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The Influence of Nerve-Impulse Flow on the Synthesis and Metabolism of 5-Hydroxytryptamine in the Central Nervous System

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Synopsis

Two inhibitors of L-5-hydroxytryptophan/L-3,4-dihydroxyphenylalanine decarboxylase were injected intraperitoneally into mice and rats. The subsequent accumulation of the normally undetectable 5-hydroxytryptophan and dopa (3,4-dihydroxyphenylalanine) in the central nervous system was investigated. The distribution of the two amino acids in rat brain was found to be similar to that of endogenous 5-hydroxytryptamine and catecholamines [noradrenaline+dopamine (3,4-dihydroxyphenethylamine)] respectively. The time-course of the accumulation was linear for the first 30min. The rate of 5-hydroxytryptophan formation was increased by L-tryptophan administration.

Section of the axons of monoamine fibre systems led to immediate changes in hydroxylation rates. The hydroxylation of L-tryptophan was decreased by about 50% in rat spinal cord, but much less in the brain. The hydroxylation of L-tyrosine was accelerated in the forebrain, where the striatal dopamine system predominates. In a cortical area, where noradrenaline is the predominating catecholamine, axotomy did not significantly influence L-tyrosine hydroxylation.

The hydroxylation of L-tryptophan was retarded by the monoamine oxidase inhibitor nialamide and by the hallucinogenic agents compound LSD-25 (lysergic acid diethylamide) and *NN*-dimethyltryptamine.

The results obtained indicate that monoamine synthesis is not regulated by one single mechanism. For 5-hydroxytryptamine both the L-tryptophan hydroxylase and the intraneuronal portion of the monoamine oxidase are probably involved in the regulation of metabolism according to physiological needs.

Introduction

There is considerable evidence that 5-hydroxytryptamine, like dopamine and noradrenaline, is formed and stored in presynaptic neuronal structures and serves as a neurohumoral transmitter in the central nervous system (for review see Andén *et al.*, 1969).

Andén *et al.* (1966) observed that, when the terminal system of the descendent spinal 5-hydroxytryptamine neurons was disconnected from its cell bodies in the brain stem, and thus deprived of the impulse flow, by cutting the spinal cord, the turnover of 5-hydroxytryptamine was slowed down, as indicated by a retarded

disappearance of the transmitter induced by an inhibitor of L-tryptophan hydroxylase. Conversely electrical stimulation of the cell bodies caused a decrease in 5-hydroxytryptamine and an increase in 5-hydroxyindol-3-ylacetate concentrations in the terminal system of the telencephalon (Sheard & Aghajanian, 1968).

When discussing the linkage between physiological activity and transmitter synthesis and turnover in a monoamine-carrying neuronal system, we have to take into account the complex chain of events taking place in the nerve terminals. In particular it should be emphasized that the nerve terminals are capable not only of synthesizing but also of degrading the transmitter, implying that part of the transmitter may be destroyed intraneuronally, by mitochondrial monoamine oxidase. In other words the increased demand induced by the nerve impulses might theoretically be met either by an increased synthesis or by a decreased intraneuronal deamination or by both mechanisms. It is difficult to decide from the observations quoted above which alternative is correct. [Admittedly some early observations on isolated spinal cords did in fact suggest an increased transmitter synthesis, induced by electrical stimulation after pretreatment with a monoamine oxidase inhibitor (Andén *et al.*, 1964, 1965).]

The present investigation deals only with the short-term changes induced by interrupting the impulse flow. Hopefully our studies will be extended to the long-term changes involving, e.g., enzyme induction and axonal transport, i.e. the type of problems discussed in this Symposium by Thoenen (1972) and Banks & Mayor (1972).

If the synthesis of 5-hydroxytryptamine is stimulated by physiological nervous activity, two possibilities have to be considered: (*a*) increased activities of the synthesizing enzymes and then, presumably, of the rate-limiting L-tryptophan hydroxylase, and (*b*) an increased uptake of L-tryptophan. This should result in increased 5-hydroxytryptamine synthesis, since the L-tryptophan hydroxylase is

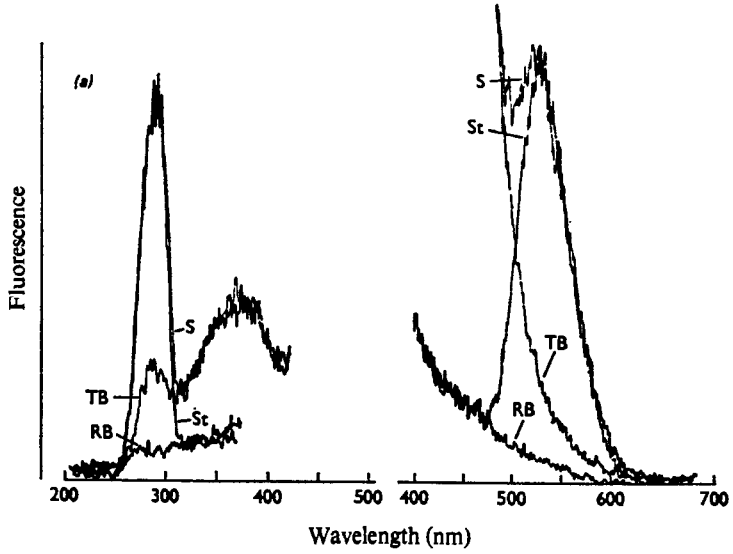

Fig. 1(*a*).

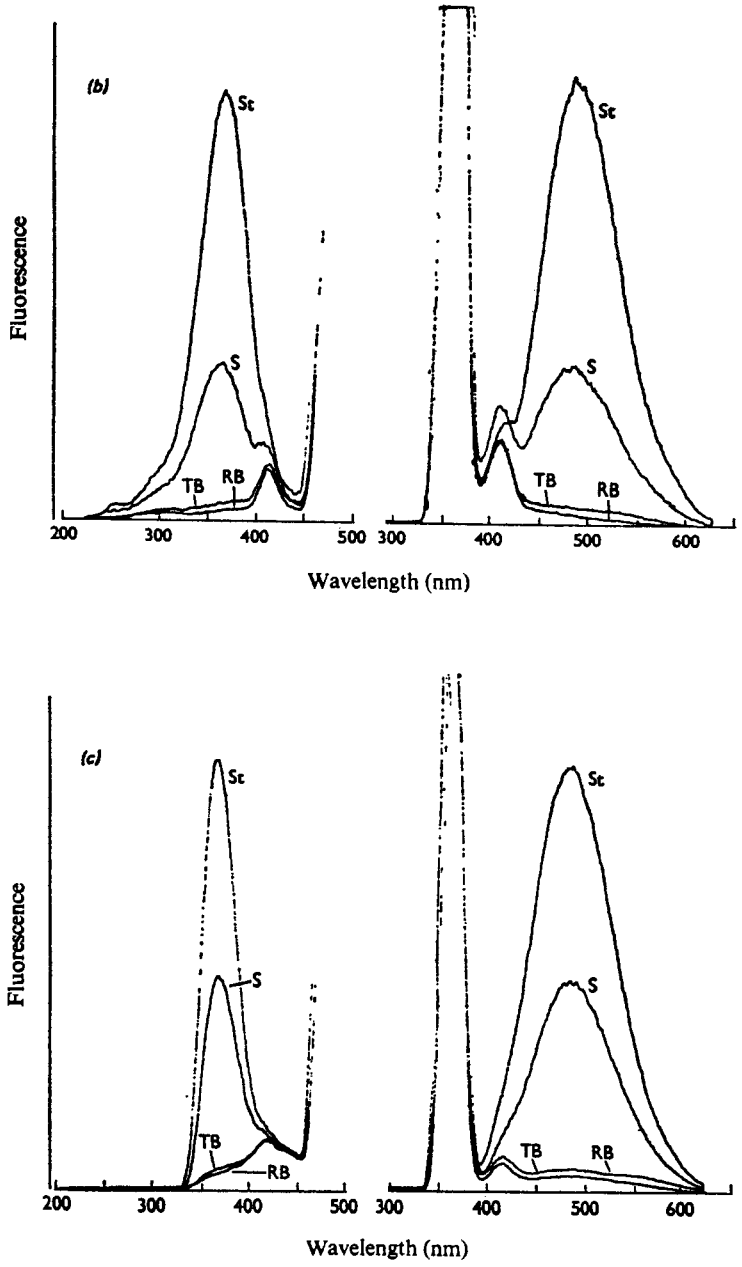

Fig. 1. *Activation and fluorescence spectra of amino acid precursors accumulating in brain after the inhibition of decarboxylase activity by compound Ro* 4-4602 (*a*) *and by compound NSD* 1015 (*b and c*)

(*a*) 5-Hydroxytryptophan in mouse brain, measured by native fluorescence (Carlsson & Lindqvist, 1970). (*b*) 5-Hydroxytryptophan in rat forebrain, measured by the fluorophore obtained by treatment with *o*-phthalaldehyde. (*c*) Dopa in rat forebrain, measured by a modified trihydroxyindole assay (Kehr *et al.*, 1972). Abbreviations: RB, reagent blank; TB, tissue blank; S, sample; St, standard (100 ng in *a* and *c*, 50 ng in *b*).

normally not saturated with its substrate (Weber & Horita, 1965; Green & Sawyer, 1966; Moir & Eccleston, 1968; Tagliamonte *et al.*, 1971; Grahame-Smith, 1971).

So far, only indirect methods have been available for investigating the rate of L-tryptophan hydroxylation *in vivo*, and they are thus of limited usefulness. In the present investigation we have measured the accumulation of the normally undetectable L-5-hydroxytryptophan, induced by an inhibitor of 5-hydroxytryptophan decarboxylase. Before discussing the influence of the nerve-impulse flow we give the results of some experiments aiming to answer the question whether or not this procedure yields reliable information on the rate of L-tryptophan hydroxylation *in vivo*.

The 5-hydroxytryptophan decarboxylase inhibitors employed inhibit also the decarboxylation of L-dopa and thus cause the accumulation of this compound too. For comparison some results on L-dopa accumulation are also given and discussed.

Experimental and Results

This investigation was made possible by some further development of our analytical techniques (Atack & Magnusson, 1970; Lindqvist, 1971; Kehr *et al.*, 1972), by means of which we can analyse a large number of amines and their precursors and metabolites in a single extract by using a single column run (on Dowex 50). For example, in some of the experiments reported below we have determined 5-hydroxyindol-3-ylacetate, tyrosine, dopa, tryptophan, 5-hydroxytryptophan, noradrenaline, dopamine, 5-hydroxytryptamine and histamine, although only some of these results are discussed here.

Our procedure is simply to inject an inhibitor of 5-hydroxytryptophan decarboxylase in a dose causing the virtually complete inhibition of the enzyme and with a rapid onset of inhibition. The initial accumulation of the substrates is assumed to indicate their rate of formation *in vivo*. We have used two different decarboxylase inhibitors, namely compound Ro 4-4602 [*N*-(DL-seryl)-*N'*-(2,3,4-trihydroxybenzyl)hydrazine] and compound NSD 1015 (3-hydroxybenzylhydrazine). So far comparisons between the two inhibitors have given very similar results with respect to substrate accumulation. However, they differ in two important respects. First, the former agent is less potent than the latter, the doses required for virtually complete inhibition of the decarboxylase in mouse brain being 800 and 100 mg intraperitoneally/kg body wt. respectively (Carlsson *et al.*, 1968; A. Carlsson & M. Lindqvist, unpublished work). Secondly, in the doses used the latter compound but not the former causes a rather pronounced inhibition of monoamine oxidase. Consequently the 5-hydroxytryptamine and catecholamine concentrations in brain will decrease after administration of the former compound but will not show any considerable changes after the latter agent.

The 5-hydroxytryptophan and dopa accumulating after the inhibition of decarboxylase activity have the same activation and fluorescence spectra as the authentic amino acids (Fig. 1). Normally none of these amino acids can be detected with certainty in brain. The chromatographic behaviour, the spectra and the

experimental circumstances under which they occur leave little doubt about the identity of the two intermediates.

Results

Distribution of 5-hydroxytryptophan and dopa in the brain

We have investigated the origin of the two substrates accumulating in the brain after the inhibition of decarboxylase activity by studying their regional distribution in the brain (Bédard *et al.*, 1971; A. Carlsson, J. N. Davis, W. Kehr, M. Lindqvist, T. Magnusson & C. V. Atack, unpublished work). It was found that the 5-hydroxytryptophan and dopa were distributed similarly to 5-hydroxytryptamine and the catecholamines (dopamine+noradrenaline) respectively. In contrast, 5-hydroxytryptophan injected intraperitoneally showed an even distribution in the brain (Figs. 2 and 3). Histochemical studies on reserpine-pretreated rats showed that the 5-hydroxytryptophan and dopa accumulating after the inhibition of decarboxylase activity were located in nerve-cell bodies and nerve terminals in areas normally containing 5-hydroxytryptamine and dopamine respectively. No accumulation of dopa in noradrenaline nerve-cell bodies and terminals could be detected histochemically, but biochemical methods indicate that such accumulation does occur, although in lower concentration than in dopamine nerve regions.

From these findings it can be concluded that the 5-hydroxytryptophan and dopa accumulating in the brain after decarboxylase inhibition have largely, if not exclusively, been formed in the brain itself, probably in the cell bodies and nerve terminals normally storing 5-hydroxytryptamine and catecholamines respectively.

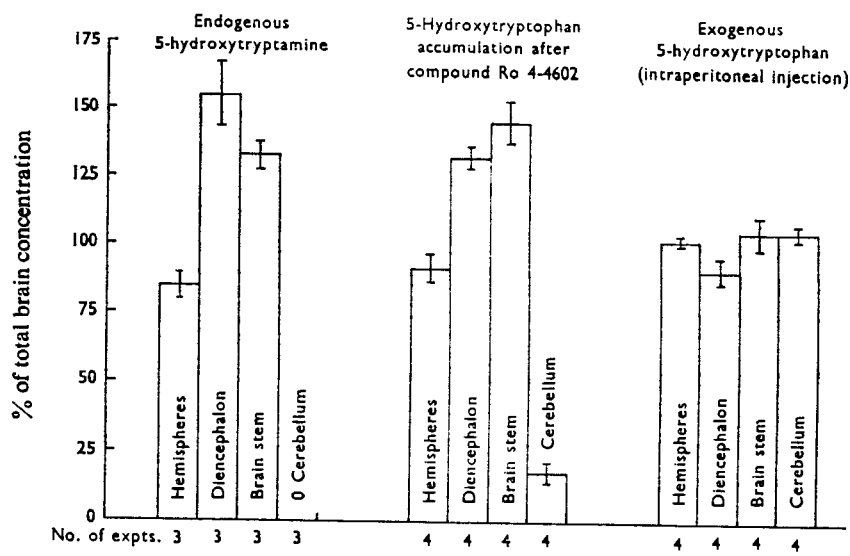

Fig. 2. *Distribution of 5-hydroxytryptamine and 5-hydroxytryptophan in rat brain (Bédard et al., 1971)*

The values given represent the means ± S.E.M.

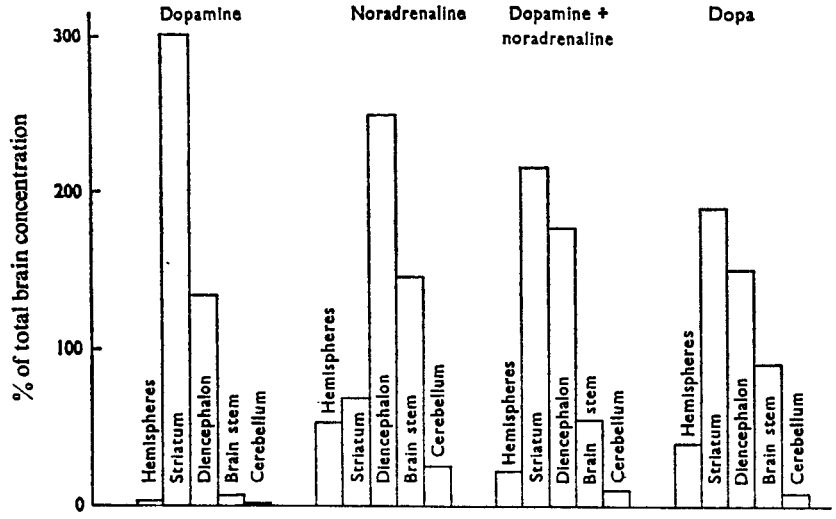

Fig. 3. *Distribution of catecholamines and dopa in rat brain after the inhibition of decarboxylase activity with compound NSD* 1015 (*A. Carlsson, J. N. Davis, W. Kehr, M. Lindqvist, T. Magnusson & C. V. Atack, unpublished work*)

The animals were treated with compound NSD 1015 (100mg intraperitoneally/kg body wt.) 30min before they were killed.

Time-course of 5-*hydroxytryptophan and dopa accumulation*

During an initial phase of about 30 min after the administration of a decarboxylase inhibitor the accumulations of 5-hydroxytryptophan and dopa are approximately linear (Fig. 4). Therefore during this stage the formation of these intermediates probably occurs at an approximately constant rate. Moreover it does not seem likely that any considerable loss of the intermediates occurs during this phase, since this would probably result in bending of the curve. The possibility therefore exists that the initial accumulation represents the true rates of hydroxylation of tryptophan and tyrosine respectively. However, this problem requires further investigation. Nevertheless, assuming that the initial accumulation represents the true rate of formation, turnover times of 1.6h and 3–5h are obtained for 5-hydroxytryptamine in rat or mouse brain and for catecholamines in rat brain respectively. These estimates are of the same order of magnitude as those reported in the literature.

Effect of L-*tryptophan administration*

It is generally assumed that tryptophan hydroxylase is normally not saturated with its substrate. We therefore investigated the accumulation of 5-hydroxytryptophan in mouse brain after the inhibition of decarboxylase activity with and without loading with L-tryptophan in various dosages. The relationship between tryptophan hydroxylation and substrate concentration thus obtained is shown in Fig. 5(*a*). Fig. 5(*b*) gives the same results presented as double-reciprocal plots. From the average of the two regression lines of Fig. 5(*b*), i.e. that of x against y and that of y against x, an apparent K_m value of 6×10^{-5} M is calculated, assuming

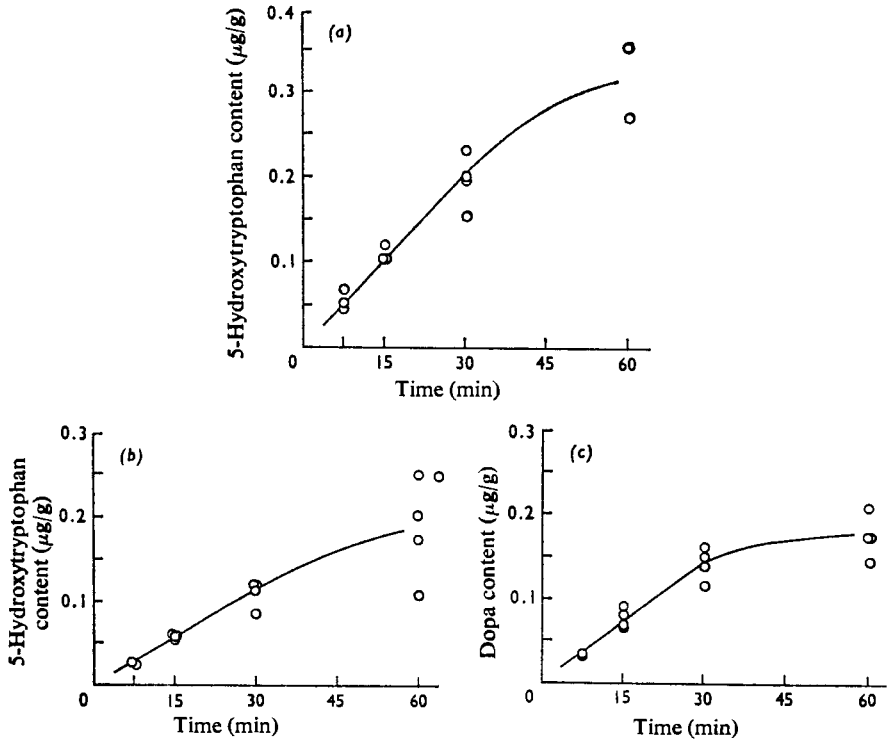

Fig. 4. *Accumulation of 5-hydroxytryptophan and dopa in brain after the inhibition of decarboxylase activity with compound NSD* 1015 (*A. Carlsson, J. N. Davis, W. Kehr, M. Lindqvist, T. Magnusson & C. V. Atack, unpublished work*)

The animals were treated with compound NSD 1015 (100mg intraperitoneally/kg body wt.) at the times indicated before they were killed. (*a*) 5-Hydroxytryptophan in mouse brain; (*b*) 5-hydroxytryptophan in rat brain; (*c*) dopa in rat brain.

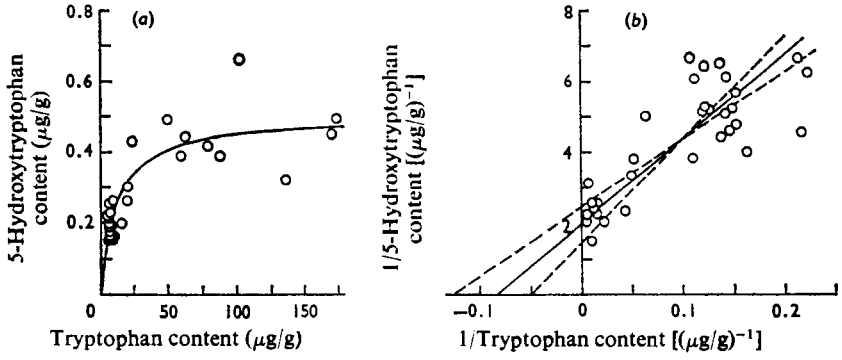

Fig. 5. *Effect of tryptophan concentration on the rate of 5-hydroxytryptophan accumulation in mouse brain* (*Carlsson & Lindqvist*, 1972)

(*a*) The animals were injected with compound Ro 4-4602 (800mg intraperitoneally/kg body wt.) 30min before being killed. Some of the animals received an intraperitoneal injection of L-tryptophan (100, 300 or 1000mg/kg body wt.) 30min before the compound Ro 4-4602. Each point represents 5-hydroxytryptophan and tryptophan values obtained from single samples of 4–12 pooled mouse brains. (*b*) Double-reciprocal plot of the relationship shown in (*a*). The continuous line is the average of the broken lines, representing the regression of x on y and of y on x. The curve in (*a*) was calculated from the continuous line in (*b*).

Table 1. *Indoles in rat spinal cord 1–2 h after transection and in non-transected controls (A. Carlsson, M. Lindqvist & T. Magnusson, unpublished work)*

All animals were treated with compound NSD 1015 (100 mg/kg body wt.) 1 h before being killed. Results are given as means ± S.E.M. ($n=3$ for transected, $n=6$ for controls); the ratios are given as medians with ranges in parentheses.

	Tryptophan (μg/g)		5-Hydroxytryptophan (ng/g)		5-Hydroxytryptamine (ng/g)		5-Hydroxyindol-3-ylacetate (ng/g)	
	Transected	Controls	Transected	Controls	Transected	Controls	Transected	Controls
Upper part (U)	4.30 ± 0.55	3.75 ± 0.31	103 ± 20	113 ± 14	282 ± 40	322 ± 12	66 ± 24	69 ± 13
Lower part (L)	4.17 ± 0.41	3.87 ± 0.39	93 ± 12	166 ± 15	688 ± 58	621 ± 34	72 ± 10	99 ± 14
Ratio U/L	1.00	0.99	1.09	0.69	0.40	0.51	1.17	0.89
	(0.91–1.17)	(0.66–1.36)	(0.89–1.37)	(0.53–0.80)	(0.37–0.45)	(0.48–0.59)	(0.37–1.22)	(0.24–1.00)

Table 2. *Indoles in rat spinal cord 6 h after transection and in non-transected controls (A. Carlsson, M. Lindqvist & T. Magnusson, unpublished work)*

All animals were treated with compound Ro 4-4602 (800 mg/kg body wt.) 2 h and L-tryptophan (300 and 150 mg/kg body wt.) 3 and 1½ h before being killed. Results are given as means ± S.E.M. ($n=3$ for transected, $n=4$ for controls); ratios are given as medians with ranges in parentheses.

	Tryptophan (μg/g)		5-Hydroxytryptophan (ng/g)		5-Hydroxytryptamine (ng/g)		5-Hydroxyindol-3ylacetate (ng/g)	
	Transected	Controls	Transected	Controls	Transected	Controls	Transected	Controls
Upper part (U)	43.4 ± 6.9	53.8 ± 6.7	454 ± 132	345 ± 99	220 ± 24	224 ± 15	131 ± 22	100 ± 14
Lower part (L)	44.5 ± 6.9	57.8 ± 9.6	337 ± 95	471 ± 118	580 ± 80	461 ± 12	143 ± 18	139 ± 17
Ratio U/L	0.96	0.96	1.26	0.72	0.39	0.49	0.88	0.72
	(0.96–1.01)	(0.83–1.06)	(1.19–1.57)	(0.64–0.79)	(0.33–0.43)	(0.40–0.57)	(0.81–1.04)	(0.69–0.75)

that the tryptophan concentration in the liquid phase surrounding the tryptophan hydroxylase is equal to that of the whole brain. This K_m value is somewhat higher than that obtained by Ichiyama *et al.* (1968), namely 2×10^{-5} M, but lower than that given by Lovenberg *et al.* (1968), namely 3×10^{-4} M. In each of these cases a partially purified tryptophan hydroxylase obtained from mammalian brain was investigated.

Acute effect of axotomy on monoamine synthesis

Spinal cord 5-hydroxytryptamine. All spinal monoamine nerve pathways are descendent, and thus transection of the spinal cord will interrupt the connexion between cell bodies and nerve terminals below but not above the lesion. This should result in immediate loss of nerve-impulse flow in the monoamine nerve pathways below the lesion. Degeneration, with loss of transmitter stores, synthesizing enzymes and so on, does not set in until after a few days. In this study we investigated the acute effect of transection, i.e. within 1–6 h. During this interval there is probably no loss of synthesizing enzymes, and the concentrations of 5-hydroxytryptamine and noradrenaline are not yet decreased but are perhaps slightly elevated (cf. Kuhar *et al.*, 1971). Nevertheless we found that the accumulation of 5-hydroxytryptophan after the inhibition of the decarboxylase activity was decreased below the lesion as compared with the concentration observed above the lesion. Some of the experiments were done with compound NSD 1015, some with compound Ro 4-4602, and in the latter case the tryptophan concentration was elevated by two injections of L-tryptophan, but the different treatment

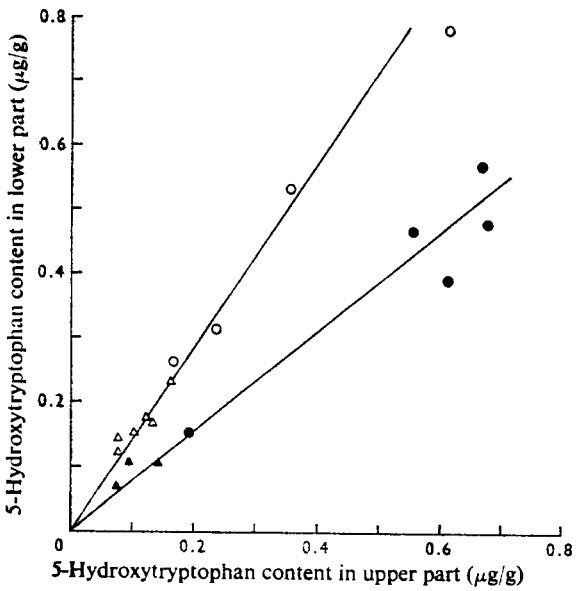

Fig. 6. *Plot of 5-hydroxytryptophan concentration in the lower part of rat spinal cord against that in the upper part 1–6 h after transection, as compared with non-transected controls (A. Carlsson, M. Lindqvist & T. Magnusson, unpublished work)*

All animals were treated with inhibitors of decarboxylase activity (as described in Tables 1 and 2): ▲ and △, compound NSD 1015; ● and ○, compound Ro 4-4602 + L-tryptophan. ▲ and ●, Transected; △ and ○, controls.

schedules did not seem to influence the main result: a relative decrease in 5-hydroxytryptophan below the lesion (Tables 1 and 2).

Fig. 6 summarizes the results obtained for spinal cord, the 5-hydroxytryptophan concentration below the lesion being plotted against that above the lesion. The slope of the regression line obtained with transected animals is clearly below that obtained with unoperated and sham-operated controls. The decrease induced by the transection is almost 50%.

Fig. 7 shows that transection of the spinal cord causes an almost 50% decrease also of the 5-hydroxyindol-3-ylacetate concentration below the lesion. In this figure the 5-hydroxyindol-3-ylacetate concentrations below the lesion were plotted against those above the lesion; these latter did not change significantly. No decarboxylase inhibitor was given in these experiments.

At 3 days after transection of the spinal cord the 5-hydroxytryptophan recovered from the lower part 1 h after the administration of compound NSD 1015 was only 10–15% of the value found in non-transected animals; no difference was found in the upper part.

Cerebral 5-hydroxytryptamine. The results obtained after a cerebral hemisection, disconnecting the forebrain monoamine nerve terminals from their cell bodies in the lower brain stem, were not entirely in agreement with the results obtained for the spinal cord. The lesion caused only a slight decrease in the 5-hydroxytryptamine accumulating after the inhibition of decarboxylase activity, statistical significance being achieved only in some of the experiments. No difference in 5-hydroxyindol-3-ylacetate concentrations (with or without inhibi-

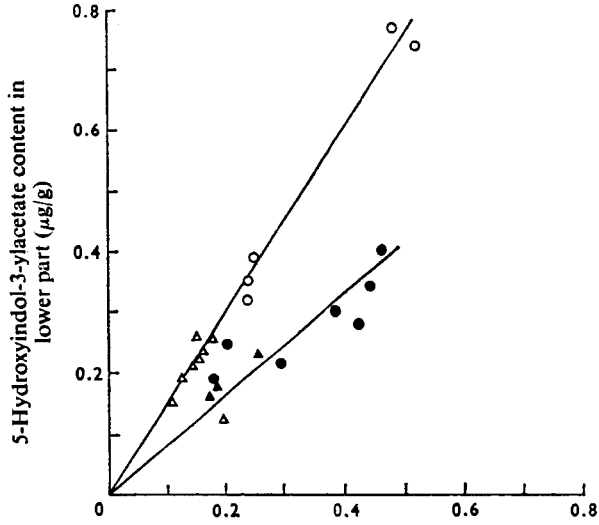

Fig. 7. *Plot of 5-hydroxyindol-3-ylacetate concentration in the lower part of rat spinal cord against that in the upper part 1–6 h after transection, as compared with non-transected controls (A. Carlsson, M. Lindqvist & T. Magnusson, unpublished work)*

Some of the animals were treated with L-tryptophan as described in Table 2 (no inhibitors of decarboxylase activity were given): ▲ and △, no L-tryptophan; ● and ○, L-tryptophan. ▲ and ●, Transected; △ and ○, controls.

tion of decarboxylase activity) was generally observed between the side with and that without a lesion, in contrast with the findings after spinal transection.

With compound Ro 4-4602 being used for the inhibition of decarboxylase activity we have used three different experimental series, with L-tryptophan loading in two of them (Bédard *et al.*, 1972). In none of them was a significant effect of the lesion on 5-hydroxytryptophan accumulation observed, even though the concentrations tended to be lower on the side with the lesion. In contrast, the disappearance of 5-hydroxytryptamine induced by the decarboxylase inhibitor was significantly less rapid on the side with the lesion, in agreement with the findings obtained by Andén *et al.* (1966), who studied the 5-hydroxytryptamine disappearance rate in the transected spinal cord after inhibition of L-tryptophan hydroxylase. This discrepancy between the results obtained for 5-hydroxytryptophan and 5-hydroxytryptamine may be explained by the occurrence of a small pool of cytoplasmic 5-hydroxytryptamine undergoing rapid deamination intraneuronally (see the Introduction). The results obtained in two of the experimental series with compound Ro 4-4602 are summarized in Table 3 (for further details see Bédard *et al.*, 1972).

In two additional experimental series we have used compound NSD 1015 for the inhibition of decarboxylase activity. The experimental conditions differed from the previous ones in certain other respects also. The intervals between hemisection and the killing of the animals were shorter (30 or 90 min instead of 3–6 h), and so was the interval between the administration of the inhibitor and the killing of the animals (30 min instead of 2 h). Further, ether instead of Nembutal anaesthesia was used. With compound NSD 1015 a slight though significant decrease (15%) in 5-hydroxytryptophan concentration was observed (Table 4) on the side with the lesion as compared with that without.

In another experimental series the effect of hemisection on the accumulation of 5-hydroxyindol-3-ylacetate induced by probenecid (200 mg/kg body wt.) was studied, in this case after L-tryptophan loading. A slight but significant decrease (20%) in the 5-hydroxyindol-3-ylacetate concentration was found on the side with the lesion as compared with that without (Bédard *et al.*, 1972).

The tryptophan concentration was always elevated on the side with the lesion. This might counteract the decrease in tryptophan hydroxylase activity induced by

Table 3. *Indoles in rat forebrain 3 h after left cerebral hemisection (Bédard et al., 1972)*

Animals were treated with compound Ro 4-4602 (800 mg/kg body wt.) alone or in combination with L-tryptophan (300 mg/body wt.) 2 and 3 h respectively before the animals were killed. Results are given as means ± S.E.M. ($n = 4$). N.S., Not significant.

	Hemisection			Hemisection + L-tryptophan		
	Left	Right	P	Left	Right	P
Tryptophan (μg/g)	13.8 ± 0.9	9.1 ± 0.7	<0.005	119.3 ± 5.0	83.8 ± 6.3	<0.005
5-Hydroxytryptophan (ng/g)	340 ± 20	410 ± 60	N.S.	590 ± 70	620 ± 50	N.S.*
5-Hydroxytryptamine (ng/g)	350 ± 30	260 ± 10	<0.005	360 ± 10	270 ± 10	<0.005
5-Hydroxyindol-3-ylacetate (ng/g)	240 ± 20	230 ± 20	N.S.	360 ± 30	300 ± 10	N.S.

* Significantly higher than after hemisection alone ($P < 0.025$).

Table 4. *Indoles in rat forebrain 30 or 90 min after left cerebral hemisection (A. Carlsson, W. Kehr, M. Lindqvist, T. Magnusson & C. V. Atack, unpublished work)*

All animals were treated with compound NSD 1015 (100mg intraperitoneally/kg body wt.) 30min before being killed. Results are given as means ± S.E.M. (n=4). N.S., Not significant.

	Tryptophan (μg/g)	5-Hydroxy-tryptophan (ng/g)	5-Hydroxy-tryptamine (ng/g)	5-Hydroxyindol-3-ylacetate (ng/g)
30min				
Left	7.2±0.4	88±6	351±23	218±26
Right	5.8±0.3	103±13	334±12	221±22
P: left versus right	N.S.	0.01*	N.S.	N.S.
90min				
Left	9.0±1.3	78±9	295±11	280±37
Right	6.2±0.4	93±10	280±27	293±26
P: left versus right	<0.01	0.01*	N.S.	N.S.

* Statistical treatment of pooled 30 and 90min groups.

the axotomy. However, the difference in 5-hydroxytryptophan concentration between the two sides did not become larger after saturation of the hydroxylase by means of L-tryptophan loading.

Cerebral catecholamines. Compound NSD 1015 (100mg/kg body wt.) was injected intraperitoneally immediately or 60min after a cerebral hemisection on the left side, performed under ether anaesthesia. The animals were killed 30min after the injection. Table 5 (same animals as in Table 4) shows concentrations of dopa, dopamine and noradrenaline in the forebrain. In the animals receiving the inhibitor immediately after the hemisection the accumulation of dopa was considerably increased on the side with the lesion. In the animals treated with the inhibitor 60min after the lesion only a slight and insignificant increase in dopa was seen on the side with the lesion. However, there was instead a considerable increase in dopamine on the side with the lesion. This difference was much smaller in the former group of animals, in which the synthesis of dopamine was inhibited shortly after the lesion had been made.

These findings indicate that section of the ascendent dopamine nerve fibres results in an increased rate of L-tyrosine hydroxylation in the cerebral dopamine nerve terminal system. This increase is very marked initially, but when the dopamine concentration has increased to a certain value the synthesis appears to slow down again. The remarkably rapid increase in forebrain dopamine after cerebral hemisection has been reported by Andén *et al.* (1972).

Table 5. *Dopa, dopamine and noradrenaline in rat forebrain 30 or 90 min after left cerebral hemisection (A. Carlsson, W. Kehr, M. Lindqvist, T. Magnusson & C. V. Atack, unpublished work)*

All animals were treated with compound NSD 1015 (100mg intraperitoneally/kg body wt.) 30min before being killed. Results are given as means ± S.E.M. (n=4). N.S., Not significant.

	Dopa (ng/g)	Dopamine (ng/g)	Noradrenaline (ng/g)
30min			
Left	554±50	1632±63	308±15
Right	195±27	1416±57	394±29
P: left versus right	<0.001	<0.05	<0.001
90min			
Left	391±35	2267±134	370±80
Right	340±30	1382± 77	437±31
P: left versus right	N.S.	<0.005	N.S.

Table 6. *Dopa, dopamine and noradrenaline in rat occipito-temporal cortex 30 or 90 min after left cerebral hemisection (A. Carlsson, W. Kehr, M. Lindqvist, T. Magnusson & C. V. Atack, unpublished work)*

All animals were treated with compound NSD 1015 (100 mg intraperitoneally/kg body wt.) 30 min before being killed. Results are given as means ± S.E.M. ($n=4$). N.S., Not significant.

	Dopa (ng/g)	Dopamine (ng/g)	Noradrenaline (ng/g)
30 min			
Left	23 ± 9	1 ± 3	156 ± 32
Right	15 ± 8	4 ± 4	192 ± 25
P: left versus right	N.S.	N.S.	<0.025
90 min			
Left	36 ± 6	34 ± 13	149 ± 39
Right	35 ± 5	19 ± 7	165 ± 14
P: left versus right	N.S.	N.S.	N.S.

In the experiment described above we also analysed the occipito-temporal cortex, i.e. the part located caudally of the hemisection. Also, in this part the terminal monoamine nerve systems are disconnected from their cell bodies by the lesion (cf. Bédard *et al.*, 1972). The predominating catecholamine in this region is noradrenaline, and thus the dopa accumulating here after the inhibition of decarboxylase activity is probably synthesized mainly in noradrenaline nerve terminals. In this region the dopa concentrations were about the same on the two sides, indicating that axotomy had no marked influence on the rate of tyrosine hydroxylation in the noradrenaline nerve terminals (Table 6; same animals as in Tables 4 and 5). Remarkably enough, the noradrenaline concentration was lower at the side of the lesion.

Table 7. *Effects of the monoamine oxidase inhibitor nialamide and the hallucinogenic agents NN-dimethyltryptamine and compound LSD-25 on the accumulation of 5-hydroxytryptophan in mouse brain induced by compound Ro 4-4602, with or without* L-*tryptophan loading (Carlsson & Lindqvist, 1972)*

All animals were treated with compound Ro 4-4602 (800 mg/kg body wt.) 30 min before being killed. The administration of each drug was as indicated in the table, with and without L-tryptophan loading (300 mg/kg body wt. 75 min before death).

	5-Hydroxytryptophan (μg/g)		
	Experimental groups (drug + compound Ro 4-4602)	Controls (compound Ro 4-4602)	*P*
Nialamide (300 mg/kg body wt. 3 h before death)	0.15	0.19	<0.01
L-Tryptophan + nialamide (100 mg/kg body wt. 1 h before death)	0.30	0.46	<0.001
NN-Dimethyltryptamine (50 mg/kg body wt. 40 min before death)	0.15	0.24	<0.01
L-Tryptophan + *NN*-dimethyltryptamine (50 mg/kg body wt. 40 min before death)	0.30	0.48	<0.01
Compound LSD-25 (1.5 mg/kg body wt. 40 min before death)	0.14	0.19	=0.01
L-Tryptophan + compound LSD-25 (1.5 mg/kg body wt. 40 min before death)	0.45	0.54	<0.001

Effect of drugs

The problem of feedback regulation of L-tryptophan or L-tyrosine hydroxylation is also being investigated, by using drugs known to interfere with various steps in the transmitter machinery. An example is shown in Table 7. The monoamine oxidase inhibitor nialamide was found to inhibit the hydroxylation of L-tryptophan, the effect being not very pronounced, though significant. The hallucinogenic agents compound LSD-25 (lysergic acid diethylamide) and *NN*-dimethyltryptamine caused similar effects. In each case the effect was observed both in the absence and in the presence of L-tryptophan loading (Carlsson & Lindqvist, 1972).

Discussion

Surprisingly, axotomy did not influence the hydroxylation of the precursor amino acid in a uniform way in the different monoamine nerve systems investigated. In the spinal 5-hydroxytryptamine nerve system this process was retarded by some 50%, whereas in the cerebral 5-hydroxytryptamine system a very slight retardation was observed. In the closely related catecholamine systems either no effect (cortical noradrenaline nerve system) or a marked acceleration (forebrain dopamine nerve system) was observed. From these observations it may be concluded that transmitter synthesis in the monoamine-carrying neuronal systems is probably not controlled by one single mechanism. Presumably different mechanisms exist, which may even oppose and balance each other. The different behaviour of the monoamine nerve systems investigated might then be explained by one mechanism playing a dominating role in one system, another in another system.

In the spinal cord the retardation of L-tryptophan hydroxylation seemed to occur almost immediately after transection. There was no significant change in the tryptophan concentrations. As mentioned above, no significant decrease of the amount of L-tryptophan hydroxylase could take place so rapidly. Nor does it seem likely that the enzyme would be inhibited to any significant degree by the slightly elevated 5-hydroxytryptamine concentration observed shortly after the transection. The possibility therefore should be considered that the nerve impulses can somehow influence the L-tryptophan hydroxylase. The fact that the enzyme activity was equally decreased by the lesion at a high as at a low degree of saturation with the substrate suggests that the change involves the $V_{max.}$ rather than the K_m. This invites the speculation that in the resting state part of the enzyme is bound in an inactive form. The nerve impulses may then cause release of the enzyme into its free and active form. Alternatively such changes may involve a cofactor rather than the enzyme.

In the brain axotomy but slightly retarded L-tryptophan hydroxylation. Whether this different outcome is in any way related to the more rapid turnover of 5-hydroxytryptamine in the brain than in the spinal cord cannot be decided from the available data. In any event, the almost unchanged rate of synthesis in spite of a marked diminution in the rate of release, and thus presumably also in the rate of extraneuronal transmitter metabolism, leads almost inevitably to the

conclusion that the rate of intraneuronal metabolism is increased after axotomy. Thus intraneuronal deamination appears to be involved in the control of transmitter metabolism. The exact nature of this control mechanism remains to be elucidated. Perhaps the degree of saturation of the granular storage mechanism is the only factor involved, implying that an increased flow of nerve impulses leads to a lower degree of saturation, and consequently to an increased uptake by the granules and a diminished intraneuronal deamination. However, the possibility cannot be excluded that, in addition, the intraneuronal monoamine oxidase activity is somehow controlled by nervous activity.

In the forebrain, which is dominated by the striatal dopamine nerve system, the rate of tyrosine hydroxylation was increased by axotomy. Since axotomy raises the forebrain dopamine concentration, this change is opposite to that of an end-product inhibition. However, it is in agreement with an earlier postulated feedback mechanism, brought into play by changes in the degree of receptor activation (Carlsson & Lindqvist, 1963; cf. Andén *et al.*, 1969). Whether the receptors involved in this feedback are located pre- or post-synaptically remains to be elucidated. It should be emphasized that this feedback mechanism appears to operate at least in part independently of the nerve-impulse flow.

The data do not exclude the possibility that end-product inhibition occurs in striatal dopamine neurons, but they indicate that another feedback mechanism is more important in this system.

The observations after axotomy did not permit any conclusions about the existence of a feedback regulation of 5-hydroxytryptamine synthesis. However, the observations with the monoamine inhibitor nialamide and the hallucinogenic agents compound LSD-25 and *NN*-dimethyltryptamine, which probably activate 5-hydroxytryptamine receptors, suggest that a certain feedback regulation of 5-hydroxytryptamine synthesis does occur, although it appears to be of limited quantitative importance. The mechanism of this regulation remains to be elucidated. The controversial problem of end-product inhibition of L-tryptophan hydroxylase (see Millard & Gál, 1971; Hamon *et al.*, 1972) is not solved by these experiments.

Thus the present investigation appears to permit the tentative conclusion that both L-tryptophan hydroxylase and intraneuronal monoamine oxidase are involved in the regulation of 5-hydroxytryptamine synthesis and metabolism in the central nervous system. The relative importance of these two enzymes may vary from one 5-hydroxytryptamine nerve system to another. In the rat forebrain deamination by intraneuronal monoamine oxidase appears to be the dominating regulatory mechanism.

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