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Developmental characteristics of brain catecholamines and tyrosine hydroxylase in the rat: effects of 6-hydroxydopamine

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Summary

1. Brain noradrenaline, dopamine and tyrosine hydroxylase were found in rat brain a few days before birth and increased progressively until reaching adult values. The most rapid period of growth for these substances seemed to occur between 7 and 18 days.

2. The intracisternal administration of 6-hydroxydopamine to rats 7 days of age reduced concentrations of noradrenaline, dopamine, and tyrosine hydroxylase by 72 hours.

3. The concentrations of noradrenaline, dopamine or tyrosine hydroxylase in rats that received 6-hydroxydopamine at 7 or 14 days of age remained markedly reduced when determined at adulthood, indicating that fibres did not continue to develop after the administration of this compound. The rats treated at 7 days also showed diminished concentrations of noradrenaline in heart.

4. Rats injected with 6-hydroxydopamine at 7 days had reduced body weight as well as a reduction of some organ weights. This growth deficit was not observed in animals that received this drug at 14 days of age.

5. The administration of ovine growth hormone to rats that received 6-hydroxydopamine at 7 days did not reverse the growth deficiency in these animals.

Introduction

Many attempts have been made to define possible functions for brain noradrenergic and dopaminergic fibres in adult animals; in the developing animal relatively few studies, however, have been performed. In 1962, Karki, Kuntzman & Brodie found that concentrations of monoamines in the guinea-pig, which is well developed at birth, were almost as high as in the adult. In contrast, the newborn rat whose functional development is poor, had low concentrations of monoamines. In an extension of this work Agrawal, Glisson & Himwich (1966, 1968) studied the progress of the development of the monoamines in the mouse and rat and confirmed that monoamines were low at birth and progressively increased to adult concentrations. More recently, Loizou & Salt (1970) have suggested that the progressive increase in the concentrations of brain monoamines in the rat is a consequence of the progressive proliferation of axon terminals.

With the view that investigating the development of monoamine systems might assist in understanding their physiological significance, our laboratory examined the development of dopamine, noradrenaline and tyrosine hydroxylase prenatally and postnatally in the developing rat. In an effort to determine if the absence of central adrenergic fibres would have an effect on the development of animals, rats were treated with 6-hydroxydopamine at various times postnatally and the effect on catecholamines, tyrosine hydroxylase and growth was determined after this treatment (Traylor & Breese, 1971).

Methods

Pregnant female Sprague-Dawley rats were obtained from Zivic-Miller Laboratories (Pittsburgh, Pa.) 10 days before gestation. Offspring and adults of both sexes of this strain were used throughout. Animals were reared and kept under control lighting conditions (10 h light and 14 h dark). Young pups were left with the mother until the time of killing (10.00 a.m.–3.00 p.m.). Some mothers were anaesthetized before delivery and the pups were removed from the uterus. In this case, the whole head of the pup was used for assay; otherwise the whole brain was used. When necessary to obtain sufficient material for analysis, two to four brains were pooled. To minimize biological variation, animals at a given age were taken from at least two litters. Adult animals between the age of 10–16 weeks were run with each analysis for comparison.

Determinations of noradrenaline and dopamine were carried out as previously described (Breese & Traylor 1970; 1971). Tyrosine hydroxylase was isolated from brain tissue according to the method of Musacchio, Julou, Kety & Glowinski (1969). Enzyme activity was determined by the method of Nagatsu, Levitt & Udenfriend (1964).

In a separate sequence of experiments, four litters of animals were equally divided. At 7 days, one-half of the rat pups received 100 μg of 6-hydroxydopamine intracisternally; the other half received saline solution containing 0·3 mg/ml of ascorbic acid. The animals were weighed daily during the first 21 days and every 2–4 days thereafter until they reached at least 60 days of age. A similar experiment was also performed on animals at 14 days except that they were given 150 μg of 6-hydroxydopamine and were killed at 44 days of age. After killing, brains were removed and split sagitally with one-half being used for dopamine and noradrenaline analysis and the other half for tyrosine hydroxylase determination. In addition, the weights of adrenal, pituitary, brain, and sex organs were recorded as well as tibia and tail lengths. In some animals treated at 7 days, noradrenaline in heart was also determined.

6-Hydroxydopamine HCl was obtained from Regis Chemical Company. The growth hormone (ovine) (NIH-GH-59) was a gift from the Endocrinology Study Section, National Institutes of Health.

Results

Development of brain noradrenaline and dopamine in the rat

Brain noradrenaline and dopamine were determined in animals from 6 days before the expected date of gestation to 80 days of age (Figs. 1 and 2). The data are ex-

pressed as a percentage of adult values for whole brain as well as per unit weight of brain. As can be seen, higher values were observed when presented on the basis of unit weight as compared with whole brain values. Adult values for noradrenaline and dopamine were derived from both male and female rats since no significant difference in the concentration of the amines was observed to occur related to the sex of the animals (Table 1). The finding is somewhat different from that reported by Agrawal *et al.* (1966) who found that noradrenaline concentrations were lower in male rats than in females.

Noradrenaline and dopamine were first clearly present about 3 days before birth (Figs. 1 and 2). While a significant rise in these catecholamines occurred between 0–7 days ($P<0{\cdot}01$), the greatest rise per unit time occurred between 7 and 18 days. From this time, until adulthood, the accumulation of amines was not as rapid and the rate remained relatively constant. In agreement with an earlier report by

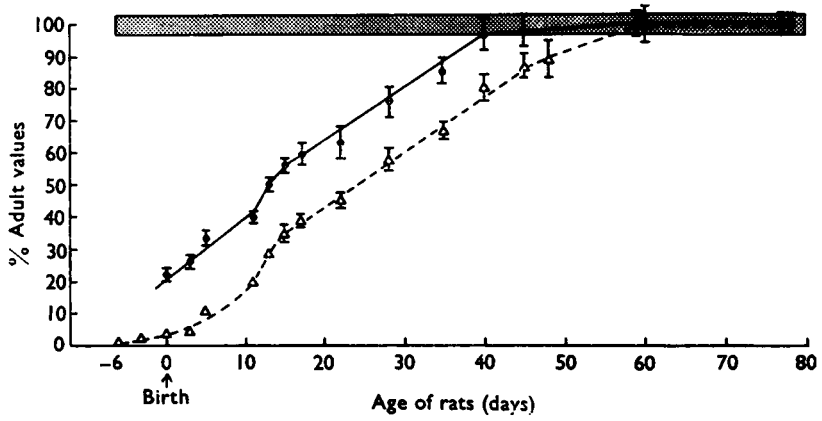

FIG. 1. Development of brain noradrenaline in the rat. (△---△), Noradrenaline per whole brain; (●——●), noradrenaline per gramme of brain tissue. Shaded section, adult value ± S.E. expressed as a percentage. Adult value for noradrenaline was 698 ± 50 ng/brain. Each point represents the mean ± S.E. of at least six determinations.

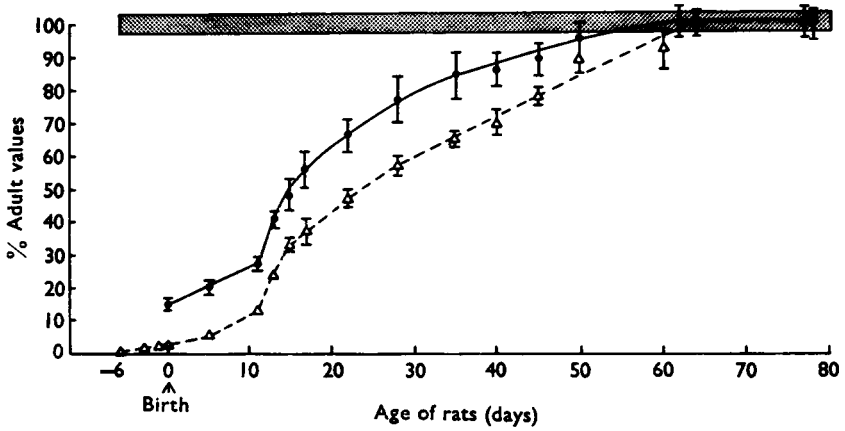

FIG. 2. Development of brain dopamine in the rat. (△---△), Amount of dopamine per whole brain; (●——●), amount of dopamine per gramme of tissue. Shaded portion, adult value ± S.E. expressed as a percentage. Adult value for dopamine was 1,189 ± 43 ng/brain. Each point represents the mean ± S.E. of at least six determinations.

Agrawal *et al.* (1966), noradrenaline values were found to reach a maximum at 40 days of age when based upon unit weight of brain (ng/g). On this basis, dopamine did not reach mature concentrations until about 50 days. Since brain continues to grow beyond these periods, total brain catecholamine concentrations did not reach maturity until rats had reached approximately 60 days of age.

Development of brain tyrosine hydroxylase

As found with noradrenaline and dopamine, tyrosine hydroxylase was present in brain 3 days before birth (Fig. 3). A triphasic curve of development as described for the catecholamines was also observed for tyrosine hydroxylase activity. The most rapid increase in activity was noted between the 7th and the 15th–18th day. However, activity of this enzyme seemed to reach maturity somewhat sooner than observed for the catecholamines (Figs. 1 and 2). In addition, no significant difference in the concentration of tyrosine hydroxylase was found to occur between control male and female rats paralleling the finding previously noted for catecholamines (Table 1).

Effect of intracisternally administered 6-hydroxydopamine on brain noradrenaline, dopamine and tyrosine hydroxylase

In an effort to determine if 6-hydroxydopamine would have a similar destructive effect on catecholamine fibres in developing animals as previously observed in adults (Breese & Traylor, 1970, 1971), animals 7 days of age were given 6-hydroxydopamine intracisternally and brain noradrenaline, dopamine and tyrosine hydroxylase deter-

TABLE 1. *Brain noradrenaline, dopamine and tyrosine hydroxylase activity in mature male and female rats*

	Noradrenaline (ng/g)	Dopamine (ng/g)	Tyrosine hydroxylase ((nmol/g)/h)
Male	372±24	530±28	4·36±0·27
Female	359±14	521±23	4·57±0·24

The male and female rats are from mixed litters and were 92 days of age. Values represent the mean ±S.E. of eight rats.

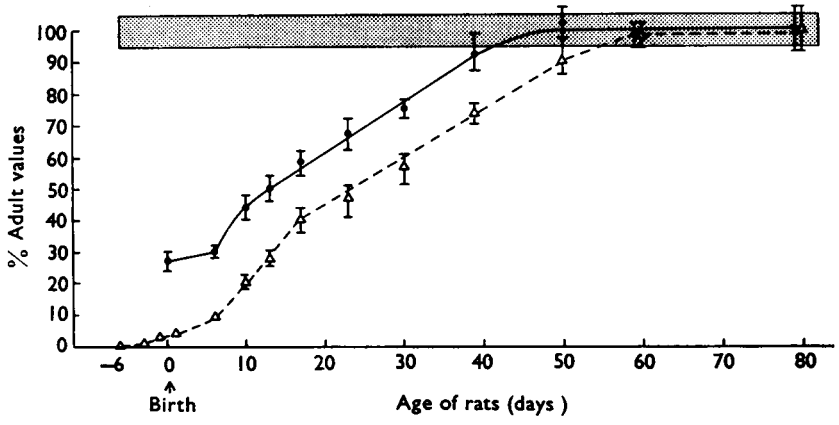

FIG. 3. Development of brain tyrosine hydroxylase in the rat. (△– – –△), Tyrosine hydroxylase activity per whole brain; (●——●), tyrosine hydroxylase activity per gramme of tissue. Shaded portion, tyrosine hydroxylase activity ((4·38±0·25 nmol/g)/h) in brains of adult animals expressed as a percentage. Each point represents the mean±S.E. of at least six determinations.

mined 3, 24, and 72 h after injection (Fig. 4). Noradrenaline was found to be severely depleted while dopamine was only moderately reduced at 3 hours. At this time, tyrosine hydroxylase was reduced by 50%. Noradrenaline remained comparably reduced during 72 hours. Dopamine was reduced to 30% of control by 24 h and to approximately 10% of control by 72 hours. Tyrosine hydroxylase was reduced approximately 90% by 24 h and remained depressed at 72 hours.

With the knowledge that 6-hydroxydopamine could reduce the amines present at 7 days, further experiments were run on developing animals to determine if such treatment would prevent further development of adrenergic fibres in the central nervous system. In this case, animals of 14 days were included in addition to the animals injected at 7 days. In an effort to have equal depletions, 100 μg was administered to 7 day old rats and 150 μg to the 14 day old animals. The results of these treatments are presented in Table 2.

In the rats treated with 6-hydroxydopamine at 7 days, noradrenaline was reduced by some 80% and dopamine by approximately 75% when killed at 85 or 102 days of age. Tyrosine hydroxylase was drastically reduced in these animals (Table 2). In

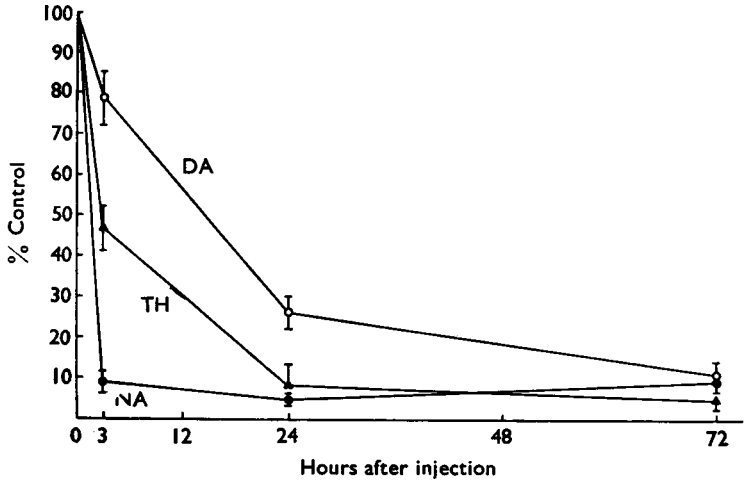

FIG. 4. Acute effects of 6-hydroxydopamine on dopamine (DA), tyrosine hydroxylase (TH) and noradrenaline (NA) in rat pups injected at 7 days of age. 6-Hydroxydopamine (100 μg) was administered intracisternally. Each point represents the mean ± S.E. of four to five determinations.

TABLE 2. *Effect of 6-hydroxydopamine on noradrenaline, dopamine and tyrosine hydroxylase in the developing rat*

Treatment	Age dosed day	Noradrenaline (ng/g)	Dopamine	Tyrosine hydroxylase ((nmol/g)/h)
Group A				
Control	7	388±25	532±56	4·60±0·15
6-OHDA	7	77± 9*	45±18*	0·09±0·03*
Group B				
Control	14	328±12	481±41	4·57±0·24
6-OHDA	14	59± 6*	54±22*	0·85±0·15*

Rats from group A received either 100 μg of 6-hydroxydopamine (6-OHDA) or vehicle intracisternally on day 7. These animals were killed between 85 and 102 days of age. Group B rats received either 150 μg of 6-OHDA or vehicle intracisternally on day 14 and were killed when the rats were 44 days of age. Values represent the mean ±S.E. of seven to fifteen male rats. The mean concentrations found for females which are not included were not significantly different from the values found in males. *$P<0.001$ when compared with corresponding control.

the animals treated at 14 days, noradrenaline and dopamine were 18 and 14% of control respectively at 44 days of age. Tyrosine hydroxylase activity was reduced by some 81%.

Effect of intracisternally administered 6-hydroxydopamine on heart noradrenaline in the developing rat

In addition to the brain chemistry, cardiac noradrenaline was determined in some of the developing animals that had been previously injected intracisternally with 6-hydroxydopamine (Table 3). Somewhat unexpectedly, it was found that the total heart concentration of noradrenaline was decreased. Since heart size was also smaller, the amount per gramme of tissue did not show this marked difference such that the difference was not significantly different in female rats (Table 3).

Effect of 6-hydroxydopamine on growth in the developing rat

In the course of examining the effect of 6-hydroxydopamine on catecholamines and enzymes in the developing rat, our attention turned to the possibility that 6-hydroxydopamine might be disrupting the growth pattern normally observed in the untreated rat. The results of this study are shown in Fig. 5. The results clearly show that 6-hydroxydopamine administered to 7 day old rats will cause a marked retardation of growth in female as well as male rats although the growth deficiency is greater in the males. While a marked difference in stature was obvious, the animals did not give an impression of being incapacitated by the drug treatment at any time after injection. Figure 6 is a photograph of a control rat and a 6-OHDA treated litter mate permitting a graphic indication of the impairment in growth observed in treated rats at 60 days of age.

The weight of the pituitary, adrenals, ovary and uterus were also significantly reduced in animals that received 6-hydroxydopamine at 7 days (Table 4). Testicular weight in the male was not significantly decreased. Brain weight was significantly reduced in male rats treated with 6-hydroxydopamine; the brains of female rats showed a tendency for decreased size but the reduction was not significantly different. Tibia length and tail length were shorter in both treated males and treated females. In contrast to the absolute organ weights, when based on weight per 100 g of body weight, no significant difference was observed between the weight of the endocrine organs of the control and 6-hydroxydopamine treated animals that previously showed a significant difference.

TABLE 3. *Effect of 6-hydroxydopamine on noradrenaline concentrations in cardiac tissue*

	Weight (g)		Noradrenaline (ng/heart)		(ng/g)	
	M	F	M	F	M	F
Control	1·37 ±0·06	0·895±0·03	803±74	578±52	586±45	646±59
6-OHDA	0·963±0·07*	0·671±0·03*	393±28*	337±28*	408±24†	502±56

Animals received 100 μg of 6-hydroxydopamine (6-OHDA) intracisternally at 7 days of age and were killed between 80 and 87 days of age. Each value represents eleven to twenty-five animals. M, Male; F, female; * $P<0·001$; † $P<0·005$.

The reduction in body weight, tibia length and tail length suggested that reduced concentrations of growth hormone might be contributing to the reduced size of these

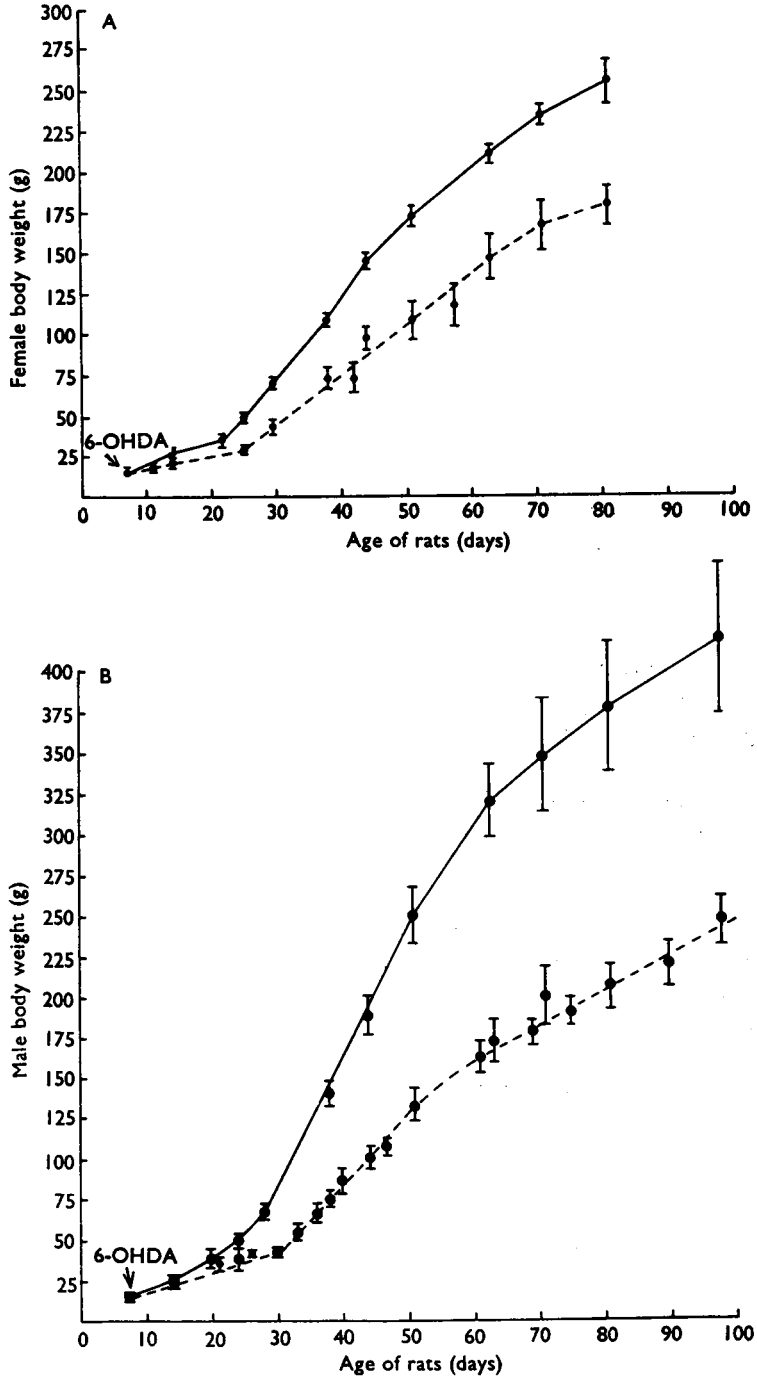

FIG. 5. Effect of 6-hydroxydopamine on body weight in female (A) and male (B) rats injected at 7 days of age. Animals received either 100 μg of 6-hydroxydopamine intracisternally or vehicle. (●——●), Control animals; (●- - -●), 6-hydroxydopamine treated animals. Each point represents six to sixteen rats. Vertical bars indicate ± S.E. of the mean.

TABLE 4. *Organ weights of animals treated with 6-hydroxydopamine at 7 days of age*

Treatment	Body weight (g)		Brain (g)		Pituitary (mg)		Adrenal (mg)		Tibia length (mm)		Tail length (mm)		Ovary (mg)	Uterus (mg)	Testis (mg)
	M	F	M	F	M	F	M	F	M	F	M	F			
Control	380 ±14	251 ±5	1·87 ±0·04	1·75 ±0·03	10·9 ±0·5	14·2 ±0·5	37·9 ±2·8	49·6 ±1·9	4·1 ±0·08	3·7 ±0·04	21·2 ±0·5	18·6 ±0·2	101·1 ±3·8	374·3 ±21·5	1422 ±240
6-OHDA	214* ±13	171* ±10	1·71† ±0·03	1·70 ±0·03	6·3* ±0·5	7·4* ±0·9	23·2* ±1·5	36·3* ±2·8	3·5* ±0·07	3·5* ±0·07	17·2* ±0·5	15·9* ±0·4	63·1* ±6·3	266·8‡ ±31·5	1313 ±90

Rat pups were treated with 100 μg of 6-hydroxydopamine (6-OHDA) at 7 days of age and were killed between 80 and 87 days of age. Values represent between eleven and seventeen animals. M, Male; F, female. * $P<0{\cdot}001$ when compared with control. † $P<0{\cdot}005$ when compared with control. ‡ $P<0{\cdot}025$ when compared with control.

animals. To test this possibility, ovine growth hormone was administered to treated animals for 25 days after injection of the 6-hydroxydopamine since this would be presumed to reverse the growth deficit if diminished growth hormone release were responsible. As shown in Table 5, this treatment did not alter the growth retardation caused by 6-hydroxydopamine treatment. The fact that the administration of growth hormone caused only a tendency for increased growth in controls could suggest that insufficient hormone was being administered. However, other investigators (Evans, Simpson, Marx & Kybreck, 1943; Greenspan, Li, Simpson & Evans, 1949) have clearly demonstrated that the doses chosen would sustain the growth of hypophysectomized rats.

In contrast to the animals treated at 7 days, it was observed that rats given a dose of 6-hydroxydopamine at 14 days of age did not show the growth deficit observed in animals injected at the younger age. As noted earlier, this treatment caused a depletion of brain noradrenaline and dopamine comparable to the animals treated at 7 days of age (Table 2).

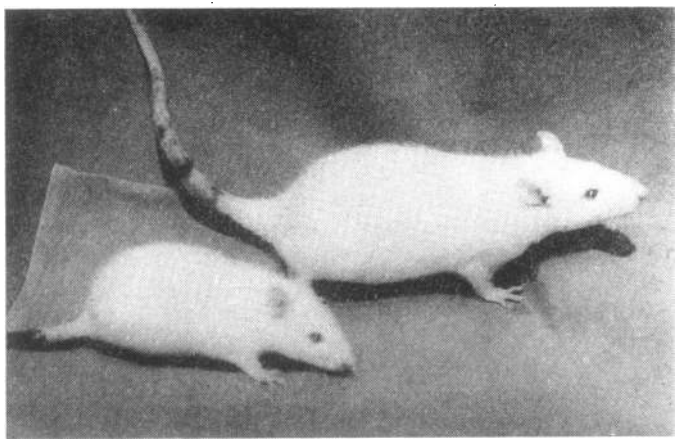

FIG. 6. Control female rat and 6-hydroxydopamine treated litter mate. The largest animal with three small black marks on its tail is the control. The small animal in the foreground with one heavy black mark is a female that received 6-hydroxydopamine at 7 days of age. Animals are approximately 60 days of age at the time of this photograph.

TABLE 5. *Effect of growth hormone in 6-hydroxydopamine treated developing rats*

Treatment	Growth hormone	Days after intracisternal injection					
		0	5	10	15	20	25
Control	Yes	20·4 ±0·8	27·7 ±1·5	39·3 ±2·9	55·6 ±1·7	88·3 ±1·1	128·5 ±3·1
6-OHDA	Yes	18·2 ±0·8	23·4* ±1·2	28·9† ±1·6	33·3* ±3·3	45·1* ±9·3	73·3* ±12·4
Control	No	20·3 ±1·3	30·1 ±1·7	42·6 ±1·3	51·4 ±1·9	77·9 ±4·2	113·0 ±11·0
6-OHDA	No	18·1 ±0·2	22·7* ±1·0	29·0† ±1·3	32·4* ±4·3	50·8* ±9·0	75·4* ±12·6

Animals were given 100 μg of 6-hydroxydopamine (6-OHDA) intracisternally at 7 days of age. Growth hormone (20 μg) was administered to animals from day 1 through day 5 after the administration of the 6-hydroxydopamine; 40 μg from day 6 through day 10; and 80 μg daily thereafter. Values refer to the mean weight (g) ± S.E. of eight to sixteen rats. * $P<0.01$ when compared with vehicle treated control. † $P<0.001$ when compared with vehicle treated control.

Discussion

In the present study the schedule of appearance of noradrenaline, dopamine as well as the rate-limiting enzyme tyrosine hydroxylase was examined in foetal and neonatal rat brain. While several previous studies have investigated the course of development of brain noradrenaline and dopamine in the rat (Agrawal *et al.*, 1966 ; Karki *et al.*, 1962 ; Loizou & Salt, 1970 ; Loizou, 1971), tyrosine hydroxylase had not previously been examined in this species. In agreement with earlier reports, noradrenaline and dopamine were found to be present in brain at birth. In fact, these amines appeared in brain approximately 3 days before birth. Tyrosine hydroxylase was also present 3 days before gestation and was seen to increase progressively until reaching adult values at near 40 days of age (Fig. 3). While not clearly evident, there was a tendency for the enzyme to precede the development of the catecholamines. McGeer, Gibson, Wada & McGeer (1967) have previously reported that tyrosine hydroxylase increased with postnatal development in kitten brain. Their data also suggested that the development of the enzyme preceded amine accumulation.

Since tyrosine hydroxylase has been associated with nerve ending particles (McGeer, Bagchi & McGeer, 1965), it is presumed that the increase in tyrosine hydroxylase postnatally may reflect terminal growth of developing nerve fibres. Holding a similar view for monoamine development, Loizou & Salt (1970) recently suggested that the increase of amines with age is a consequence of the proliferation of axon terminals. The rapid increase of amines and tyrosine hydroxylase between 7 and 18 days also coincides with the increased number of synaptic areas (Aghajanian & Bloom, 1967). However, the possible relationship between these two events remains obscure.

Earlier studies have established that 6-hydroxydopamine will destroy catecholamine-containing fibres in brain (Bloom, Algeri, Groppetti, Revuelta & Costa, 1969 ; Breese & Traylor, 1970 ; Uretsky & Iversen, 1970). To determine if this compound would have a similar effect in developing animals, 6-hydroxydopamine was administered to developing animals at 7 or 14 days of age. Amine concentrations and tyrosine hydroxylase fell immediately after injection and remained severely reduced as these animals matured, indicating that further growth of adrenergic neurones was not occurring.

Several investigators (Karki *et al.*, 1962 ; Loizou, 1971) have suggested that development of monoamines might be responsible for certain functional processes in the mammalian species. With the knowledge that 6-hydroxydopamine could prevent further development of adrenergic fibres, attention was given to the effects of 6-hydroxydopamine on functional development of treated animals. Rats were injected at 7 or 14 days of age. Although comparable depletions were observed after these treatments, quite different results were observed on growth. The animals treated at 7 days showed a marked reduction in body weight while the weight of animals treated at 14 days was not altered. This observation could be likened to the fact that treatment of immature rats with sex hormones during a critical period of development cause alterations in sexual behaviour at adulthood (Barraclough, 1961). While it is our impression that such treated animals may not move as well as controls, little more can be implied since the behaviour of these animals has yet to be evaluated fully.

In adults the administration of 6-hydroxydopamine does not alter cardiac amines after intracisternal administration (Breese & Traylor, 1970). In the present study, injection of 6-hydroxydopamine into brain to 7 day old rats reduced cardiac amines significantly. Lytle, Shoemaker, Cottmann & Wurtman (1971) have recently confirmed this observation in rats injected with 6-hydroxydopamine at birth. At present, it is uncertain whether this reduction of noradrenaline in heart is the result of the growth deficit observed in these animals or to a direct effect of 6-hydroxydopamine on peripheral adrenergic fibres with the amine reaching the peripheral circulation after intracisternal injection. If the latter, the result would be similar to the effect of 6-hydroxydopamine on peripheral fibres as described after peripheral administration of 6-hydroxydopamine by Angeletti & Levi-Montalcini (1970).

There is much literature to suggest that the control of growth hormone release from the pituitary is modulated by central adrenergic fibres (Müller, Sawano, Arimura & Schally, 1967 ; Müller, Pra & Pecile, 1968 ; Blackard & Heidingsfelder, 1968). It was, therefore, logical that our first viewpoint related to the growth deficient in 6-hydroxydopamine treated rats would include reduced growth hormone release as a possible factor. The reduced pituitary size and the shorter tibia and tail lengths in the 6-hydroxydopamine treated rats would support this contention. However, in the present study administration of growth hormone did not alter the growth deficit in the animals treated at 7 days with 6-hydroxydopamine. This would seem to discount a deficit of growth hormone as a possible explanation for the diminished growth. Nevertheless, in view of the massive literature supporting the view that adrenergic fibres play a role in growth hormone release, it would seem advisable to measure plasma growth hormone directly before completely discounting

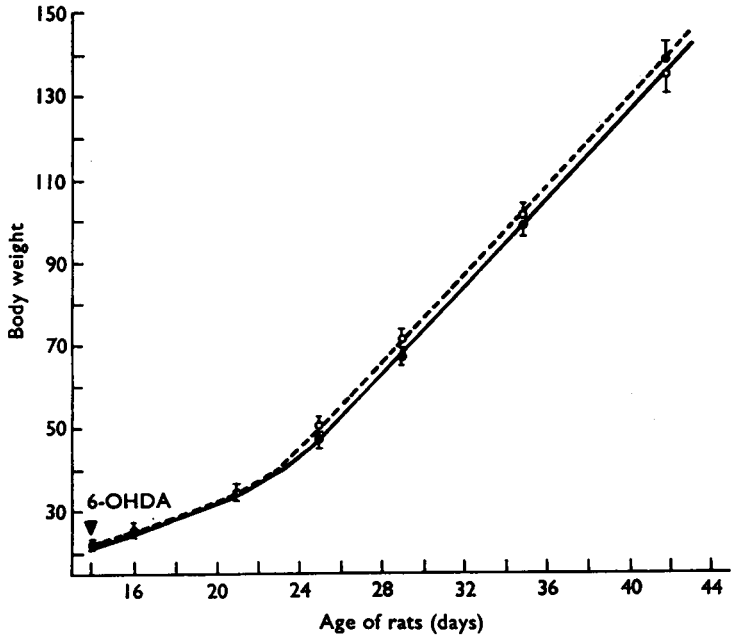

FIG. 7. Effect of 6-hydroxydopamine on body weight of rats injected at 14 days of age. Animals received 150 μg intracisternally at 14 days of age. Values represent equal numbers of males and females for both the control (●——●) and the 6-hydroxydopamine treated groups (○- - -○). Each point represents the mean ± S.E. of eight to fourteen rats.

that a deficiency of this hormone could be present in 6-hydroxydopamine treated rats. Furthermore, it is not inconceivable that multiple factors could contribute to the diminished growth of these animals. For example the reduced size of the animals could also be due to diminished food consumption. Lytle *et al.* (1971) have recently implied a nutritional component in animals with decreased weight that were treated with 6-hydroxydopamine at birth. Some evidence is presently available linking noradrenaline and food and water intake (Grossman, 1960). However, data on food and water intake in adult animals treated with 6-hydroxydopamine are confusing. While Bloom *et al.* (1969) reported that adults had normal intake of food and water after treatment, Stein & Wise (1971) found reduced intake of liquid after 6-hydroxydopamine. However, in the present study treatment of animals at 14 days of age did not alter growth, suggesting that food or water intake was not diminished (Fig. 7). Further work is certainly indicated to determine the mechanism(s) of the diminished growth of animals treated with 6-hydroxydopamine.

The recent report by Black, Bloom, Hendry & Iversen (1971) that the destruction of adrenergic fibres can influence the development of cholinergic fibres in sympathetic ganglion may be pertinent to the finding that 6-hydroxydopamine treatment caused diminished growth. Since it is known that the numbers of synaptic contacts are rapidly increasing in brain at the time 6-hydroxydopamine was administered, it is possible that this treatment may actually be interfering with proper development of other types of fibres as previously observed in sympathetic ganglia (Black *et al.*, 1971). This possibility is presently being investigated.

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