenal, to help identify how changes in uptake and storage can occur, and to determine the role of neural input in the maturation of the gland.

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METHODS

Albino Wistar rats (Hilltop Lab Animals, Scottdale, Pa.) were sacrificed by decapitation at 10-day intervals from birth until 50 days of age. The adrenal glands were removed, cleaned of connective tissue and fat, pooled as shown in Table 1, and treated as follows.

TABLE 1. NUMBERS OF ADRENAL GLANDS USED PER SINGLE DETERMINATION IN EACH TYPE OF EXPERIMENT

Age (days)	Catecholamines, dopamine β-hydroxylase and ATP determinations; distribution studies	Insulin-induced secretion	Secretion *in vitro*
1	8	2	1
10	8	2	
20	6	2	
30	4	2	
40	2	2	
50	2	2	½

Determination of total catecholamines and dopamine β-hydroxylase. The glands were homogenized (glass-to-glass) in 3–5 ml of distilled water, and an aliquot was deproteinized by adding perchloric acid (final concentration, 3·5%) and centrifuged for 10 min at 26,000 *g*. The supernatant was then analyzed for catecholamines (trihydroxyindole method[10]). Portions, 0·4 ml, of the homogenates were used for analysis of dopamine β-hydroxylase (periodate oxidation method[11]) using ^{3}H-tyramine as a substrate. *para*-Hydroxymercuribenzoate (PMB) in concentrations ranging from 0·05 to 5 mM was used to inactivate endogenous inhibitors.[12] In all cases, the activity is reported at the optimal PMB concentration for each sample.

Differential centrifugation. Adrenal glands were homogenized in 2·5 ml of 0·3 M sucrose containing 10^{-5} M iproniazid (monoamine oxidase inhibitor) buffered at pH 7 with 0·025 M Tris. The suspension was centrifuged at 800 *g* to remove debris, and the pellet (fraction A) was resuspended in 5 ml of 3·5% perchloric acid, centrifuged and analyzed for catecholamines. One ml of the supernatant was centrifuged for 10 min at 26,000 *g* and the pellet (fraction P) was resuspended in 2 ml of 3·5% perchloric acid. The 26,000 *g* supernatant (fraction S) was added to an equal volume of 7% perchloric acid, and then both fractions P and S were centrifuged and analyzed for catecholamines. A flow sheet of this procedure as well as the procedure for discontinuous sucrose density gradient centrifugation appears in Fig. 1.

Discontinuous density gradient centrifugation and ATP determination. One ml of the 800 *g* supernatant was layered over 2·5 ml of 1·6 M sucrose (buffered at pH 7 with 25 mM Tris) and centrifuged at 140,000 *g* for 2 hr in the No. 40 rotor of the Beckman model L2 ultracentrifuge. This procedure separates intact storage vesicles from most contaminating particles,[13] from membranes of lysed vesicles,[14] and to some extent from intact vesicles of lower density.[14–16] The 0·3 M sucrose layer (fraction B, including the interface of the two sucrose layers) and the 1·6 M sucrose layer (fraction C) were diluted with perchloric acid (final concentration, 3·5%), centrifuged and analyzed for catecholamines. The 140,000 *g* pellet (fraction D) was resuspended in 2 ml of distilled water and centrifuged at 26,000 *g* to remove the vesicle membranes, which

contain an ATPase.[17,18] The supernatant was then used for ATP determinations by a modification[14] of the method of Strehler and Totter[19] (firefly method); an aliquot was also deproteinized and analyzed for catecholamines.

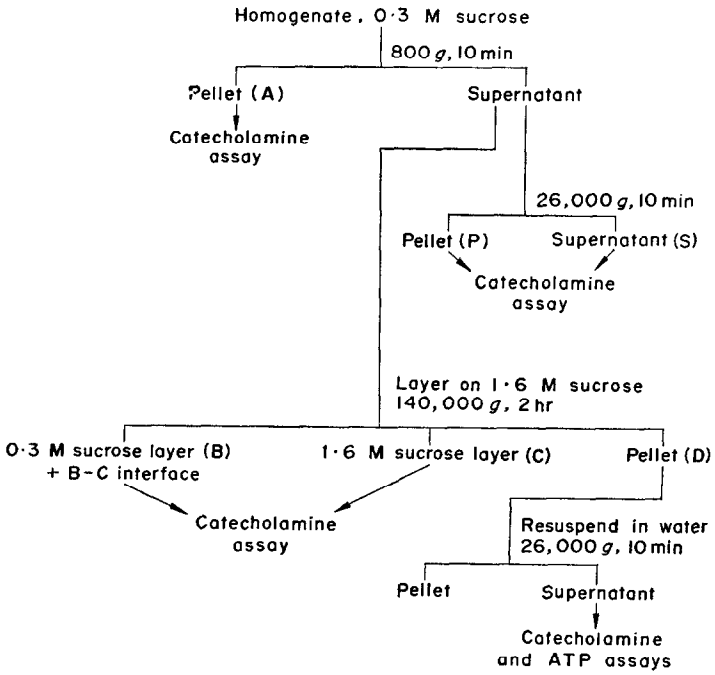

FIG. 1. Subcellular fractionation of adrenal homogenates.

Insulin-induced secretion. Rats were given insulin (20 i.u./kg, s.c.) and sacrificed 3 hr later. The adrenals were homogenized in 2·5 ml of 3·5% perchloric acid, centrifuged and the supernatants analyzed for catecholamines.

Secretion in vitro. Adrenal glands were sliced in half and immersed in either Locke's solution or Ca^{2+}-free Locke's solution and incubated at 37° with shaking. The medium was changed at half-hour intervals and was deproteinized and analyzed for catecholamines. The rate of spontaneous release of catecholamines under these conditions was high initially, but by the end of the third incubation period the rate of release was reduced to much lower levels (Table 2). For measurement of secretion *in vitro*, the studies were conducted during the fourth incubation period, using the third period as a control. In the fourth period, the Locke's solution was replaced with fresh Locke's solution, with high-K^+ Locke's solution (150 mM K^+, 5·6 mM Na^+) or with Ca^{2+}-free, high-K^+ Locke's solution (in glands previously incubated in Ca^{2+}-free Locke's solution). At the end of the half-hour period, the media were deproteinized and analyzed for catecholamines.

Statistical methods. Data are reported as means $\pm$ standard errors. Levels of significance are calculated by Student's *t*-test.[20]

TABLE 2. SPONTANEOUS RELEASE OF CATECHOLAMINES FROM ISOLATED RAT ADRENAL GLANDS*

Age (days)	Incubation period						
	1	2	3	4	5	6	7
1 (3)	459 ± 87†	66 ± 11	21 ± 4	13 ± 5	6·6 ± 2·2	6·6 ± 3·3	7·7 ± 4·4
50 (4)	21 ± 2‡	8·2 ± 1·1	5·1 ± 0·8	3·8 ± 0·4	3·4 ± 0·3	2·5 ± 0·2	2·2 ± 0·2

* All incubations were in Locke's solution at 37°; media were changed at half-hour intervals. Numbers in parentheses refer to the number of determinations at each age. Each incubation contained ½ gland for adult rats or 1 gland for neonates. Values are reported as means ± standard errors.

† pmoles/gland/30-min incubation period.

‡ nmoles/gland/30-min incubation period.

Materials. Tyramine-G-^{3}H was obtained from New England Nuclear Corp., regular insulin (80 i.u./ml) from Squibb, and buffered firefly lantern extract from Worthington Biochemicals. The epinephrine bitartrate and disodium ATP used for standards were obtained from Winthrop Laboratories and P-L Biochemicals respectively.

RESULTS

Catecholamines, dopamine β-hydroxylase and ATP. Catecholamine levels increased steadily from about 3 per cent of adult levels at birth to 75 per cent at 40 days of age (Fig. 2). Although the largest relative increase in catecholamines was obtained between

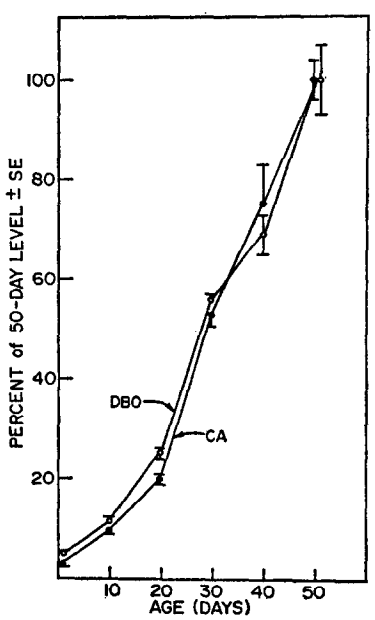

FIG. 2. Catecholamine (CA) levels (●) and dopamine β-hydroxylase (DBO, ○) in homogenates of whole adrenal glands expressed as percentages of 50-day values. Points represent mean ± S.E. of six determinations for ages 1–40 days, 17 determinations for age 50 days. Fifty-day values were: CA, 56·8 ± 2·2 nmoles/gland; DBO, 0·97 ± 0·07 nmole octopamine formed/gland/hr.

1 and 10 days (3- and 4-fold increment), the largest absolute increases occurred after 20 days. Dopamine β-hydroxylase levels displayed a similar growth characteristic, but from birth to 20 days of age the relative levels of the enzyme were higher than those of catecholamines ($P < 0.01$, Fig. 2). The ratios of catecholamines to dopamine β-hydroxylase at these time periods were about 20–40 per cent below adult levels, indicating an excess of dopamine β-hydroxylase compared to catecholamines (Table 3).

TABLE 3. RATIOS OF CATECHOLAMINES (CA), DOPAMINE β-HYDROXYLASE (DBO) AND ATP IN DEVELOPING RAT ADRENAL GLANDS*

Age (days)	Whole gland CA/DBO (nmoles/unit)	Purified vesicles CA/ATP (nmoles/nmole)
1	37 ± 5 (6)	4·07 ± 0·24 (6)
	$P < 0.001$	$P > 0.5$
10	51 ± 1 (6)	4·44 ± 0·08 (6)
	$P < 0.01$	$P < 0.001$
20	46 ± 3 (6)	3·85 ± 0·16 (6)
	$P < 0.005$	$P > 0.2$
30	57 ± 1 (6)	4·16 ± 0·08 (6)
	$P > 0.1$	$P > 0.1$
40	67 ± 4 (6)	4·00 ± 0·12 (6)
	$P > 0.2$	$P > 0.9$
50	62 ± 3 (17)	3·99 ± 0·06 (20)

* Values are given as mean ± S.E. The numbers in parentheses denote the number of determinations. Significance is by comparison with 50-day values.

In contrast, the age-dependent increases in catecholamines and ATP in the purified vesicles were identical, except at 10 days (Fig. 3), at which time the catecholamine to ATP ratio was slightly elevated (Table 3). At all other times the ratio was not significantly different from the expected value of 4.

TABLE 4. SUBCELLULAR DISTRIBUTION OF CATECHOLAMINES IN HOMOGENATES OF DEVELOPING RAT ADRENALS—DIFFERENTIAL CENTRIFUGATION*

Age (days)	A 800 *g* pellet	S 26,000 *g* supernatant	P 26,000 *g* pellet	No. of determinations
1	18·7 ± 0·7	22·7 ± 0·7	58·5 ± 1·3	12
	$P > 0.8$	$P < 0.001$	$P < 0.001$	
10	19·4 ± 0·4	17·4 ± 0·5	62·8 ± 2·1	6
	$P > 0.1$	$P > 0.1$	$P > 0.2$	
20	19·4 ± 0·9	16·5 ± 0·6	64·1 ± 1·4	6
	$P > 0.2$	$P > 0.5$	$P > 0.5$	
30	19·8 ± 0·6	15·3 ± 0·7	64·4 ± 2·0	6
	$P > 0.1$	$P > 0.2$	$P > 0.5$	
40	18·6 ± 0·6	15·3 ± 0·7	65·7 ± 1·3	6
	$P > 0.9$	$P > 0.2$	$P > 0.5$	
50	18·5 ± 0·5	16·3 ± 0·5	65·0 ± 0·6	24

*Values are given as mean ± S.E. of the percentage of total catecholamines. Significance is determined by comparison with 50-day values.

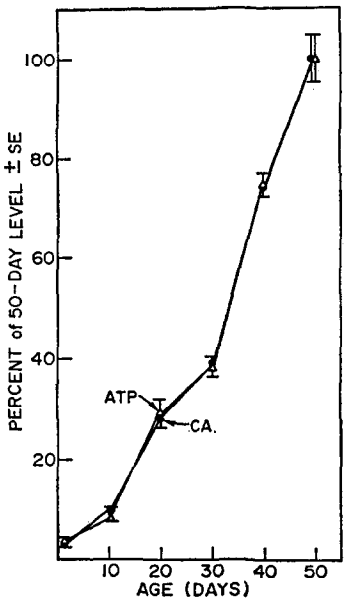

FIG. 3. Catecholamine (CA) levels (●) and ATP (△) in purified vesicles expressed as percentages of 50-day values. Points represent mean ± S.E. of six determinations for ages 1–40 days, 20 determinations for age 50 days. Fifty-day values were: CA, 28·6 ± 1·3 nmoles/gland; ATP, 7·17 ± 0·32 nmoles/gland.

Subcellular distribution—differential centrifugation (*Table* 4). In adult rats, approximately 19 per cent of the catecholamines was found in the 800 *g* pellet (A), 16 per cent in the 26,000 *g* supernatant (S) and the remaining 65 per cent in the 26,000 *g* pellet (P). At birth, the distribution of catecholamines was different from that of adults; a greater proportion was found in fraction S and less in fraction P, and this difference from adults disappeared by 10 days of age. At 20–40 days, there was no distribution difference compared to adults.

Subcellular distribution—discontinuous sucrose density gradient centrifugation (*Table* 5). In adult rats, 21 per cent of the catecholamines was found in the light sucrose layer (B) and about half of the catecholamines was found in the pellet (D). The smallest amount was found in the 1·6 M sucrose layer (C). At birth, there was little change in the percentage in fraction B, but there was a decrease of 7 per cent of the total catecholamines in fraction D, part of which represented an increase in fraction C. In contrast, at 10 and 20 days, decreases in fraction D reflected increases in catecholamines in fraction B amounting to 7–9 per cent of the total (1·5-fold increase in fraction B). Differences in distribution on the discontinuous gradient disappeared by 30–40 days of age.

Insulin-induced secretion from the adrenal medulla (*Table* 6). Insulin-induced hypoglycemia results in a reflex sympathetic discharge. In adult rats, insulin caused secretion of about one-fourth of the adrenal catecholamine stores in 3 hr. Similar degrees of depletion were observed in rats from ages 10 to 40 days. However, 1-day-old rats did not respond to insulin, despite the fact that the animals appeared to be in hypoglycemic shock.

TABLE 5. SUBCELLULAR DISTRIBUTION OF CATECHOLAMINES IN HOMOGENATES OF DEVELOPING RAT ADRENALS—DENSITY GRADIENT CENTRIFUGATION*

Age (days)	A 800 *g* pellet	B 0·3 M sucrose +B–C interface	C 1·6 M sucrose	D 140,000 *g* pellet	No. of determinations
1	18·8 ± 0·6	23·4 ± 0·9	15·8 ± 0·8	41·9 ± 1·5	12
	P > 0·5	P > 0·05	P < 0·001	P < 0·001	
10	20·2 ± 0·4	30·2 ± 1·1	12·2 ± 0·6	39·4 ± 1·0	6
	P > 0·1	P < 0·001	P < 0·02	P < 0·001	
20	19·4 ± 0·9	28·3 ± 1·3	10·5 ± 1·1	42·3 ± 2·0	6
	P > 0·8	P < 0·001	P > 0·9	P < 0·005	
30	20·4 ± 0·6	23·2 ± 0·9	13·1 ± 0·8	42·8 ± 1·5	6
	P > 0·1	P > 0·1	P < 0·01	P < 0·002	
40	19·2 ± 0·6	19·3 ± 1·5	12·3 ± 0·6	48·2 ± 1·5	6
	P > 0·9	P > 0·2	P < 0·02	P > 0·5	
50	19·2 ± 0·5	21·3 ± 0·8	10·5 ± 0·3	49·2 ± 0·9	24

* Values are given as mean ± S.E. of the percentage of total catecholamines. Significance is determined by comparison with 50-day values.

Secretion in vitro (*Fig.* 4). When adrenal glands from adult rats were incubated in Locke's solution, there was little difference in release of catecholamines between the third and fourth half-hour washes. When high potassium concentrations were used in the fourth wash, however, there was a doubling of the release of catecholamines. The increased release with potassium was prevented by incubation in calcium-free medium. Glands from 1-day-old rats also demonstrated potassium-induced release, which was prevented in calcium-free Locke's solution.

TABLE 6. INSULIN-INDUCED SECRETION OF CATECHOLAMINES FROM ADRENAL MEDULLAE OF DEVELOPING RATS*

Age (days)	Catecholamines (nmoles/gland)		Secretion (%)
	Control	3 hr post-insulin	
1	1·5 ± 0·1 (6)	1·5 ± 0·1 (6)	0 ± 4 P > 0·9
10	8·4 ± 0·5 (4)	5·7 ± 0·4 (5)	32 ± 5 P < 0·005
20	13·2 ± 0·5 (5)	9·8 ± 0·1 (4)	26 ± 1 P < 0·001
30	29 ± 1 (5)	20 ± 1 (5)	30 ± 5 P < 0·002
40	45 ± 2 (4)	29 ± 2 (4)	35 ± 5 P < 0·005
50	69 ± 2 (3)	51 ± 2 (2)	26 ± 3 P < 0·02

* Values are given as mean ± S.E. The numbers in parentheses denote the number of determinations. Significance is determined by comparison with control values.

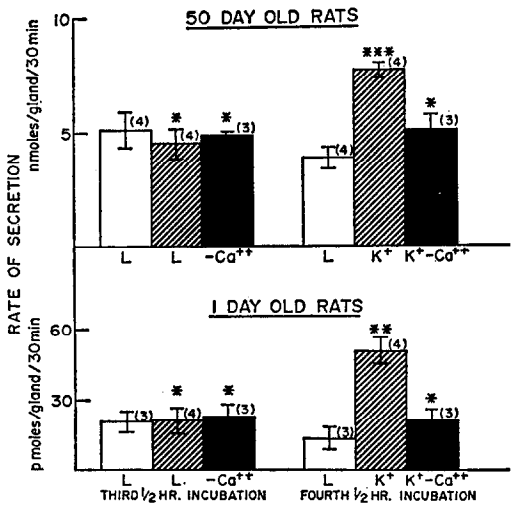

FIG. 4. Potassium-induced release of catecholamines from adrenal glands incubated at 37° in Locke's solution. Clear bars denote control glands (incubated in Locke's solution (L) throughout). Bars with diagonal lines denote glands incubated in Locke's solution (L) and switched to high potassium Locke's solution (K^+) in the fourth half-hour period. Shaded bars denote glands incubated in calcium-free Locke's solution ($-Ca^{2+}$) and switched to high potassium, calcium-free Locke's solution (K^+–Ca^{2+}) in the fourth period. Decreases in controls between period 3 and 4 are not significant ($P > 0.05$). Numbers in parentheses refer to the number of determinations for each type of incubation. A single asterisk = not significant ($P > 0.05$) compared to incubation in normal Locke's solution during same incubation period; the double asterisk = $P < 0.01$; and the triple asterisk = $P < 0.001$.

DISCUSSION

Between birth and 20 days of age, changes occur in the distribution of catecholamines on sucrose density gradients. There is an excess of catecholamines in fraction B (0·3 M sucrose layer) compared to adult rats, while the percentage of catecholamines in fraction D (140,000 *g* pellet) is lower than in adults (Table 5). These differences can arise from at least two sources: first, there may be a population of lighter vesicles which do not sediment in 1·6 M sucrose; second, a greater proportion of vesicles may be lysed during homogenization (increased fragility). To determine which was the case, the 800 *g* supernatants of adrenal homogenates in isotonic sucrose were spun at 26,000 *g* to sediment storage vesicles, partially filled vesicles and broken vesicle membranes. The proportion of catecholamines in the 26,000 *g* supernatant thus represents the fraction of catecholamines released into the medium by lysis of vesicles (Table 4). If the catecholamines in this fraction (S) are subtracted from the catecholamines in fraction B (values in Table 5 minus values in Table 4), we obtain the percentage of catecholamines associated with intact vesicles, which are less dense than normal and hence do not sediment in 1·6 M sucrose ("light" vesicles). The values derived are shown in Table 7. At birth, the percentage of catecholamines in light vesicles is lower than in the adult, which indicates that the high percentage of catecholamines in fraction B in the neonate represents increased fragility rather than decreased vesicle density; indeed, the low value for light vesicles suggests that the vesicles are more dense at birth or that the light vesicles of the neonate are more fragile and are preferentially destroyed by homogenization. The increased lysis may

TABLE 7. PERCENTAGE OF CATECHOLAMINES IN INTACT STORAGE VESICLES FOUND IN FRACTION B ("LIGHT" VESICLES)*

Age (days)	Total catecholamines in "light" vesicles (%)	No. of determinations
1	0·7 ± 1·1	12
	P < 0·005	
10	12·8 ± 1·2	6
	P < 0·001	
20	11·8 ± 1·4	6
	P < 0·001	
30	7·9 ± 1·1	6
	P > 0·05	
40	4·0 ± 1·7	6
	P > 0·5	
50	5·0 ± 0·9	24

* Values are given as mean ± S.E. Significance is determined by comparison with 50-day values.

reflect greater mechanical fragility or osmotic fragility. Since the vesicles of the neonates appear to contain higher catecholamine contents per vesicle,[9] the latter factor may play a major role.

In comparison to the situation in neonates, at 10–20 days, the percentage of catecholamines in light vesicles is more than twice normal, and there is no increase in fragility. These observations confirm earlier studies[9] showing that vesicles labeled with radioactive amines equilibrate on continuous density gradients at densities above above normal at birth, but below normal at 10–20 days. Since light vesicles probably contain lower than normal catecholamine contents,[9,15,16,21,22] they represent a greater proportion of the total number of vesicles than the figures for catecholamines indicate.

In some samples, the fraction of catecholamines in the 1·6 M sucrose layer (C) was elevated somewhat, which may be due to several factors: (1) failure of the vesicles to form a hard pellet because of the small amounts of material at the earlier ages, (2) increased leakage of catecholamines as they pass through the 1·6 M sucrose, or (3) the presence of vesicles of lighter density.

If, as has been suggested, age-dependent differences in catecholamine content are at least in part responsible for differences in vesicle density,[9] then alterations should occur in the relative amounts of catecholamines and dopamine β-hydroxylase (DBO, an enzyme marker for storage vesicles).[23] There was a lower CA/DBO ratio in adrenals from 1-, 10- and 20-day-old rats, which in the case of the latter two age groups confirms the hypothesis that there are light vesicles present which are relatively deficient in catecholamines. At birth, however, the proportion of light vesicles is below normal, and one would not expect to find a low CA/DBO ratio, The extra DBO at birth which is not associated with intact vesicles thus may represent enzyme which has not yet been incorporated into vesicles or it may represent empty vesicle membranes left behind after secretion of the vesicle contents.[10,14,15,22,24] It was therefore important to establish whether the neonatal adrenal medulla is capable of secretion. After insulin-induced hypoglycemia, there was no release of adrenal catecholamines in neonates, but secretion was observed at all other ages. This indicates that there is little or no

neural input to the adrenal at birth, which may reflect either lack of effective innervation of the organ or inability of the gland to release amines upon depolarization of the chromaffin cells. Neonatal adrenals incubated *in vitro* did release catecholamines in response to depolarizing concentrations of potassium and the release was calcium-dependent, indicating that secretion (rather than increased leakage) was occurring, just as it does upon neural stimulation.[25–27] Thus, the absence of splanchnic stimulation is probably due to ineffective inervation of the adrenal. Indeed, although neural tissue is detectable in the adrenal at birth, there are marked proliferative changes in the neurons in the ensuing weeks.[28]

Even though there is no neural input at birth, the studies *in vitro* suggest that the adrenal medulla still can secrete amines in response to certain stimuli. For example, fetal and neonatal calves, which also lack functional adrenal innervation, can secrete amines in response to anoxia but do not respond to splanchnic stimulation.[6] Thus, it is likely that in rats the excess DBO at birth represents vesicle membranes left behind after non-neurogenic amine secretion during the stress of birth (while in older rats the extra DBO represents vesicles with low catecholamine contents). The vesicle membranes are either destroyed or recycled within 1 or 2 days after amine secretion.[15]

The level of neural input also plays a role in the regulation of the synthesis of vesicle constituents. In adult animals, increased neural stimulation results in accelerated synthesis of vesicles followed by increases in catecholamine synthesis,[10,14,15] and in developing rats denervation of the adrenal attenuates age-dependent increases in tyrosine hydroxylase, catecholamines and dopamine β-hydroxylase.[5] Similarly, the present study tends to confirm that the developmental differences in vesicle density result from neurally mediated changes in vesicle and catecholamine synthesis rates; the unique properties (higher density, increased fragility, non-neurogenic secretion etc.) of storage vesicles in the neonate are associated with lack of effective innervation, and these properties change when neural stimulation becomes significant (by 10 days of age). The lack of neural input at birth probably results in a low rate of vesicle synthesis and a low rate of secretion such that the vesicles become maximally loaded with soluble constituents (catecholamines, ATP, soluble proteins) and, as a result, these vesicles are more dense on the average than in adults and fewer "light vesicles" are present. On the other hand, the high level of neural input at subsequent times probably causes increased vesicle turnover, with the resultant formation of partially filled vesicles similar to those formed in adult rats whose adrenal catecholamines have been depleted by massive neurogenic stimulation.[14,15] Similarly, the uptake and storage properties of the vesicles at 10 and 20 days resemble those of the partially filled, newly formed vesicles in catecholamine-depleted adults.[9,14,15]

During the course of development, there is little change in the catecholamine/ATP ratio. Thus, the vesicles which are catecholamine-deficient (10–20 days) are also equally ATP-deficient, which implies that the accumulation of ATP by the storage vesicles plays a determining role in the degree of loading with catecholamines. This contrasts with the situation in massively stimulated adult adrenals, where the resynthesis of catecholamines is probably rate-limiting.[15] At 10 days of age, the catecholamine/ATP ratio was elevated by 10 per cent, and at this age the vesicles bind catecholamines in a somewhat less stable manner,[9] which probably reflects loose binding of the excess catecholamines to vesicle proteins and membranes. The observation that catecholamine levels exceed the stable binding capacity confirms the view that at 10

days catecholamine synthesis is not rate-limiting in the development of amine stores.

The presence of vesicles deficient in ATP as well as in catecholamines should result in changes in the specificity of the uptake and storage of amines. Metaraminol, which probably does not enter the ATP storage complex but rather is bound loosely within the vesicles,[14,29,30] should be incorporated into immature, ATP-deficient vesicles to nearly the same extent as in mature vesicles. Epinephrine, which does complex with ATP, should be incorporated to a lesser extent in immature vesicles. Previous studies[9] have demonstrated that the predicted age-dependent change in amine specificity does occur. Furthermore, the change in specificity correlates with the developmental changes in the subcellular distribution of catecholamines and relative catecholamine, ATP and DBO contents. These age-dependent alterations probably affect the response of the adrenal to pharmacological agents; for example, infant rats are more susceptible to catecholamine depletion by reserpine and tetrabenazine than are adults.[31]

The maturation of the adrenal medulla in the rat can be summarized as follows:

(1) At birth. Neural input is absent, resulting in a low rate of vesicle turnover. The vesicles become fully loaded with soluble constituents and are more dense than in adults. In addition, the vesicles are more fragile and non-neurogenic secretion can occur in response to certain forms of stress.

(2) At 10–20 days. Neural input is high, resulting in a high rate of vesicle turnover with formation of ATP- and catecholamine-deficient vesicles of lower density (low catecholamine/DBO ratios). The ATP deficiency results in decreased specificity for epinephrine vs metaraminol and may contribute to decreased catecholamine binding stability at 10 days.

(3) At 30–40 days. All parameters approach normal adult values.

In contrast to these changes in the properties of the storage vesicles, the overall development (on a per gland basis) of catecholamines, ATP and DBO is quite similar (Figs. 2 and 3). Furthermore, the magnitudes of the changes in vesicle content and properties in developing rats are not as large as in insulin-treated adult rats, where catecholamine/ATP and catecholamine/DBO ratios may be as low as one-third of normal during the recovery process.[14,15] However, it should be noted that in these massively stimulated adult rats, catecholamine stores are being regenerated within 4 days, while in the developing rats the same order of increase is spread over 50 days. The present studies do indicate, though, that significant developmental changes occur in the content and properties of the storage vesicles and the rates of synthesis of the components are influenced by neural input.

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