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Effect of inhibition of catecholamine synthesis on central catecholamine-containing neurones in the developing albino rat

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Summary

1. Tyrosine hydroxylase is thought to be the rate limiting enzyme step in catecholamine biosynthesis. Inhibition of this enzyme using α-methyl-*p*-tyrosine resulted in a time dependent depletion (and repletion) of formaldehyde induced fluorescence in catecholamine-containing neurones of the central nervous system in developing and adult rats.

2. Dopamine-containing neurones were depleted faster and more completely than noradrenaline-containing neurones.

3. The extent of depletion caused by α-methyl-*p*-tyrosine in the initial 6–9 h period was more or less comparable in young and adult rats from the age of 1 week onwards ; this suggests that catecholamine turnover increases with age and parallels the increase in catecholamine levels.

4. The extent of depletion (and repletion) 18 h after administration of the inhibitor varied in animals of different age.

5. Administration of a monoamine oxidase inhibitor just before administration of α-methyl-*p*-tyrosine resulted in a reduction of the extent of depletion caused by the latter drug, indicating that monoamine oxidase is important for the breakdown of catecholamines in rats of all ages.

6. It is suggested that the catecholamine-containing neurones of the newborn are biochemically as well as functionally differentiated before completion of morphological differentiation.

Introduction

In the albino rat the development of catecholamine (CA)-containing neurones and the appearance of CA in the central nervous system (CNS) are predominantly postnatal events (Loizou, in preparation ; Loizou & Salt, 1970). The presence of intraneuronal CA at birth suggests the intraneuronal localization of the enzymes involved in CA biosynthesis. Tyrosine hydroxylase is thought to be the rate limiting enzyme in the biosynthetic pathway for CA (Levitt, Spector, Sjoerdsma & Udenfriend, 1965 ; Nagatsu, Levitt & Udenfriend, 1964 ; Udenfriend, Zaltzman-Nirenberg, Gordon & Spector, 1966 ; Udenfriend, 1968). This investigation is concerned with the effect of inhibition of tyrosine hydroxylase, on the intraneuronal content of CA-containing neurones in the CNS of the developing rat. It was thought that such a study could give information as to the relative rates of turnover of CA in developing and adult

rats. The interaction of inhibition of monoamine oxidase (MAO), an enzyme involved in intraneuronal degradation of CA, with that of tyrosine hydroxylase was also studied in order to determine indirectly whether MAO is involved in CA degradation in neonatal rats.

Methods

Albino rats of a laboratory inbred Wistar strain were used. The methyl ester hydrochloride of DL-α-methyl-*p*-tyrosine (H44/68, Hassle, Sweden) was used to deplete endogenous CA stores by inhibition of tyrosine hydroxylase. The drug was administered subcutaneously in newborn to 2 week old rats and intraperitoneally in the older rats at a dose of 250–300 mg/kg as a 5% solution in 0·9% saline at pH 4–6. Groups of two to five rats aged 0–3 days, 1, 2, 3, 4, 5 weeks and adult were killed 6–9 h or 18 h after H44/68. In addition four adult rats were killed 2–4 h after H44/68 and four rats injected at birth were killed 1 or 7 days afterwards. Nialamide (Niamid, Pfizer) was administered subcutaneously or intraperitoneally at a dose of 200–250 mg/kg 10–30 min before H44/68, to another group of rats consisting of neonatal (six), 1 week old (two), 2 weeks old (three), 5 weeks old (two) and adult rats (two). The brains and spinal cords of these animals were always compared with those from rats given H44/68 alone for the same period of time.

The brain and spinal cord from experimental and normal animals were studied with the fluorescence histochemical technique of Falck & Hillarp for the demonstration of biogenic amines (Falck & Owman, 1965 ; Loizou, in preparation). With this technique, CA form a fluorophore which emits a green to yellow-green light in the fluorescence microscope.

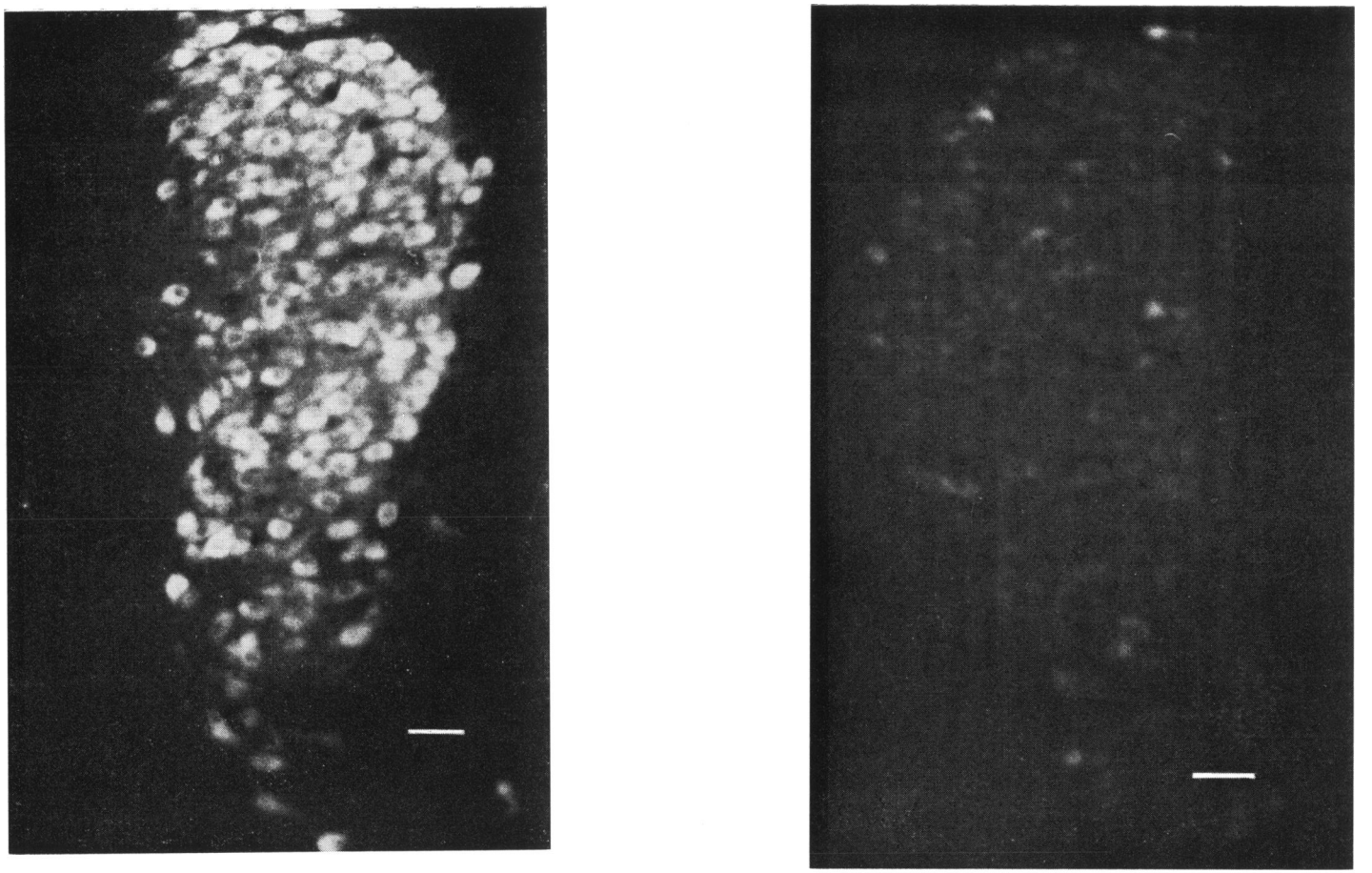
Results

Effect of H44/68 and nialamide on neonatal and 1 week old rats

CA-induced fluorescence in the CNS of newborn rats is limited to the CA-containing cell body groups A1–A10, to presumed dopamine (DA)-containing terminals in the telencephalon and a few scattered noradrenaline (NA)-containing terminals in the brain stem, hypothalamus and spinal cord. An increased number and fluorescence intensity of cell bodies as well as an increased density of terminals are seen in the 1 week old rat (Dahlstrom & Fuxe, 1964 ; Fuxe, 1965 ; Loizou, 1969).

Administration of H44/68 caused an almost complete disappearance of fluorescence from all CA-containing cell bodies and terminals in the brain and spinal cord after 6–9 h (Fig. 1a, b). There were slight individual variations in the extent of depletion.

Treatment of rats with nialamide 20–25 min before H44/68 resulted in a slight to marked reduction in the loss of fluorescence from CA-containing terminals caused by H44/68 alone. Nialamide, however, did not influence the loss from CA-containing cell bodies, almost all of which showed a loss of fluorescence comparable to that of animals treated with H44/68 alone. The efficacy of nialamide treatment was noted by the increase in the fluorescence induced in the 5-hydroxytryptamine (5-HT)-containing neurones, all of which had very strong yellow fluorescence in their cell bodies and processes, in all animals treated with nialamide +H44/68.



(a) (b)

FIG. 1. Locus coeruleus (a) normal 1 week old rat; (b) 1 week old rat 7 h after treatment with H44/68, 250 mg/kg subcutaneously. Calibration 50 μm.

The depletion of terminal and cell body fluorescence caused by H44/68 alone in newborn rats remained virtually complete after 18 and 24 h. Seven days after H44/68 administration to newborn rats, the rats had normal fluorescence in all CA and 5-HT-containing neurones.

In contrast to the newborn rats, there was a virtually complete repletion of all CA induced fluorescence in cell bodies and terminals of 1 week old rats 18 h after H44/68.

Two week old rats

H44/68 produced variable degrees of diminution of CA-fluorescence in 2 week old rats after 6 hours. CA-containing cell bodies (groups A1–A13 of Dahlstrom & Fuxe) were slightly to clearly depleted of fluorescence but nearly all of them had enough fluorescence in their cytoplasm to be visible (in contrast to 1–7 day old rats). The DA-containing terminals were more affected (clearly to completely) than the NA-containing terminals. Of the latter, those in the spinal cord and the ones of fine-medium thickness in the brain showed more pronounced reduction in density and intensity ; the fluorescence of thick NA-containing terminals was only reduced in intensity. The effect of H44/68 was more marked after 9 hours.

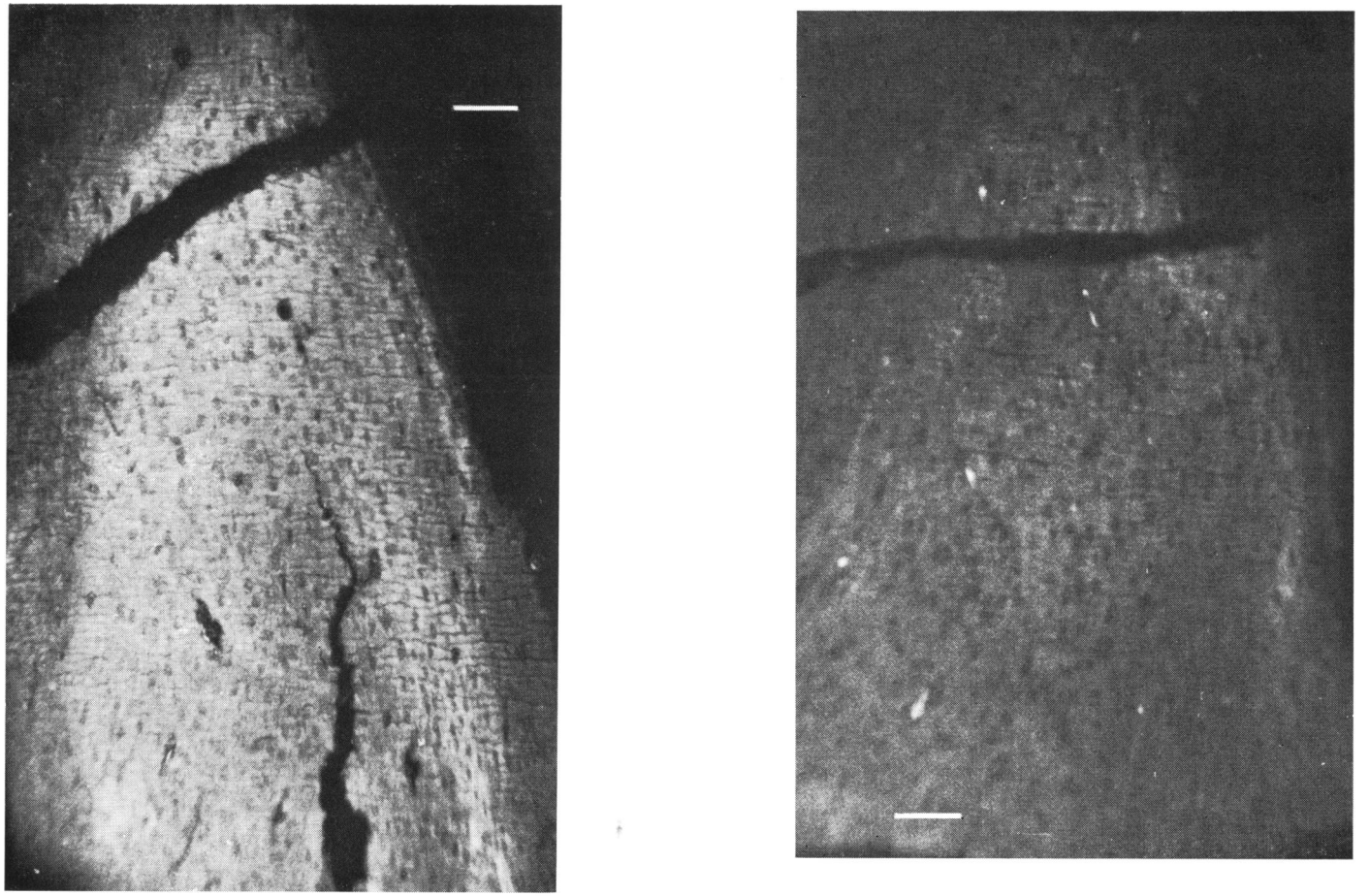
After 18 h the fluorescence in the DA-containing terminals had recovered greatly or completely in density and intensity with the exception of those in the median eminence—infundibular stem area. NA-containing terminals in the spinal cord had markedly recovered, but NA-containing terminals in the brain still showed lower than normal density and intensity—that is, they were mostly in the depleted state. All CA-containing cell bodies (except those of groups A11–A13) had recovered normal fluorescence. Treatment of 2 week old rats with nialamide 10 min before H44/68 resulted in a very pronounced reduction of the effect of H44/68 on CA-containing terminals, but not on cell bodies.

Three, four and five week old and adult rats

The density and intensity of CA-containing terminals in the 3–5 week old rats approached the adult pattern. H44/68 (6 h) produced a marked loss in their fluorescence ; in general the DA-containing terminals were more affected (strongly to completely) than the NA-containing terminals, and of the latter the thicker varicosities showed only slightly reduced intensity of fluorescence. CA-containing cell bodies (groups A1–A13) were clearly to completely depleted of their fluorescence.

There was a variable degree of depletion 18 h after H44/68. In the 3 week old rats nearly all the CA-containing cell bodies showed recovery of their fluorescence intensity to normal levels, and there was a higher density of CA-containing fluorescent varicosities than at 6 h after H44/68. In the 4 week and 5 week old rats half the animals showed a sustained fluorescence loss from CA-containing cell bodies and terminals similar or more pronounced than that seen 6 h after H44/68, while the other animals showed definite signs of slight to marked recovery both in intensity of fluorescence and density of CA-containing nerve terminals, and fluorescence intensity of all cell bodies.

In adults H44/68 produced a gradual depletion of CA fluorescence in nerve terminals and cell bodies 2–18 h after its administration (Figs. 2a, b). The DA-containing nerve terminals were depleted faster than the NA-containing ones, and of the latter the thicker ones were only reduced in intensity of fluorescence. All



(a) (b)

FIG. 2. Dopamine-containing terminals of nucleus accumbens septi, adult rats. (a) Normal; (b) 6 h after treatment with H44/68, 250 mg/kg intraperitoneally. Calibration 64 μm.

CA-containing cell groups were completely depleted after 8 h ; but after 18 h cell bodies of groups A1, A2 and A12 were beginning to recover, groups A4–A7 had almost normal intensity, while those of groups A8–A11 were still depleted.

In the 5 week old and adult rats treated with nialamide before H44/68 there was a very marked reduction in the depletion caused by H44/68 alone in terminal fluorescence. Cell bodies of groups A8–A12, however, were markedly to completely depleted, but most of those of groups A1–A7 were only slightly depleted of their fluorescence.

H44/68 had no effect on 5-HT-containing neurones at any stage of development.

Discussion

The observed depletion (and repletion) of the fluorescence of CA-containing neurones of the CNS after inhibition of tyrosine hydroxylase indicates that these neurones can synthesize their CA stores from tyrosine from the time of birth.

At a systemic dose of 250 mg/kg, H44/68 can produce a very marked time dependent depletion of CA stores in the brain of rats, suggesting that inhibition of tyrosine hydroxylase is very marked (Corrodi & Hanson, 1966 ; Spector, Sjoerdsma & Udenfriend, 1965). DA stores are depleted faster than NA stores and the latter not completely (Corrodi & Hanson, 1966). Similar observations were made on the rats of this study, suggesting that DA stores ' turn over ' faster than NA stores.

In newborn rats there was little if any recovery of fluorescence 18–24 h after H44/68 in contrast to the 1 week old rats, which showed a very marked to complete recovery. This observation could be interpreted to mean that tyrosine hydroxylase is inhibited for a longer period of time in newborn than 1 week old rats, probably due to persistence of the inhibitor in the brain for a longer period of time. The 1 week old rats differed from the older rats in that they had almost completely normal CA induced fluorescence 18 h after H44/68, while the latter were still deficient in fluorescence to a greater or lesser extent.

The degree of loss of CA fluorescence caused by H44/68 after 6–9 h was more or less comparable in the young and adult animals, from the age of 1 week onwards ; since there is an increasing content of CA with age, this observation suggests that the size of the CA pool is not altogether important in determining the extent of its depletion, at least in the initial period when tyrosine hydroxylase is maximally inhibited. This could be so if the ' turnover rate ' (Brodie, Costa, Dlabac, Neff & Smookler, 1966) of CA increases proportionally to the increase in the size of the CA store. This in fact has been shown recently by Coppola (1969) in the case of CA in the hypothalamus. In adult animals electrical stimulation in the pons or mesencephalon accelerates the depletion of CA-containing terminals caused by H44/68 (Loizou, unpublished results) in agreement with the view that conduction of impulses to the terminal is important in the release and depletion of terminal CA (Anden, 1967 ; Anden, Corrodi, Dahlstrom, Fuxe & Hökfelt, 1966). It is difficult to know whether the impulse flow is of the same order or of different magnitude in the CA-containing neurones of the 1 week old and of adult animals since other factors involved in the catabolic process could show variations with age; for example, the form of storage, activity of catabolic enzymes, efficacy of the membrane-pump uptake mechanism. If it is assumed that depletion of terminal CA after tyrosine

hydroxylase inhibition depends on an intact impulse flow, it can be inferred that CA-containing neurones in the newborn show impulse conduction.

MAO activity shows a predominantly postnatal development, reaching adult levels by about the third week of life (Baker & Quay, 1969), but since MAO is not found exclusively in the CA-containing neurones, it is not possible to infer anything conclusive about its contribution to the 'turnover rate' without more direct experiments. However, assuming that tyrosine hydroxylase was fully inhibited, the experiments on nialamide +H44/68 treated animals clearly demonstrated that intraneuronal MAO is important in effecting the depletion of CA-containing terminals caused by H44/68 at all stages of development studied. This has also been shown histochemically, and biochemically in adult animals (Corrodi, Fuxe & Hökfelt, 1967).

It would appear therefore, that all the requirements for transmission are present in the CA-containing neurones of the newborn—presence of transmitter in terminals, conduction of impulses and release of transmitter, re-uptake through the cell membrane and inactivation by MAO even though the neurone is morphologically immature and its terminals contain very little CA (Loizou, in preparation).

A related situation has been reported for developing axons in the spinal cord of rats (Crain, Bornstein & Petterson, 1968; Crain & Petterson, 1967) and monkeys (Bodian, 1968) which show evidence of function as soon as they develop discernible synaptic junctions with other neurones.

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