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Simultaneous Measurement of Tyrosine and Tryptophan Hydroxylase Activities in Brain *in Vivo* Using an Inhibitor of the Aromatic Amino Acid Decarboxylase

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Summary. DOPA and 5-HTP accumulated *in vivo* in rat brain after decarboxylase inhibition with NSD 1015 (3-hydroxybenzylhydrazine). This accumulation was linear for the first 30 min and occurred in several brain regions over a wide range of NSD 1015 doses. After a peripheral decarboxylase inhibitor much less, if any, DOPA or 5-HTP accumulated in the brain. The accumulation of DOPA was prevented by H 44/68 (methylester of α-methyl para-tyrosine), a tyrosine hydroxylase inhibitor. DOPA, which accumulated before H 44/68 was given, appeared stable for at least 20 min. There were no significant changes in the levels of NA, DA, 5-HT or tryptophan shortly after NSD 1015 administration, but a rise in tyrosine was noted. Increased brain tyrosine after L-tyrosine administration did not alter the DOPA accumulation, however. These data as well as the distribution of the accumulated amino acids suggest that the accumulation of DOPA and 5-HTP after decarboxylase inhibition occurs intraneuronally, that the decarboxylase enzyme is completely inhibited, and that the accumulated products are not appreciably metabolized or transported from the region studied. Amine synthesis rates and rate constants were calculated from the data and compare well with similar values determined by other methods. Thus this accumulation appears to be a reliable measure of the *in vivo* hydroxylation of tyrosine and tryptophan.

Key words: DOPA — 5-Hydroxytryptophan — Tyrosine Hydroxylase in Rat Brain — Tryptophan Hydrolyxase in Rat Brain — NSD 1015 (3-hydroxybenzylhydrazine).

In a series of papers we have reported on the accumulation of 5-hydroxytryptophan (5-HTP) in brain after administration of an inhibitor of the aromatic amino acid decarboxylase (Carlsson and Lindqvist, 1970; Bédard *et al.*, 1971; Bédard *et al.*, 1972). Dihydroxyphenylalanine (DOPA) accumulates as well after inhibition of this enzyme (Cegrell *et al.*,

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1970; Bédard *et al.*, 1971). We have now developed a sensitive and accurate method for the determination of DOPA in tissues (Kehr *et al.*, 1972). This provides us with the opportunity of measuring simultaneously the accumulation of 5-HTP and DOPA after decarboxylase inhibition.

In our earlier studies we used N^1-(DL-Seryl)-N^2-(2,3,4-trihydroxybenzyl)hydrazine (Ro 4-4602) as the inhibitor of the decarboxylase enzyme. In the present study, we have used 3-hydroxybenzyl hydrazine HCl (NSD 1015), a centrally more potent inhibitor. In this study we present evidence that the accumulation of DOPA and 5-HTP after NSD 1015 administration is a measure of the *in vivo* activity of tyrosine and tryptophan hydroxylase.

Elegant techniques for estimating brain neurotransmitter synthesis and turnover have been developed in recent years, especially by Brodie and his colleagues (Brodie *et al.*, 1966; Tozer *et al.*, 1966; Neff *et al.*, 1971). We hope that the proposed method offers an addition to these techniques.

Materials and Methods

Male Sprague-Dawley rats weighing 180 to 250 g were used throughout. In a few experiments on the distribution of DOPA, hooded rats of either sex were used. The animals were fed on commercially available pellets (Anticimex, Stockholm, Sweden). In some experiments whole rat brain was dissected into 3 parts, the corpus striatum, including the olfactory tubercles ("striatum"), the rest of the cerebral hemispheres ("hemispheres"), and the rest of the brain. In other experiments the brains were divided into 5 parts (see Results, section 3). During the dissection the brains were kept on a cold glass plate over ice. Immediately after dissection, the parts were frozen on dry ice. Data from these experiments are expressed as concentration per g wet weight of hemisphere or striatum. Concentrations in whole brain were calculated from the three parts. Three rat brains were pooled for a single determination.

The extraction, column separation and fluorimetric assay of the substances reported here was performed by methods previously described (Atack and Magnusson, 1970; Lindqvist, 1971; Bédard *et al.*, 1972; Kehr *et al.*, 1972). Briefly, the specimens were homogenized in perchloric acid in the presence of EDTA and $Na_2S_2O_5$. After neutralization, the supernates were passed onto Dowex 50 X-4 columns and the elution of DOPA, 5-HTP, tyrosine, tryptophan, noradrenaline (NA), dopamine (DA), serotonin (5-HT), and 5-hydroxyindoleacetic acid (5-HIAA) was carried out. Fluorimetric assays were used throughout for determination of the eluates. For the determination of DA a modification of the method of Carlsson and Waldeck (1958) was used (Atack, to be published).

NSD 1015 was obtained from Smith & Nephew Research Ltd, Gilston Park, Harlow, England and chlorpromazine hydrochloride from Leo Ltd., Helsingborg, Sweden.

Results

1. *Accumulation of DOPA and 5-HTP in Brain after NSD 1015 Pretreatment*

Fig. 1 illustrates the accumulation of both DOPA and 5-HTP during the first 60 min after intraperitoneal administration of 100 mg/kg of

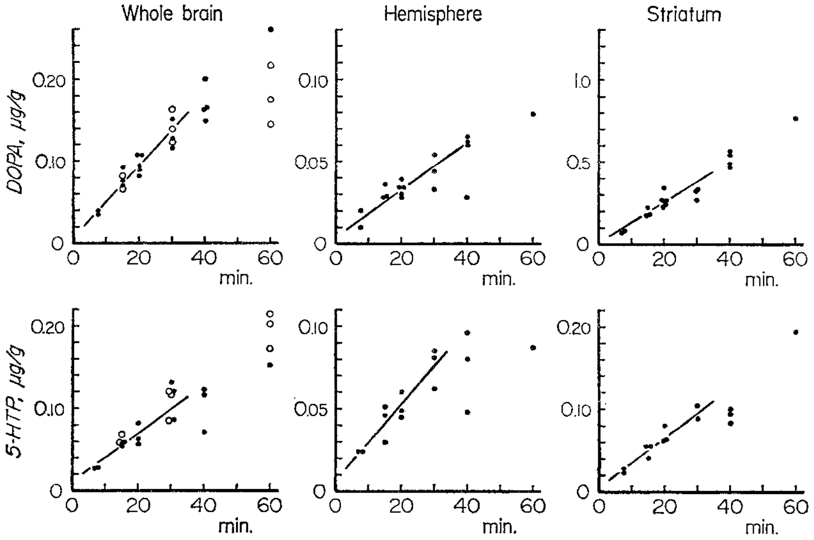

Fig. 1. Accumulation of 5-HTP and DOPA *in vivo* after decarboxylase inhibition in rat brain. DOPA and 5-HTP are plotted *vs.* time after the administration of NSD 1015, 100 mg/kg i.p. The brains were dissected into hemispheres, striatum and the "rest" (see Methods) and whole brain data were calculated from all three parts. Each closed dot represents a value from three such pooled brain parts. DOPA and 5-HTP were determined in the same brain. The open dots represent a few experiments in which whole brain DOPA and 5-HTP levels were determined without dissection

NSD 1015. The accumulation of both amino acids appears linear during the first 30 min, after this period the 5-HTP accumulation seems to slow somewhat.

2. *Effect of Various Doses of NSD 1015 on DOPA and 5-HTP Levels in Brain*

Fig. 2 shows the accumulation of DOPA and 5-HTP 30 min after the i.p. injection of either 50, 100, or 200 mg/kg of NSD 1015. There was no significant difference between the levels of the amino acids with any of the NSD doses. This applied to the hemispheres and striatum as well as whole brain.

3. *Distribution of DOPA and Catecholamines in Brain after Treatment with NSD 1015*

In three experiments rats were given NSD 1015, 100 mg/kg i.p., and the animals were killed 30 or 60 min later. The brains were divided into the following parts: corpus striatum (including olfactory tubercles), rest

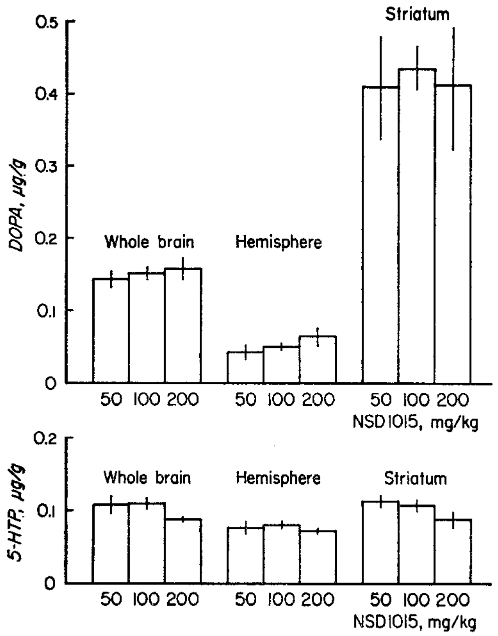

Fig. 2. Effect of various doses of NSD 1015 on DOPA and 5-HTP accumulation in rat brain. The animals were killed 30 min after the i.p. injection of NSD 1015. Shown are the means ± S.E.M. ($n = 3$). Each analysis was performed on 3 pooled brain parts (see Methods). Analysis of variance (*F* test) showed no significant difference between any of the DOPA or the 5-HTP levels accumulated

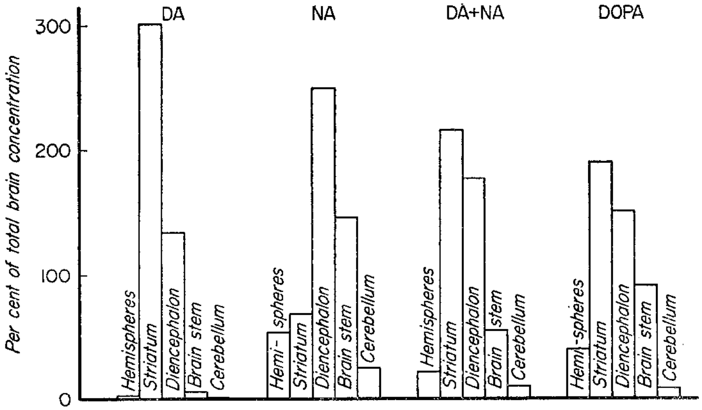

Fig. 3. Distribution of catecholamines and DOPA in rat brain 30 min after NSD 1015, 100 mg/kg i.p. The levels of DA, NA or DOPA in each brain part were expressed as per cent of the level calculated for the whole brain

of the hemispheres, diencephalon, lower brain stem, and cerebellum. These parts were analyzed for DOPA, NA, and DA. 5-HT and 5-HTP were also measured, but only the catecholamine data will be shown as the indole data were in close agreement with previously published observations (Bédard *et al.*, 1971).

The results of these 3 experiments were similar as regards the distribution of DOPA and catecholamines. Fig. 3 shows the result of one such experiment. The concentration of each constituent in the different parts is expressed in per cent of the concentration calculated for whole brain. As can be seen from the figure, the distribution of DOPA differs from that of DA and that of NA but is very similar to that of the sum (DA + NA).

4. *Attempts to Detect DOPA in Rat Corpus striatum*

Two attempts have been made to detect DOPA in the corpus striatum of untreated rats. The corpora striata of 9 or 10 rats were dissected immediately after decapitation and analyzed for DOPA as described above.

The sample readings obtained were 5 and 2%, respectively, below the readings of the tissue blanks, corresponding to levels of −2 and −1 ng/g. In a corresponding experiment on rats treated with chlorpromazine (5 mg/kg) and NSD 1015 (100 mg/kg) 100 and 60 min before death, respectively, 4200 ng/g was obtained in the corpus striatum.

In our earlier paper (Kehr *et al.*, 1972) we concluded that the DOPA level of the normal rat brain is below 10 ng/g. The present observations indicate that this conclusion is valid for the rat corpus striatum as well. We were thus unable to confirm the tentative findings of 200 to 300 ng/g in rat corpus striatum reported by Romero *et al.* (1972). However, our conclusion is drawn with reservation for a rapid postmortem loss of DOPA.

5. *Effect of NSD 1015 on Brain Monoamines*

The effect of the i.p. injection of NSD 1015, 100 mg/kg, on the levels of NA, DA, and 5-HT was studied. Fig. 4 illustrates the amine levels for 40 min after NSD 1015. There was no significant change in any of the levels in whole brain or in hemisphere and striatum. After 40 min there appears to be a tendency for a drop in levels. However, these late changes were not statistically significant (analyses of variance, F test).

6. *The Effect of NSD 1015 on Tryptophan and Tyrosine Levels*

Fig. 5 shows the levels of whole brain tryptophan and tyrosine during the 40 min after an i.p. injection of NSD 1015, 100 mg/kg. There was no significant change in levels of tryptophan, but a clear and significant

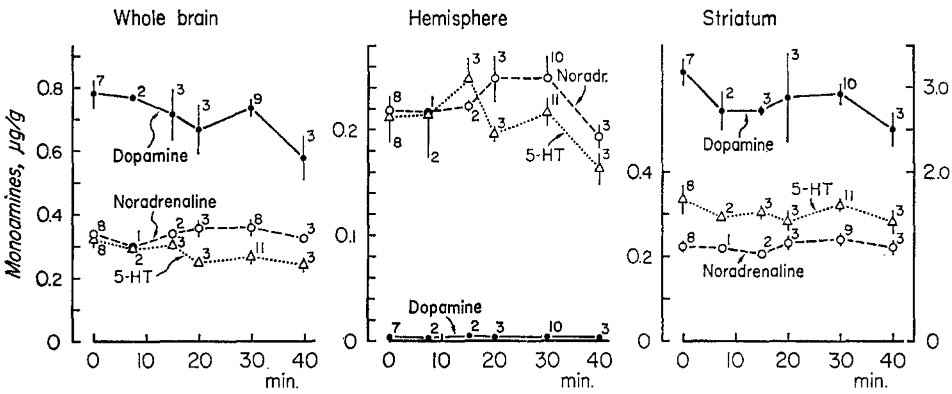

Fig. 4. Rat brain monoamines at various intervals after decarboxylase inhibition (NSD 1015, 100 mg/kg i.p.). The brains were dissected as described in Methods and the whole brain levels were calculated from the parts. Three rat brains were pooled for each determination. All three amines were determined in the same pooled brain specimen. The small numbers next to the values on the graph represent the number of pooled specimens determined. Where appropriate the standard error of the mean is indicated by a vertical line. Analysis of variance (*F* test) was applied to all three amines in both brain regions and whole brain. There was no significant difference in amine level between any of the times studied

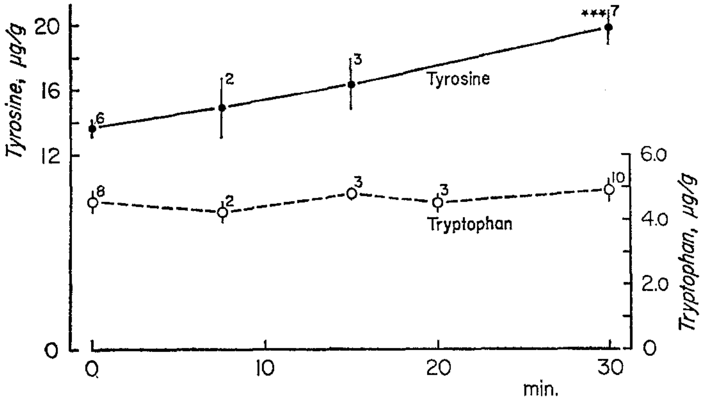

Fig. 5. Rat brain tyrosine and tryptophan at various intervals after decarboxylase inhibition with NSD 1015 (100 mg/kg i.p.). The data refer to whole brain and are calculated from brain parts (see Methods) and each determination represents 3 pooled rat brains. The numbers next to the points on the graphs represent the number of such determinations in that point. The standard error of the mean is indicated by a small vertical line. Analysis of variance (*F* test) showed no significant difference in tryptophan level at any of the times studied. The 30 min tyrosine value was significantly different from the controls ($P < 0.001$) by this analysis of variance

rise in brain tyrosine. These measurements were also carried out in brain parts and similar results were obtained; there was a rise in tyrosine but not tryptophan in both hemispheres and striatum. The correlation coefficient of the tyrosine rise in whole brain was statistically highly significant ($P < 0.001$).

7. *Effect of Oral Administration of Tyrosine on the Accumulation of DOPA after NSD 1015*

Because the brain tyrosine was significantly elevated after NSD 1015, it became important to determine if this elevation had any effect on the DOPA accumulation 30 min after NSD 1015. Accordingly, L-tyrosine as a suspension was given orally to rats in doses of 100, 300 or 1000 mg/kg. Thirty min later, NSD 1015, 100 mg/kg was given i.p. and the animals were sacrificed 30 min after the NSD 1015, that is 60 min after the tyrosine was given. Table 1 shows the results. Although there was a clear and significant rise in whole brain tyrosine ($P < 0.001$) there was no change in the amount of DOPA accumulated.

Table 1. Effect of L-tyrosine on brain DOPA accumulation after NSD 1015

Treatment	Brain tyrosine (μg/g ± SEM)	Brain DOPA (μg/g ± SEM)
NSD + Saline	27 ± 2.5	0.14 ± 0.013
NSD + 100 mg/kg, L-tyrosine	44 ± 5.0	0.15 ± 0.014
NSD + 300 mg/kg, L-tyrosine	60 ± 6.9	0.14 ± 0.008
NSD + 1000 mg/kg, L-tyrosine	73 ± 5.7	0.14 ± 0.015

Six rats in each group received either tyrosine or saline orally 1 h and 100 mg/kg of NSD 1015 30 min before death.

8. *Effect of Mk 486 (L-α-hydrazino-α-methyl-β-[dihydroxyphenyl]-proprionic acid), a Peripheral Decarboxylase Inhibitor, on Brain DOPA and 5-HTP*

Mk 486 is a potent inhibitor of aromatic amino acid decarboxylase, but it does not readily penetrate into the brain parenchyma (Porter *et al.*, 1962). This agent was injected i.p. into six rats in a dose of 100 mg/kg. The rats were killed 30 min later and the brains were analyzed for DOPA and 5-HTP. The levels obtained were 6 to 12 ng/g for both amino acids. Whether these low values do indeed represent DOPA or 5-HTP cannot be stated as yet. The data indicate that an inhibitor of peripheral decarboxylase is far less efficient in raising brain levels of DOPA and 5-HTP than an inhibitor of both central and peripheral decarboxylase.

9. Effect of H 44/68 on the DOPA Accumulation after NSD 1015

H 44/68 (the methyl ester of α-methyl-para-tyrosine), a potent inhibitor of tyrosine hydroxylase, was given after DOPA had accumulated from NSD 1015 pretreatment in order to study the stability of the accumulated DOPA. NSD 1015, 100 mg/kg was given i.p. first, after 20 min a supra-maximal i.p. dose of H 44/68 (500 mg/kg) was given to some of the animals. After another 20 min, the animals were sacrificed. A separate control group was sacrificed 20 min after NSD 1015.

The levels of DOPA in these animals are illustrated in Fig. 6. The DOPA levels 20 min after H 44/68 (and 40 min after NSD 1015) were statistically indistinguishable from the DOPA levels at the time the H 44/68 was given (20 min after NSD 1015). The H 44/68 appears to stop the production of DOPA and the concentration of accumulated DOPA remains approximately constant. Both the hemisphere and striatum showed the same results as whole brain. In all cases there was a highly significant difference in accumulated DOPA ($P < 0.001$) between NSD 1015 alone (40 min) and NSD 1015 (40 min) + H 44/68 (20 min). Kehr *et al.* (1972) had previously shown that when H 44/68 is administered *before* NSD 1015 the accumulation of DOPA was prevented.

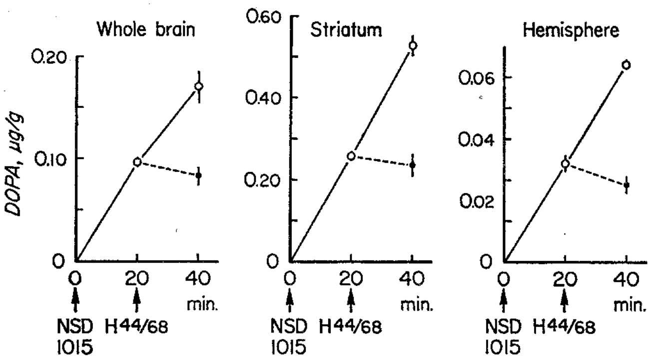

Fig. 6. Effect of α-methyltyrosine methylester HCl (H 44/68) on the *in vivo* DOPA accumulation after decarboxylase inhibition in rat brain. All groups received NSD 1015 100 mg/kg i.p. at zero time. The first group was sacrificed at 20 min. The second group received H 44/68, 500 mg/kg i.p. 20 min after the NSD 1015. The third group was given saline, i.p. 20 min after the NSD 1015. The second and third group were killed 40 min after the NSD 1015. This was 20 min after the H 44/68 for the second group. The means ± S.E.M. of three such experiments (each on 3 pooled brains) are plotted. Analysis of variance (*F* test) was performed and the third group (40 min after NSD 1015 alone) was significantly different from the first group (20 min after NSD 1015) ($P < 0.005$). The third group was also significantly different from the second group (40 min after NSD 1015, 20 min after H 44/68) ($P < 0.001$). There was no significant difference between the first and second groups

Discussion

1. Does the Accumulation of DOPA and 5-HTP after NSD 1015 Measure the in Vivo *Hydroxylation of Tyrosine and Tryptophan, Respectively?*

If this method measures the hydroxylation of tyrosine and tryptophan, the dose of NSD 1015 employed must cause complete inhibition of the brain aromatic amino acid decarboxylase. Secondly, the amino acids DOPA and 5-HTP should accumulate in a linear manner if the activity of the hydroxylase enzymes does not change during the period of measurement. The accumulated amino acids should be of central origin and they should be relatively stable; they should not be degraded by another biochemical mechanism or physically transported out of the region studied. Finally, and of less significance, factors which are known to affect the *in vitro* hydroxylase activity should be shown to have the same qualitative effect on the *in vivo* hydroxylation.

The data presented in this study support the proposition that we indeed measure the *in vivo* hydroxylation of tyrosine and tryptophan in brain.

It seems quite clear that the aromatic amino acid decarboxylase is completely inhibited. Our dose-response curve showed the same accumulation of DOPA and 5-HTP with several different doses of NSD 1015, suggesting that the 100 mg/kg of NSD 1015 employed is a supramaximal dose of the drug and completely blocks the decarboxylase enzyme. Earlier data in mouse brain indicated complete decarboxylase inhibition with 100 mg/kg of NSD 1015 as well (Carlsson, 1964; Carlsson *et al.*, 1968). We compared the blockade of decarboxylase produced by NSD 1015 with that caused by Ro 4-4602, a structurally somewhat different inhibitor. Our results showed the same accumulation of 5-HTP in mouse brain with both inhibitors (unpublished observations). These three sets of data all support the conclusion that we have completely inhibited the decarboxylating enzyme.

We have studied the normal, untreated rat brain and brain parts and have been unable to demonstrate measurable quantities of DOPA or 5-HTP, both probably being less than 10 ng/g. After 100 mg/kg of NSD 1015 there is initially an approximately linear accumulation of both DOPA and 5-HTP in whole brain. Because tyrosine hydroxylase is present in two different types of neuronal systems, those that store DA and those that store NA, we have measured the accumulation of DOPA in the corpus striatum and hemisphere. The striatum contains predominantly DA neurons and the hydroxylation rate obtained from this area probably reflects the rate of this type of neuron. On the other hand, the hemisphere is almost free of DA (see Fig. 4) and appears to have mainly

NA-storing catecholamine neurons both histochemically and biochemically. In both regions we have demonstrated a linear accumulation of DOPA during the first 30 min after NSD 1015.

In an earlier biochemical and histochemical study, we reported the distribution of 5-HTP in the brains of Ro 4-4602 treated rats (Bédard *et al.*, 1971). This study led to the conclusion that the 5-HTP had been formed almost exclusively in the 5-HT storing neurons (cell bodies and fibre systems) of the brain. Our earlier study also gave histochemical evidence of DOPA accumulation in DA-storing neurons of the brain, whereas no DOPA could be detected in NA-storing neurons, perhaps due to insufficient sensitivity of the histochemical technique in this case. The present distribution study indicated that DOPA does in fact accumulate both in DA and NA neurons; the distribution of DOPA differed clearly from either that of DA or NA but was markedly similar to the sum of the catecholamines (DA + NA). This is in contrast to the even distribution of exogenous L-DOPA (Romero *et al.*, 1972). Therefore the DOPA accumulated in rat brain after decarboxylase inhibition must have been formed locally, rather than being derived from the blood stream. The observation with a peripheral decarboxylase inhibitor, Mk 486, further supports this view.

H 44/68, a potent and specific blocker of the *in vitro* tyrosine hydroxylase enzyme was found to prevent the NSD 1015-induced DOPA accumulation. The experiments reported with H 44/68 demonstrate that the DOPA accumulated after NSD 1015 is stable for at least 20 min. This suggests that the DOPA is not readily available for transport out of the brain and is not appreciably degraded by another enzymatic process. These data apply to both DA-storing neurons in the striatum and NA-storing neurons in the cerebral hemispheres. There is evidence that exogenously administered DOPA is metabolized to 3-O-methyl DOPA as well as to deaminated products (Kuruma *et al.*, 1970). Since the accumulated DOPA appears stable in our experiments, it probably accumulates in a cellular compartment where it is not exposed to catechol-O-methyl transferase. Evidence for the stability of accumulated 5-HTP after decarboxylase inhibition has been presented (Carlsson and Lindqvist, 1972).

We found a significant rise in brain tyrosine after administration of NSD 1015. However, administration of tyrosine before decarboxylase inhibition had no effect on the accumulation of DOPA. This result was expected as the tyrosine hydroxylase enzyme is presumably saturated with amino acid substrate under normal physiological conditions (Udenfriend, 1966). The rise in tyrosine could be due to blockade of brain and/or hepatic tyrosine aminotransferase. Such a rise in plasma

Table 2. Amine levels, synthesis rates and rate constants determined by the NSD 1015 method and compared to other methods. The amine levels in nmoles/g, the amine synthesis rate in nmoles/g/h and the amine synthesis rate constant in h^{-1} are given for NA, DA and 5-HT as determined by different methods in different brain regions. The "NSD method" refers to the results obtained in this study. Thirty min after NSD 1015, 100 mg/kg i.p. 0.050 ± 0.004 (12) μg/g of DOPA accumulated in pooled rat brain hemisphere [mean ± SEM (*n*)], 0.43 ± 0.030 (12) μg/g of DOPA accumulated in striatum and 0.109 ± 0.008 (18) μg/g of 5-HTP accumulated in whole brain. Each determination was performed on 3 pooled rat brains (see Methods) and *n* is the number of such determinations

Method	Brain area	Normal amine level nmoles/g	Synthesis rate nmoles/g/h	Amine synthesis rate constant h^{-1}
Noradrenaline				
1. NSD method	Hemisphere	1.3	0.50	0.39
2. ^{3}H-Tyrosine injection (Neff *et al.*, 1971)	Teledienceph.	2.1	0.59	0.28
3. ^{3}H-Tyrosine infusion (Neff *et al.*, 1969)	Whole brain	2.8	0.71	0.25
4. α-MT (Brodie *et al.*, 1966)				
Marland farm rats	Whole brain	1.8	0.22	0.12
NIH rats	Whole brain	2.5	0.43	0.17
Dopamine				
1. NSD method	Striatum	20.8	4.4	0.21
2. ^{3}H-Tyrosine injection (Neff *et al.*, 1971)	Teledienceph.	5.7	1.9	0.34
3. ^{3}H-Tyrosine infusion (Neff *et al.*, 1969)	Whole brain	5.4	1.4	0.26
4. α-MT (Brodie *et al.*, 1966)				
Marland farm rats	Whole brain	4.9	1.4	0.28
NIH rats	Whole brain	7.5	2.8	0.37
5-HT				
1. NSD method	Whole brain	1.8	1.0	0.54
2. ^{3}H-Tryptophan (Neff *et al.*, 1971)	Teledienceph.	1.9	1.1	0.56
	Brainstem	2.9	2.2	0.75
	Whole brain[a]	2.1	1.3	0.60
3. 5-HT rise after MAO inhibition (Lin *et al.*, 1969)	Whole brain	2.4	1.7	0.71
4. 5-HIAA drop after MAO inhibition (Lin *et al.*, 1969)	Whole brain		1.5	
5. 5-HIAA rise after probenecid (Lin *et al.*, 1969)	Whole brain		1.6	
(Meek and Werdinius, 1970)	Whole brain		1.2	

[a] Calculated on the assumption that the brain stem represents $^1/_5$ of the weight of the whole brain without cerebellum.

tyrosine has been demonstrated after a structurally similar hydrazine compound (Hempel and Männl, 1968).

Thus, our results suggest that the accumulation of DOPA and 5-HTP 30 min after an intraperitoneal dose of NSD 1015, 100 mg/kg, is an accurate measurement of the *in vivo* activity of the tyrosine and tryptophan hydroxylase, respectively.

2. Comparison of the NSD 1015 Method of Measuring Tyrosine and Tryptophan Hydroxylase with other Methods for Measuring Catecholamine and 5-HT Turnover

In Table 2, the amine concentrations, synthesis rates and rate constants are calculated from our data and compared to those from other methods. The amine concentration is expressed in nmoles/g tissue and is calculated from our control, untreated animals (see Fig.4). The synthesis rate is in nmoles/g/h of either DOPA or 5-HTP accumulated after NSD 1015 pretreatment. These synthesis rates should represent the rates of hydroxylation of tyrosine and tryptophan, respectively. Finally, the rate constant of synthesis for a given amine is calculated by dividing the appropriate precursor synthesis rate by the concentration of the amine. This constant is in h^{-1}. We have not routinely corrected our values for recovery. As we have about the same recovery for DOPA, 5-HTP, and the amines, our uncorrected values are not appreciably affecting our rate constants.

Theoretical Considerations. The other methods listed in Table 2 and the one described here using NSD 1015 are approximations of the production and utilization of neurotransmitter. However, different methods are based on different assumptions. For instance, the methods utilizing tracer doses of isotopically labelled precursors depend on the measurement of the precursor pool and often the assumptions of a single neurotransmitter pool must be made (for discussion see Neff *et al.*, 1971 and Sedvall *et al.*, 1968). It seems likely that the plasma pool of precursor (usually tyrosine or tryptophan) most accurately reflects the true intraneuronal precursor pool; however, it remains an approximation. As a result, data calculated from isotope measurements in non-steady state conditions depend on the assumption that the unmeasured precursor pool does not change during the experiment. The number of metabolically active pools of neurotransmitter is a debated subject. Two pools have been proposed in monoamine neurons; a slowly metabolized storage pool, and a rapidly metabolized pool containing newly synthesized neurotransmitter which is preferentially released by neuronal impulses (Kopin *et al.*, 1968; Javoy and Glowinski, 1971).

Measurement of catecholamine turnover utilizing blockade of synthesis with α-methyl-para-tyrosine (α-MT) is based on the assumption that this drug does not change the turnover of the amine studied and further that the metabolism of the catecholamines is not altered as the levels of the amines decline. If more than one metabolically active pool exists, the α-MT method would measure the slowly metabolized pool with greater accuracy than rapidly metabolized pools. If there is a great difference between the metabolism of such pools, the latter may be lost with this method.

Finally the measurement of 5-HT accumulation after a MAO inhibitor or 5-HIAA disappearance after probenecid are also based on the assumptions that the drugs employed do not affect the synthesis or the disposition of the substance measured.

A distinct advantage of our method utilizing NSD 1015 is that we measure the product of hydroxylation of tyrosine and tryptophan. It is most likely that these are the rate-limiting steps in catecholamine and 5-HT synthesis, respectively. Physiological adjustments in amine metabolism are probably made by alterations in the activity of these enzymes. Synthesis rates determined by the NSD 1015 method should reflect the hydroxylase activity without requiring measurement of the level of neurotransmitter and regardless of the number of metabolically active pools. An additional advantage is that the NSD 1015 method actually involves just one measurement of either DOPA or 5-HTP. This allows greater flexibility in the measurement of monoamine metabolism, e.g., when steady-state conditions have been altered. Estimations of synthesis utilizing isotopes require fluorimetric and isotopic estimations of both brain tissue and plasma. Methods using probenecid or enzyme blockers require at least two measurements at different time intervals.

The major weakness in the NSD 1015 method lies in the fact that both catecholamine neurotransmitters have as their probable rate-limiting step the hydroxylation of tyrosine . We are thus unable to differentiate between the two rates of transmitter synthesis in areas where both NA and DA neurons are found. However, in general NA and DA occur in different regions.

We must make the assumption that NSD 1015 does not itself affect tyrosine or tryptophan hydroxylase activity. This seems reasonable as our synthesis rates are not affected by a wide dose range of NSD 1015. In addition it must be assumed that NSD 1015 has no *indirect* effects on hydroxylation rates. NSD 1015 has a mild MAO inhibitory effect (Bavin, 1960; personal communication), thus the levels of the monoamines did not change significantly for 30 min in rat brain after NSD 1015 administration. There may be a change in the intracellular distribution of the amines, but the linear accumulation of DOPA and 5-HTP argue against any such change in intracellular levels of the amines or their precursors having an effect on hydroxylase activity.

Comparison of Results. Strict comparisons are not always possible, since data from identical brain parts are not available. By studying the rat cerebral hemispheres, we found a DOPA accumulation of 0.50 nmoles/g/h. This is slightly lower than the 0.59 that Neff *et al.* (1971) reported in rat telediencephalon. It is higher than or agrees with measurements on whole brain using α-MT. Our hemisphere NA level is lower, and consequently, our calculated rate constant higher than those reported by others for telediencephalon and whole brain.

In the rat corpus striatum, a predominantly DA neuronal area, the synthesis rate of DOPA was much higher, 4.43 nmoles/g/h. The rate constant, 0.21 h^{-1}, was lower than the rate constant for central NA neurons calculated in the hemispheres as 0.39 h^{-1}. Our rate constant for DA is somewhat lower than that calculated by the isotope method of Neff *et al.* (1971). However, their rate constant is based on telediencephalon, while ours is on striatum alone.

Finally we calculated the synthesis of 5-HTP from our whole brain data as being 1.0 nmoles/g/h. This is in fairly good agreement with the isotope data of Neff *et al.* (1971) if these data are recalculated as whole brain. Using our laboratory's strain of Sprague-Dawley rats, Meek and Werdinius (1970) calculated the synthesis rates of 5-HIAA as 1.2 nmoles/g/h. Since this value is corrected for 5-HIAA recovery, it agrees quite well with our uncorrected level of 1.0. The variation in rat strains may explain some of the different synthesis rates others have obtained.

This comparison with other data indicates that the values obtained with NSD 1015 are similar to those obtained with other procedures. For a closer comparison experiments using the different techniques will have to be run in parallel in the same laboratory and on the same strain of animals.

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