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Some problems encountered in attempting to estimate catecholamine turnover using labelled tyrosine

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Rats were given [^{3}H]tyrosine intravenously. The decrease of [^{3}H]noradrenaline (^{3}H-NA) and [^{3}H]dopamine (^{3}H-DA) from 2 to 6 h after the [^{3}H]tyrosine injection was estimated in the cerebral cortex, brain stem, caudate nucleus, spinal cord and adrenal medulla. Half-lives were estimated with or without the tyrosine hydroxylase inhibitor H 44/68 or the dopamine-β-hydroxylase inhibitor FLA-63, given 2 h after the [^{3}H]tyrosine. The influence of the inhibitors on endogenous noradrenaline was also studied. H 44/68 did not change the half-life of ^{3}H-NA in the central nervous system but FLA-63 reduced it considerably. FLA-63 reduced endogenous noradrenaline more than did H 44/68. The mechanism responsible for the faster turnover of noradrenaline after FLA-63 has not yet been elucidated. In the adrenal medulla the [^{3}H]adrenaline + ^{3}H-NA level rose significantly from 2 to 6 h after the injection of [^{3}H]tyrosine. The synthesis inhibitors prevented this rise, indicating that appreciable amounts of labelled precursors remain for a long time after the injection of the labelled tyrosine. Some problems encountered with the different methods for turnover determinations are discussed on the basis of these data.

Several methods have been used to estimate the turnover of catecholamines. For example, the rate of disappearance of a labelled amine after either administration of the amine itself (Brodie, Costa & others, 1966; Costa & Neff, 1966) or of a labelled precursor (Udenfriend & Zaltman-Nirenberg, 1963; Burack & Draskóczy, 1964; Nybäck, Borzecki & Sedvall, 1968; Sedvall, Weise & Kopin, 1968) has been estimated. Another approach has been to estimate the rate of disappearance of the endogenous amines after synthesis inhibition (Brodie & others, 1966; Costa & Neff, 1966; Andén, Corrodi & Fuxe, 1969; Neff, Ngai & others, 1969). Other methods have been used like measuring the accumulation of labelled amines after infusion or injection of labelled tyrosine (Gordon, Spector & others, 1966; Neff & others, 1969) or changes in the levels of the catecholamine metabolites (c.f. Carlsson & Lindqvist, 1963; Andén Roos & Werdinius, 1964; Sharman, 1969; Guldberg, 1969).

In a previous publication (Persson, 1969), turnover estimations using tritium-labelled tyrosine or dopa of high specific activity, were made and discussed. In the heart, the half-lives of the catecholamines were long in comparison to the times obtained after administration of the labelled amine itself. One explanation of this may be the difficulty in separating the synthesis and elimination processes from each other. Refilling of the labelled amine stores may occur from remaining labelled precursors and that will result in an apparently delayed disappearance. This

methodological problem may be circumvented by giving a synthesis inhibitor at a suitable time interval to stop the refilling. In the present investigation a tyrosine hydroxylase inhibitor (H 44/68) and a dopamine-β-hydroxylase inhibitor (FLA-63) have been used for this purpose.

EXPERIMENTAL

Male albino rats, 150–200 g, were used; all experiments were made at an ambient temperature of 30°. No hypothermia occurred when the synthesis inhibitors were given. The following drugs were used: L-tyrosine, ring 3,5-^{3}H (specific activity 36 Ci/mmol), the inhibitor of dopamine-β-hydroxylase, FLA-63 [bis(4-methyl-1-homopiperazinylthiocarbonyl) disulphide, Svensson & Waldeck, 1969; Carlsson, Corrodi, Florvall, Ross & Sjöberg, to be published] and the methylester of α-methyl-tyrosine (H 44/68), which inhibits the tyrosine hydroxylase (cf. Corrodi & Hanson, 1966). The brain was dissected as described by Persson (1969) and the cerebral cortex, brain stem, caudate nucleus, spinal cord and adrenal medulla analysed. Analyses were made on tissues collected and pooled from two rats. ^{3}H-NA and ^{3}H-DA were measured as described by Persson & Waldeck (1968). The ^{3}H-NA fraction from the adrenal medulla also contained [^{3}H]adrenaline (^{3}H-A) (Häggendal, 1962).

The isotope substances were obtained from The Radiochemical Centre, Amersham, England.

RESULTS

Rats received 5 μg/kg of [^{3}H]tyrosine intravenously and 2 h later 40 mg/kg of FLA-63 or 250 mg/kg of H 44/68 intraperitoneally. After another 4 h the animals were killed and the [^{3}H]catecholamines in the various tissues determined. Control animals received only [^{3}H]tyrosine and were killed 2 or 6 h later. In analogous experiments the endogenous noradrenaline levels were determined 4 h after administration of the synthesis inhibitors.

Effect of FLA-63 and H 44/68 on the disappearance of ^{3}H-NA, ^{3}H-DA and endogenous noradrenaline in different parts of the brain.

The results are presented as the mean values ± s.e. The apparent half-lives were calculated by regression analysis, assuming that the disappearance of the amines was a single exponential process (Table 1).

The results show that H 44/68 probably did not change the rate of disappearance of ^{3}H-NA. In the caudate nucleus the half-life of ^{3}H-DA was estimated to be about 3·5 h and this was reduced to 2 h after H 44/68 but the difference was not statistically significant. FLA-63 enhanced the disappearance rate of ^{3}H-NA significantly in the brain stem ($P < 0{\cdot}001$) and in the spinal cord ($P < 0{\cdot}05$). In the cortex there was a similar difference, though this was not significant. FLA-63 did not change the rate of disappearance of ^{3}H-DA in the caudate nucleus. After administration of H 44/68 or FLA-63 the endogenous noradrenaline levels decreased at about the same rate (or possibly faster) than did the ^{3}H-NA (Table 2).

The ^{3}H-DA levels in cortex, brain stem and the spinal cord were estimated but because of a relatively large scatter the data have been omitted. ^{3}H-NA in the caudate nucleus was also determined, but the large excess of ^{3}H-DA in this tissue made these results uncertain.

Table 1. *Effect of FLA-63 and H 44/68 on the disappearance of [^3H]noradrenaline formed from [^3H]tyrosine in different parts of the CNS and of [^3H]dopamine in the caudate nucleus of the rat.* [^{3}H]Tyrosine, 5 μg/kg was given i.v. to rats and 2 h later FLA-63, 40 mg/kg or H 44/68, 250 mg/kg i.p. After another 4 h the animals were killed and the [^{3}H]amine levels in the different parts of the CNS determined. Control animals received [^{3}H]tyrosine and were killed 2 and 6 h later. Figures in brackets denote the number of experiments, each comprising two animals

Tissue		Control 2 h	Control 6 h	FLA-63	H 44/68
		[^{3}H]noradrenaline, fmol/g, and apparent half lives h			
Cortex	mean	14·6 (6)	9·3 (5)	4·6 (4)	7·8 (4)
	s.e.	2·54	0·74	0·92	0·36
	T 1/2		7·3	2·4	5·0
Brain stem ..	mean	33·2 (5)	20·4 (6)	7·0 (4)	20·2 (5)
	s.e.	3·05	2·17	1·37	1·51
	T 1/2		5·5	1·7	5·6
Spinal cord ..	mean	17·4 (6)	9·7 (6)	4·6 (5)	9·9 (5)
	s.e.	1·26	0·59	0·54	0·62
	T 1/2		4·8	2·1	4·9
		[^{3}H]dopamine, fmol/g, and apparent half lives h			
Caudate nucleus ..	mean	461 (6)	213 (6)	225 (5)	14 (5)
	s.e.	45	24	43	34
	T 1/2		3·5	3·6	2·0

fmol = 10^{-15} mol.

Table 2. *Effect of FLA-63 and H 44/68 on the disappearance of noradrenaline in different parts of the CNS of the rat.* FLA-63, 40 mg/kg or H 44/68, 250 mg/kg was given i.p. to rats. 4 h later the animals were killed and the different parts of the CNS determined for noradrenaline

Tissue		Noradrenaline, μg/g, and apparent half lives h		
		Control	FLA-63	H 44/68
		n=4	n=5	n=5
Cortex	mean	0·24	0·013	0·10
	s.e.	0·012	0·005	0·005
	T 1/2		—	3·2
Brain stem	mean	0·65	0·090	0·39
	s.e.	0·048	0·015	0·037
	T 1/2		1·4	5·4
Spinal cord.. ..	mean	0·35	0·060	0·15
	s.e.	0·034	0·009	0·021
	T 1/2		1·5	3·1

Effect of FLA-63 and H 44/68 on the disappearance of [^3H]catecholamines formed from [^3H]tyrosine in the adrenal medulla of the rat

The ^{3}H-NA + ^{3}H-A of the adrenals increased from 2 to 6 h after the [^{3}H]tyrosine injection in the absence of a synthesis inhibitor ($P < 0{\cdot}05$) (Fig. 1). FLA-63 and H 44/68 inhibited this increase ($P < 0{\cdot}001$ and $P < 0{\cdot}01$, respectively). The ratio ^{3}H-DA/^{3}H-NA rose after FLA-63. The ^{3}H-DA levels of the adrenal medulla appeared to decrease, though not significantly, from 2 to 6 h in animals not given synthesis inhibitors ($0{\cdot}05 < P < 0{\cdot}10$). After FLA-63 treatment the ^{3}H-DA level

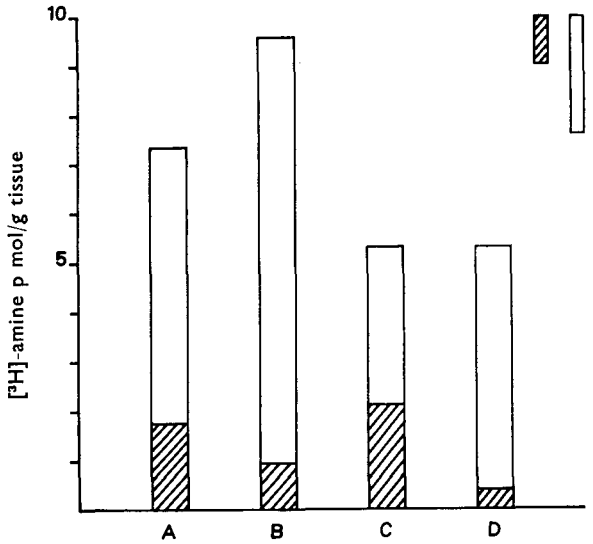

FIG. 1. Effect of FLA-63 and H 44/68 on the disappearance of [³H]catecholamines formed from [³H]tyrosine in the adrenal medulla of the rat. [³H]Tyrosine, 5 μg/kg was given i.v. to rats and 2 h later FLA-63, 40 mg/kg or H 44/68, 250 mg/kg, i.p. After another 4 h the animals were killed and [³H]noradrenaline + [³H]adrenaline and [³H]dopamine in the adrenals estimated. Control animals received [³H]tyrosine and were killed 2 and 6 h later. The means (based on 4—6 determinations) and differences ($P<0.05$) are given. Open columns: [³H]noradrenaline + [³H]adrenaline. Hatched columns [³H]dopamine. A: control 2 h, B: control 6 h, C: FLA-63, D: H 44/68.

was significantly higher than in the control; an opposite trend, though not significant, was observed after H 44/68.

DISCUSSION

Although much used for estimations of catecholamine formation and turnover, the isotope and synthesis inhibitor methods appear to require further analysis before their accuracy can be regarded as settled. Brodie & others (1966) reported a very good agreement for noradrenaline turnover rate in heart using an isotope method or a tyrosine hydroxylase inhibitor. On the other hand Westfall & Osada (1969) found obvious discrepancies with the above-mentioned methods and with measuring the conversion of labelled tyrosine to labelled noradrenaline in estimating the noradrenaline turnover in heart before and after adrenalectomy. With the central nervous system, only indirect comparisons can be made. Generally, a faster turnover of dopamine than of noradrenaline in brain is indicated, also some regional differences occur (see Persson, 1969). The aim of the present study was to compare these two methods for the estimation of catecholamine turnover and to examine further the methodological problems associated with the isotope technique (see Introduction). We found that administration of a tyrosine hydroxylase inhibitor 2 h after the injection of [³H]tyrosine did not change the rate of disappearance of ³H-NA. Sedvall & Nybäck (personal communication) agree. This cannot, however, be taken as evidence that the isotope method gives a true indication of turnover. The reasons for this are evident from the following discussion.

Inhibition of tyrosine hydroxylase and of dopamine-β-hydroxylase induced different rates of noradrenaline depletion. The disappearance of both ³H-NA and

endogenous noradrenaline was more rapid when dopamine-β-hydroxylase was inhibited. Goldstein & Nakajima (1967) reported similar findings. In a preliminary report we (Persson & Waldeck, 1970) have presented some experimental support for the view that the activity of the noradrenaline-containing neurons is affected by the activity in the dopamine neurons. When both dopamine and noradrenaline syntheses are inhibited, the turnover of the latter amine appears to be slowed. When only the dopamine-β-hydroxylase is inhibited, the influence of the dopamine neurons on the noradrenaline neurons is undisturbed. Such a mechanism would explain the difference in action of H 44/68 and FLA-63 on ^{3}H-NA and endogenous noradrenaline depletion. Another possible mechanism might be an incomplete inhibition of noradrenaline synthesis by H 44/68 but a more complete inhibition by FLA-63. However, when H 44/68 or FLA-63 were injected into mice in the same doses as have been used here, the synthesis of ^{3}H-NA from [^{3}H]tyrosine was less than 10% of normal after 30 min (Svensson & Waldeck, 1969, and unpublished data). It is unlikely that FLA-63 has a releasing action of its own, since noradrenaline disappears very slowly in the transected spinal cord after injection of this drug (Andén, personal communication). Preliminary experiments with DL-^{3}H-NA do not indicate any releasing effects of FLA-63 from mouse heart [Persson & Waldeck, unpublished].

The tyrosine hydroxylase inhibition as a method for estimation of noradrenaline turnover therefore seems to have the disadvantage of slowing noradrenaline turnover. On the other hand, when isotope precursor methods are used the extent to which the disappearing isotope-labelled amines are replaced by remaining labelled precursors is not known. Both methods therefore indicate too slow a turnover of noradrenaline. Another possible source of error is an unequal labelling of storage pools, since much experimental work (Hillarp, 1960; Lundborg, 1963; Häggendal & Lindqvist, 1963; Andén & Henning, 1966; see Kopin, Breese & others, 1968) indicates the presence of a small functionally active pool with high turnover and a large pool from which the amines are slowly released. During the comparatively long time intervals used for estimating the disappearance of the labelled amines in these experiments, only the turnover of the large pool would then be reflected in the half-lives obtained with [^{3}H]tyrosine. The estimations of the catecholamine turnover in the brain with the present methods are therefore not very accurate.

There are no statistically significant differences in the turnover of ^{3}H-DA with or without the synthesis inhibitors. But the invalidations of the methods discussed above may also apply to the estimations of dopamine turnover. We have not found any indication that the dopamine neurons depend on the noradrenaline neurons.

In the adrenal medulla, [^{3}H]adrenaline + ^{3}H-NA increased rather than decreased between 2 and 6 h after the administration of [^{3}H]tyrosine. This may in part be due to conversion of intermediate ^{3}H-DA since this amine appeared to decrease during this time interval, a decrease which failed to occur when the dopamine-β-hydroxylase had been blocked. A supply of [^{3}H]tyrosine or [^{3}H]dopa, or both, during this time interval has also to be taken into account, since the total [^{3}H]catecholamine content was reduced after synthesis inhibition. Whether this probable supply of precursor comes from the blood stream or from stores in the adrenal medulla remains to be elucidated. In any event it would appear that the synthesis of ^{3}H-A + ^{3}H-NA from [^{3}H]tyrosine is not completed within 2 h of the injection and that any con-

clusions about the exact turnover rates of catecholamines from the present data are hazardous. According to Burack & Drascóczy (1964) who used [^{3}H]dopa as catecholamine precursor, and to Brodie & others (1966), who used a tyrosine hydroxylase inhibitor, the catecholamines in the adrenal medulla had a very slow turnover with half-lives of about a week.

It may be concluded that there are many uncertainties about catecholamine turnover estimations with the present methods. They are, however, valuable for comparative studies.

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