PHARMACOLOGICAL REVIEWS
Vol. 24, No. 2

Printed in U.S.A.

Regulation of Monoamine Metabolism in the Central Nervous System

A. CARLSSON, W. KEHR, M. LINDQVIST, T. MAGNUSSON AND C. V. ATACK
Department of Pharmacology, University of Göteborg, Göteborg, Sweden

THIS Symposium is mainly devoted to the topic of the catecholamines of the peripheral sympathetic system. The present paper will deal with the central nervous system and will include a discussion not only of the catecholamines but also of 5-hydroxytryptamine (5-HT). This deviation from the main topic may seem justified since the peripheral and central monoamine-carrying neuronal systems are closely related. Observations made on any one of these systems are thus likely to throw light also on the others.

The emphasis of this paper will be on the synthesis of monoamines and its regulation. It is generally assumed that the first step in their synthesis is rate limiting, and therefore this step is of particular interest from the regulatory point of view. However, so far only indirect techniques have been available for investigating this step *in vivo*. During the last 1 or 2 years we have been working on more direct techniques, *i.e.*, to measure the accumulation of 5-hydroxytryptophan (5-HTP) and dopa induced by inhibitors of the aromatic amino acid decarboxylase. This investigation has been made possible by some further development of our analytical techniques (4, 17, 19), by means of which we can analyze a large number of amines and their precursors and metabolites in a single extract, with a single column run (Dowex 50). For example, in some of the experiments to be reported here we have estimated 5-hydroxyindoleacetic acid (5-HIAA), tyrosine, dopa, tryptophan, 5-HTP, noradrenaline, dopamine, 5-HT and histamine, although only some of these data will be discussed here.

Most of our data available so far deal with the formation of 5-HTP but beginning a few months ago the method for determining dopa has also been in use. We are thus able to include some data on the formation of this intermediate in this report.

Our procedure is simply to inject an inhibitor of the aromatic amino acid decarboxylase with a rapid onset of action in a dose causing the virtually complete inhibition of the enzyme. The initial accumulation of the substrates is assumed to indicate their rate of formation *in vivo*. We have used two different decarboxylase inhibitors, *i.e.*, Ro 4-4602 = [N-(DL-seryl)-N′-(2,3,4-trihydroxybenzyl)] hydrazine and NSD 1015 = 3-hydroxybenzyl hydrazine. So far comparisons between the two inhibitors have given very similar results with respect to substrate accumulation. However, they differ in two important respects. Firstly, 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/kg intraperitoneally, respectively (10, 13). Secondly, in the doses used the latter compound but not the former causes a rather pronounced inhibition of monoamine oxidase. Consequently, the 5-HT and catecholamine levels in brain will decrease after administration of the former compound but will not show any considerable changes after the latter agent.

The 5-HTP and dopa accumulating after decarboxylase inhibition 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

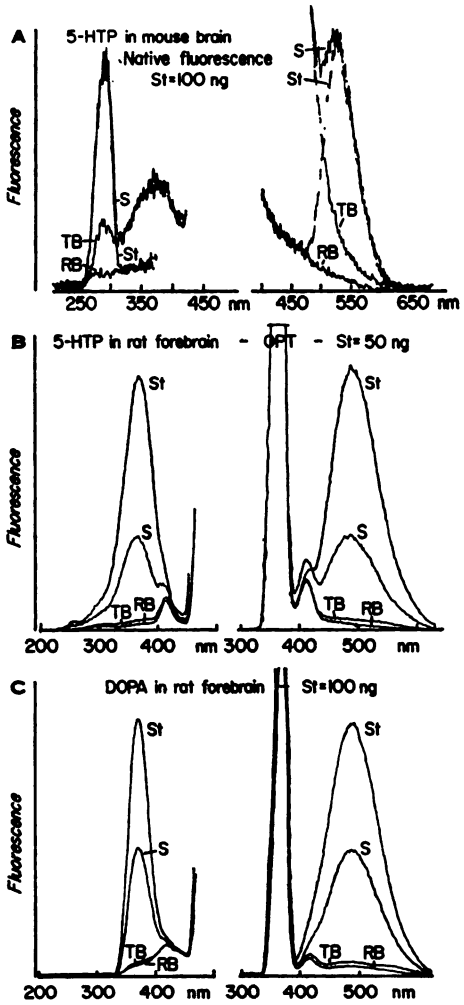

FIG. 1. Activation and fluorescence spectra of amino acid precursors accumulating after decarboxylase inhibition. A. 5-HTP in mouse brain, native fluorescence (from J. Pharm. Pharmacol. 22: 726–727, 1970). B. 5-HTP in rat forebrain, fluorophore obtained by treatment with orthophthalaldehyde. C. Dopa in rat forebrain, THI assay (17).

chromatographic behavior, the spectra and the experimental circumstances under which they occur, leave little doubt about the identity of the two intermediates.

We have investigated the origin of the two substrates accumulating in the brain after decarboxylase inhibition by studying their regional distribution in the brain (6, 8). It was found that the 5-HTP and dopa were distributed similarly to 5-HT and the catecholamines (dopamine plus noradrenaline), respectively. In contrast, 5-HTP, injected intraperitoneally, showed an even distribution in the brain (figs. 2 and 3). Histochemical studies on reserpine-pretreated rats showed that the 5-HTP and dopa accumulating after decarboxylase inhibition were located in nerve cell bodies and nerve terminals in areas normally containing 5-HT and dopamine (DA), respectively. No accumulation of dopa in noradrenaline (NA) cell bodies and terminals could be detected histochemically, but biochemical data indicate that such accumulation does occur, although in lower concentration than in dopamine regions.

From these data it can be concluded that the 5-HTP 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-HT and catecholamines, respectively.

During an initial phase of about 30 min after the administration of a decar-

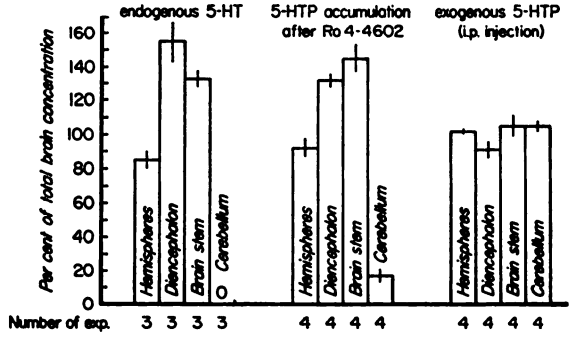

FIG. 2. Distribution of 5-HT and 5-HTP in rat brain (6).

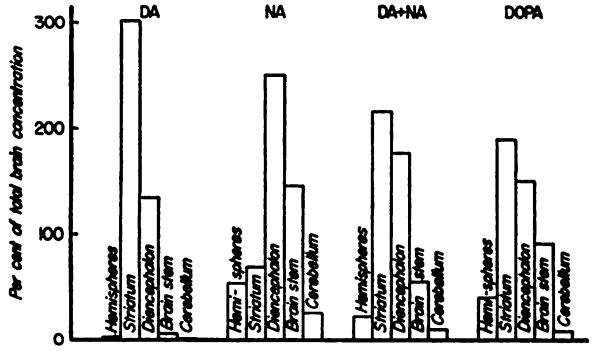

FIG. 3. Distribution of catecholamines and dopa in rat brain 30 min after NSD 1015, 100 mg/kg intraperitoneally (8).

boxylase inhibitor the accumulations of 5-HTP 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 rate of hydroxylation of tryptophan and tyrosine, respectively. However, this problem requires further investiation. Assuming that the initial accumulation represents the true rate of synthesis, turnover times of 1.6 hr and 3 to 5 hr are obtained for 5-HT 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 (*e.g.*, see 14).

It is generally assumed that tryptophan hydroxylase is normally not saturated with its substrate. We therefore investigated the accumulation of 5-HTP in mouse brain after decarboxylase inhibition with or without loading with L-tryptophan in varying dosage. The relationship between tryptophan hydroxylation and substrate concentration thus obtained is shown in figure 5A. Figure 5B gives the same data presented as double reciprocal plots. From the average of the two regression lines of figure 5B, *i.e.*, that of x against y and that of y against x, an apparent $K_m = 6 \times 10^{-5}$ M is calculated, assuming that the tryptophan concentration in the liquid phase surrounding the tryptophan hydroxylase is equal to that of the whole brain. This K_m is somewhat higher than that obtained by Ichiyama *et al.* (15), *i.e.*, 2×10^{-5} M, but lower than that given by Lovenberg *et al.* (20), *i.e.*, 3×10^{-4} M. In both these cases a partially purified tryptophan hydroxylase obtained from mammalian brain was investigated.

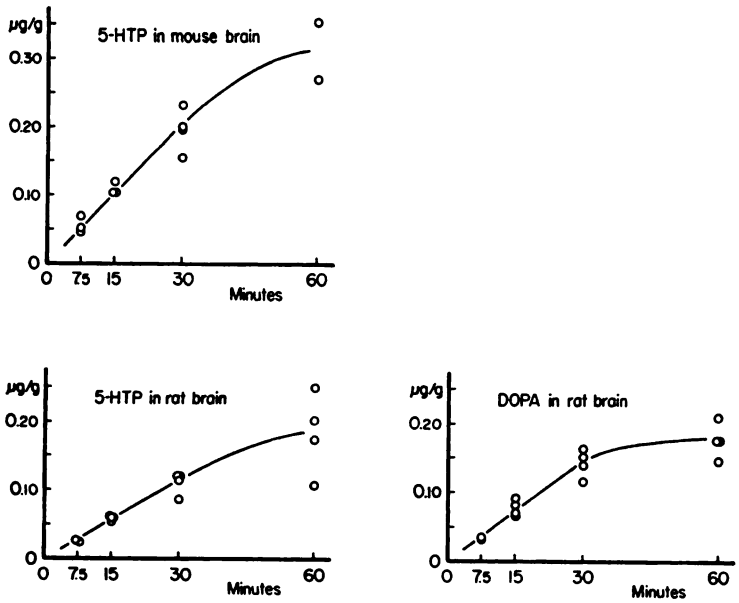

FIG. 4. Accumulation of 5-HTP and dopa after decarboxylase inhibition with NSD 1015, 100 mg/kg intraperitoneally (8).

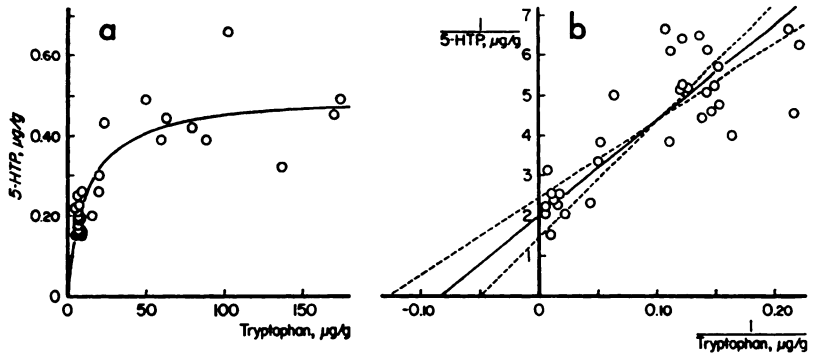

FIG. 5a. The effect of tryptophan concentration on the rate of 5-HTP accumulation in mouse brain (10). The animals were given intraperitoneal injections of Ro 4-4602, 800 mg/kg and killed 30 min later. Some of the animals received an intraperitoneal injection of L-tryptophan (100, 300 or 1000 mg/kg) 30 min before Ro 4-4602. Each point represents 5-HTP and tryptophan values (μg/g) obtained from single samples of 4 to 12 pooled mouse brains.

b. Double reciprocal plot of the same relationship. The solid line of figure 4B is the average of the broken lines, representing the regression of x upon y and y upon x, respectively. The curve of figure 5A was calculated from the solid line of figure 5B.

The Acute Effect of Axotomy on Monoamine Synthesis

A. Spinal cord 5-HT. All spinal monoamine pathways are descendent, and thus transection of the spinal cord will interrupt the connection between cell bodies and nerve terminals below but not above the lesion. This should result in immediate loss of impulse flow in the monoamine 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 to 6 hr. During this interval there is probably no loss of synthesizing enzymes, and the levels of 5-HT and noradrenaline are not yet reduced, and perhaps slightly elevated (*cf.* 18). Nevertheless, we found that the accumulation of 5-HTP after decarboxylase inhibition was reduced below the lesion as compared to the concentration observed above the lesion. Part of the experiments were done with NSD 1015, part with Ro 4-4602. In the latter case the tryptophan level was elevated by two injections of L-tryptophan, but the different treatment schedules did not seem to influence the main result, namely a relative reduction of 5-HTP below the lesion (tables 1 and 2).

Figure 6 summarizes the spinal data, the 5-HTP below the lesion being plotted against the 5-HTP above the lesion. The slope of the regression line obtained from transected animals is clearly below that of unoperated and sham operated controls. The reduction induced by the transection is almost 50%.

The fact that the tryptophan hydroxylase activity, when measured by the present procedure *in vivo*, was equally reduced by the lesion at a low and a high degree of saturation with substrate, suggests that the change of the enzyme involves the V_{max} rather than K_m. This invites the speculation that in the resting state part of the enzyme is bound in an inactive form. The impulse flow may then

TABLE 1

*Indoles in rat spinal cord 1 to 2 hr after transection (n = 3) or in non-transected controls (n = 6). All animals treated with NSD 1015, 100 mg/kg, 1 hr before death**

	Tryptophan		5-HTP		5-HT		5-HIAA	
	Transected	Control	Transected	Control	Transected	Control	Transected	Control
	μg/g		ng/g		ng/g		ng/g	
Upper part†	4.30 ±0.551	3.75 ±0.308	103 ±20	113 ±14	282 ±40	322 ±12	66 ±24	69 ±13
Lower part†	4.17 ±0.406	3.87 ±0.392	93 ±12	166 ±15	688 ±58	621 ±34	72 ±10	99 ±14
Ratio U/L‡	1.00 0.91–1.17	0.99 0.66–1.36	1.09 0.89–1.37	0.69 0.53–0.80	0.40 0.37–0.45	0.51 0.48–0.59	1.17 0.37–1.22	0.89 0.24–1.00

* Unpublished data of Carlsson, Lindqvist and Magnusson.
† Mean ± S.E.M.
‡ Median and range.

TABLE 2

*Indoles in rat spinal cord 6 hr after transection (n = 3) or in non-transected controls (n = 4). All animals treated with Ro 4-4602, 800 mg/kg, 2 hr + L-tryptophan, 300 + 150 mg/kg, 3 and 1½ hr before death**

	Tryptophan		5-HTP		5-HT		5-HIAA	
	Transected	Control	Transected	Control	Transected	Control	Transected	Control
	μg/g		ng/g		ng/g		ng/g	
Upper part†	43.4 ±6.93	53.8 ±6.67	454 ±132	345 ±99	220 ±24	224 ±15	131 ±22	100 ±14
Lower part†	44.5 ±6.89	57.8 ±9.64	337 ±95	471 ±118	580 ±80	461 ±12	143 ±18	139 ±17
Ratio U/L‡	0.96 0.96–1.01	0.96 0.83–1.06	1.26 1.19–1.57	0.72 0.64–0.79	0.39 0.33–0.43	0.49 0.40–0.57	0.88 0.81–1.04	0.72 0.69–0.75

* Unpublished data of Carlsson, Lindqvist and Magnusson.
† Mean ± S.E.M.
‡ Median and range.

induce release of the bound enzyme into its free and active form. Alternatively, such changes may involve a cofactor rather than the enzyme.

Figure 7 shows that transection of the spinal cord causes an almost 50% reduction of the 5-HIAA concentration below the lesion. In this figure the 5-HIAA concentrations below the lesion were plotted against the 5-HIAA concentrations above the lesion which did not change significantly. No decarboxylase inhibitor was given in these experiments.

Three days after transection of the spinal cord the 5-HTP recovered from the lower part 1 hr after NSD 1015 was only 10 to 15% of the value found in non-transected animals; no difference was found in the upper part.

B. Cerebral 5-HT. The results obtained after a cerebral hemisection, discon-

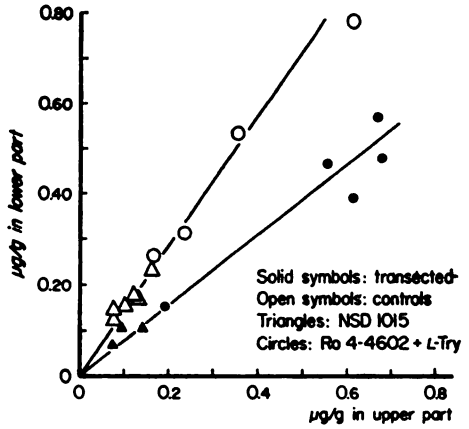

FIG. 6. 5-HTP concentration in lower *versus* upper part of rat spinal cord 1 to 6 hr after transection, as compared to non-transected controls. All animals were treated with decarboxylase inhibitors, the same data as in tables 1 and 2 (12).

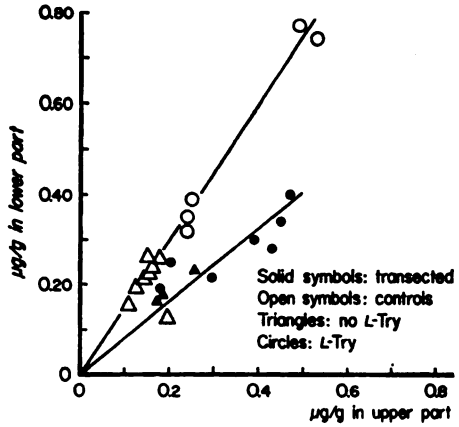

FIG. 7. 5-HIAA concentration in lower *versus* upper part of rat spinal cord 1 to 6 hr after transection, as compared to non-transected controls. Some of the animals were treated with L-tryptophan as described in table 2. No decarboxylase inhibitors were given (12).

necting the forebrain monoamine nerve terminals from their cell bodies in the lower brainstem, were not entirely in agreement with the spinal data. The lesion caused only a slight decrease in the 5-HTP accumulating after decarboxylase inhibition, statistical significance being achieved only in part of the experiments. No difference between lesioned and unlesioned side in 5-HIAA levels (with or without decarboxylase inhibition) was generally observed, in contrast to the findings after spinal transection.

With Ro 4-4602 for decarboxylase inhibition, we have three different experimental series, with L-tryptophan loading in two of them (7). In none of them was a significant effect of the lesion on 5-HTP accumulation observed, even though

the levels tended to be lower on the lesioned side. In contrast, the disappearance of 5-HT induced by the decarboxylase inhibitor, was significantly less rapid on the lesioned side, in agreement with earlier data of Andén *et al.* (3), who studied the 5-HT disappearance rate in the transected spinal cord after inhibition of tryptophan hydroxylase. This discrepancy between 5-HPT and 5-HT data may be explained by the occurrence of a small pool of cytoplasmic 5-HT undergoing rapid deamination intraneuronally (see below). The data of two of the experimental series, with Ro 4-4602, are summarized in table 3 (for further details see ref. 7).

In two additional experimental series we have used NSD 1015 for decarboxylase inhibition. The experimental conditions differed from the previous ones also in certain other respects. The intervals between hemisection and sacrifice was lower (60 or 90 min instead of 3 to 6 hr), and so was the interval between the administration of the inhibitor and sacrifice (30 min instead of 2 hr). Furthermore, ether instead of Nembutal anesthesia was used. With NSD 1015 a slight though significant decrease (15%) in 5-HTP on the lesioned as compared to the unlesioned side was observed (table 4).

TABLE 3

*Indoles in forebrain, 3 hr after left cerebral hemisection; animals were treated with Ro 4-4602, alone or in combination with L-tryptophan (n = 4)**

	Hemisection 3 hr			Hemisection 3 hr + L-Tryptophan		
	Left	Right	P	Left	Right	P
Try, μg/g	13.8 ± 0.9	9.1 ± 0.7	<.005	119.3 ± 5.0	83.8 ± 6.3	<.005
5-HTP, ng/g	340 ± 20	410 ± 60	N.S.	590 ± 70	620 ± 50	N.S.†
5-HT, ng/g	350 ± 30	260 ± 10	<.005	360 ± 10	270 ± 10	<.005
5-HIAA, ng/g	240 ± 20	230 ± 20	N.S.	360 ± 30	300 ± 10	N.S.

* Bédard, Carlsson and Lindqvist, 1972 (7).

† Significantly higher than after hemisection alone (P < .025).

TABLE 4

*Indoles in rat forebrain 30 or 90 min after left cerebral hemisection; NSD 1015 100 mg/kg given intraperitoneally 30 min before death (n = 4)**

	Tryptophan	5-HTP	5-HT	5-HIAA
	μg/g	ng/g	ng/g	ng/g
30 min				
Left	7.2 ± 0.4	88 ± 6	351 ± 23	218 ± 26
Right	5.8 ± 0.3	103 ± 13	334 ± 12	221 ± 22
P: left-right	N.S.	<.01†	N.S.	N.S.
90 min				
Left	9.0 ± 1.3	78 ± 9	295 ± 11	280 ± 37
Right	6.2 ± 0.4	93 ± 10	280 ± 27	293 ± 26
P: left-right	<.01	<.01†	N.S.	N.S.

* Unpublished data of Carlsson, Kehr, Lindqvist, Magnusson and Atack.

† Statistical treatment of pooled 30 and 90 min groups.

In another experimental series the effect of hemisection on the accumulation of 5-HIAA induced by probenecid (200 mg/kg) was studied, in this case after L-tryptophan loading. A slight but significant reduction (20%) of the 5-HIAA level on the lesioned side, as compared to the unlesioned side was found (7).

The tryptophan level was always elevated on the lesioned side. This might counteract the decrease in tryptophan hydroxylase activity induced by the axotomy. However, the difference in 5-HTP between the two sides did not become larger after saturating the hydroxylase by means of L-tryptophan loading.

From these hemisection studies we conclude that cutting the ascending 5-HT axons probably results in a slight reduction (about 15%) in the rate of hydroxylation of tryptophan in the forebrain terminal system. Thus, even in the probable absence of an impulse flow the synthesis of 5-HTP (and 5-HT) appears to continue at an almost normal rate in this system. Since very little release of 5-HT probably occurs under these conditions, it must be assumed that the main part of 5-HT formed will be deaminated intraneuronally. The impulse flow will cause an increase in the relase and extraneuronal metabolism of 5-HT. This in turn might be expected to reduce the degree of saturation of the intraneuronal storage granules, followed by increased uptake from the cytoplasm and reduced intraneuronal metabolism. A regulatory mechanism involving monoamine oxidase may possibly contribute to this shift. Since more 5-HT may be synthesized than is drawn from the stores by the nerve impulses, the synthesis and the depletion of the store after synthesis inhibition may vary independently of each other.

C. Cerebral catecholamines. NSD 1015, 100 mg/kg, was injected intraperitoneally immediately or 60 min after a cerebral hemisection on the left side, performed under ether anesthesia. The animals were killed 30 min after the injection. Table 5 (same animals as in table 4) shows the levels of dopa, DA and NA in the forebrain. In the animals receiving the inhibitor immediately after the hemisection the accumulation of L-dopa was considerably increased on the lesioned side. In the animals treated with the inhibitor 60 min after the lesion, only a slight and insignificant increase in L-dopa was seen on the lesioned side.

TABLE 5

*Dopa, dopamine (DA) and noradrenaline (NA) in rat forebrain (ng/g) 30 or 90 min after left cerebral hemisection; NSD 1015 100 mg/kg given intraperitoneally 30 min before death (n = 4)**

	Dopa	DA	NA
30 min			
Left	554 ± 50	1632 ± 63	308 ± 15
Right	195 ± 27	1416 ± 57	394 ± 29
P: left-right	<.001	<.05	<.001
90 min			
Left	391 ± 35	2267 ± 134	370 ± 80
Right	340 ± 30	1382 ± 77	437 ± 31
P: left-right	N.S.	<.005	N.S.

* Unpublished data of Carlsson, Kehr, Lindqvist, Magnusson and Atack.

TABLE 6

*Dopa, dopamine (DA), and noradrenaline (NA) in rat occipitotemporal cortex (ng/g) 30 or 90 min after left cerebral hemisection; NSD 1015 100 mg/kg given intraperitoneally 30 min before death (n = 4)**

	Dopa	DA	NA
30 min			
Left	23 ± 9	1 ± 3	156 ± 32
Right	15 ± 8	4 ± 4	192 ± 25
P: left-right	N.S.	N.S.	<.025
90 min			
Left	36 ± 6	34 ± 13	149 ± 39
Right	35 ± 5	19 ± 7	165 ± 14
P: left-right	N.S.	N.S.	N.S.

* Unpublished data of Carlsson, Kehr, Lindqvist, Magnusson and Atack.

However, there was instead a considerable increase in DA on the lesioned side. This difference was much smaller in the former group of animals, in which the synthesis of DA was inhibited shortly after the lesion.

These data indicate that cutting the ascendent DA fibers results in an increased rate of tyrosine hydroxylation in the cerebral DA terminal system. This increase is very marked initially, but when the DA has increased to a certain level the synthesis appears to slow down again. The remarkably rapid increase in forebrain DA after cerebral hemisection has recently been reported by Andén *et al.* (1).

In the experiment described above, we also analyzed the occipitotemporal cortex, *i.e.*, the part located caudally to the hemisection. Also in this part the terminal monoamine systems are disconnected from their cell bodies by the lesion (*cf.* 7). The predominating catecholamine in this region is NA, and thus the dopa accumulating here after decarboxylase inhibition is probably synthesized mainly in NA nerve terminals. In this region the dopa level was about the same on the two sides, indicating that axotomy had no marked influence on the rate of tyrosine hydroxylation in the NA nerve terminals (table 6, same animals as in tables 4 and 5). Remarkably enough, the NA level was lower at the side of the lesion.

Discussion

Various regulatory mechanisms have been proposed to control the synthesis of monoamine transmitters. In the case of NA, feedback inhibition of tyrosine hydroxylase by the products dopa, DA or NA has been suggested to play a major regulatory role (for review, see 14).

In the present investigation we have found that after decarboxylase inhibition dopa accumulates in rat brain at an approximately constant rate during the first 30 min, reaching levels which are more than 10% of the total catecholamine levels (DA + NA) occurring in rat brain. It is hardly probable that as much as 10% of the catecholamines can occur free in the cytoplasm in the normal brain,

owing to the high activity of the monoamine oxidase and the granular storage mechanism. Against such an assumption is the virtually complete disappearance of the catecholamines after the blockade of the granular storage mechanism by reserpine. The different normally occurring catechol derivatives appear to be roughly equipotent as tyrosine hydroxylase inhibitors (16, 22). Taken together, these facts argue against the hypothesis that dopa, DA or NA should cause any significant feedback inhibition of tyrosine hydroxylase under normal conditions. However, after inhibition of monoamine oxidase the situation may be different. Twelve years ago we presented evidence that the catecholamine synthesis in brain slows down considerably some time after injection of a monoamine oxidase inhibitor, when the catecholamine levels were considerably increased (11), and similar observations have later been reported by many others. The possibility remains that in this case we are dealing with a direct product inhibition of the tyrosine hydroxylase.

Nine years ago we proposed an entirely different mechanism of feedback regulation (9). We presented evidence that catecholamine release and metabolism is stimulated by chlorpromazine and haloperidol and from the unchanged catecholamine level we inferred that also catecholamine synthesis is stimulated by these drugs. We suggested that the effects observed were due to a receptor blockade by these agents. In other words, according to this hypothesis a feedback control of catecholamine neurons is exerted *via* the degree of receptor activation. Since then numerous observations have been published, supporting such a mechanism (for review, see 2).

Some of the present observations appear to be most easily explained on the basis of this hypothesis. We found that cutting the DA axons by means of a cerebral hemisection markedly increased the rate of tyrosine hydroxylation in the forebrain. This activation cannot be due to release of a direct end-product inhibition, because the axotomy actually raised the DA level. A more reasonable explanation appears to be that the interruption of the impulse flow leads to diminished release of DA into the synaptic cleft and to a depletion of DA at the receptor sites. The lowered receptor activation somehow gives rise to feedback information causing an increased rate of tyrosine hydroxylation, analogous to the observations on chlorpromazine and haloperidol quoted above.

The mechanism involved in this hypothetical feedback regulation remains obscure. The receptors involved might be located either pre- or postsynaptically. In the latter case the feedback information must be assumed to cross the synaptic cleft, perhaps in the shape of a chemical messenger. In the former case a simpler mechanism would be possible. For example, diminished receptor activation might directly cause release of bound and inactive tyrosine hydroxylase. It should be pointed out that for obvious reasons the feedback information is probably not mediated *via* the impulse flow in this case.

The fact that the rate of tyrosine hydroxylation appeared to slow down again when the DA level was elevated, might be due to a direct end-product inhibition. However, further work is needed to clarify this problem.

While the DA terminal system in the forebrain appeared to respond to axotomy

by an increased transmitter synthesis, other terminal systems showed entirely different reactions. In the NA system of the occipitotemporal cortex no significant change in tyrosine hydroxylation rate was found. Paradoxically, the NA level was reduced, which should mean an increased release and metabolism of NA, since the change occurred when the synthesis was blocked. This may invite the speculation that the lesion caused increased firing of a fiber system making excitatory axo-axonal synaptic contacts with the NA terminals. The lesions caused a decreased rate of tryptophan hydroxylation in the 5-HT terminal systems, the effect being much less marked in the forebrain than in the spinal cord.

These observations suggest that the synthesis of monoamine transmitters is not controlled by a single regulatory mechanism, but perhaps rather by a complex system, whose different components may even work in opposite directions, balancing each other. The data suggest that the relative importance of these components may vary in different neuronal systems.

In the present investigation axotomy of monoamine-carrying fiber systems was performed by means of a spinal transection or a frontal cerebral hemisection. The advantage of these non-selective lesions is that a high degree of completeness is obtained. In our series of investigations completeness has been checked by following the depletion of monoamines after chronic lesions (1, 7, 21). In the spinal cord the depletion is virtually complete. This is true also of the forebrain DA after cerebral hemisection, while the depletion of forebrain NA and 5-HT approaches 90 %. The obvious disadvantage with non-selective lesions is the risk that the monoamine metabolism may be influenced by cutting other fiber systems and perhaps also non-nervous structures. In fact certain changes observed in the present studies are probably due to damage of structures other than the monoamine fiber systems. For example, an elevation of 5-HIAA in the brain was observed equally on both sides after hemisection, and certain observations indicated that this might be due to a retarded efflux of the acid out of the brain. Such a retardation has recently been demonstrated in the present laboratory: transection of the spinal cord caused a marked retardation of the pargyline-induced disappearance of 5-HIAA below as well as above the lesion (5). However, this change was seen 24 but not 3 hr after the lesion.

Another change induced by the cerebral hemisection is probably unrelated to the cutting of monoamine fibers: an increase in tryptophan levels on the side of the lesion. This increase was observed also after chronic lesions leading to degeneration of the monoamine fiber systems. The difference between the two sides was still marked after L-tryptophan loading, leading to considerably increased tryptophan levels (7). It cannot be decided as yet whether the lesion caused an increased tryptophan net uptake or an inhibition of its metabolism. The tyrosine levels seem to undergo similar changes (unpublished data), suggesting a more generalized change of amino acid uptake or metabolism. It is tempting to speculate that this apparent change in amino acid uptake or metabolism, induced by a cerebral lesion, is analogous to the stimulation of DA synthesis discussed above. In other words, the lesion might lead to a reduced concentration of a hypothetical

amino acid or peptide transmitter in the synaptic cleft, resulting in a feedback-induced stimulation of amino acid uptake or inhibition of its metabolic breakdown. Our data do not exclude the possibility that also in monoamine nerve terminals the amino acids may be influenced by axotomy, leading to increased monoamine synthesis.

Conclusion

Cerebral or spinal lesions, involving monoamine-carrying neuronal pathways, leads to complex immediate changes in the monoamine metabolism, with considerable variation between the different terminal systems. In the present investigation emphasis has been placed on the first step in the monoamine synthesis, *i.e.*, the hydroxylation of tyrosine or tryptophan. This step has been investigated by following the accumulation of dopa and 5-HTP, induced by the administration of a decarboxylase inhibitor. Axotomy was found to cause (a) an increase in the rate of tyrosine hydroxylation in the forebrain where DA is the predominating catecholamine, (b) no significant change in the tyrosine hydroxylation in cortical tissue where NA predominates, and (c) a decrease in the rate of tryptophan hydroxylation, the effect being more pronounced in the spinal cord than in the forebrain.

The data do not support the view that direct product inhibition of the tyrosine hydroxylase plays an important regulatory role in central catecholamine neurons *in vivo*. Instead, a feedback mechanism operating *via* the degree of receptor activation in the synaptic cleft may control the synthesis. It appears, however, that different mechanisms are involved in this control, occurring in varying proportions in different terminal systems.

REFERENCES

1. Andén, N.-E., Bédard, P., Fuxe, K. and Ungerstedt, U.: Early and selective increase in brain dopamine levels after axotomy. Experientia (Basel), 28: 300–302, 1972.
2. Andén, N.-E., Carlsson, A. and Häggendal, J.: Adrenergic mechanisms. Annu. Rev. Pharmacol. 9: 119–134, 1969.
3. Andén, N.-E., Fuxe, K. and Hökfelt, T.: The importance of nerve impulse flow for the depletion of the monoamines from central neurons by some drugs. J. Pharm. Pharmacol. 18: 630–632, 1966.
4. Atack, C. V. and Magnusson, T.: Individual elution of noradrenaline (together with adrenaline), dopamine, 5-hydroxytryptamine and histamine from a single, strong cation exchange column by means of mineral acid-organic solvent mixtures. J. Pharm. Pharmacol. 22: 625–627, 1970.
5. Atack, C. V and Modigh, K.: To be published.
6. Bédard, P., Carlsson, A., Fuxe, K. and Lindqvist, M.: Origin of 5-hydroxytryptophan and L-DOPA accumulating in brain following decarboxylase inhibition. Naunyn-Schmiedebergs Arch. Pharmakol. Exp. Pathol. 269: 1–6, 1971.
7. Bédard, P., Carlsson, A. and Lindqvist, M.: Effect of a transverse cerebral hemisection on 5-hydroxytryptamine metabolism in the rat brain. Naunyn-Schmiedebergs Arch. Pharmakol. Exp. Pathol. 272: 1–15, 1972.
8. Carlsson, A., Kehr, W., Lindqvist, M., Magnusson, T. and Atack, C. V.: To be published.
9. Carlsson, A. and Lindqvist, M.: Effect of chlorpromazine or haloperidol on formation of 3-methoxytyramine and normetanephrine in mouse brain. Acta Pharmacol. Toxicol. 20: 140–144, 1963.
10. Carlsson, A. and Lindqvist, M.: To be published.
11. Carlsson, A., Lindqvist, M. and Magnusson, T.: On the biochemistry and possible functions of dopamine and noradrenaline in brain. *In* Adrenergic Mechanisms, ed. by J. R. Vane, G. E. W. Wolstenholme, and M. O'Connor, pp. 432–439, Churchill, London, 1960.
12. Carlsson, A., Lindqvist, M. and Magnusson, T.: To be published.
13. Carlsson, A., Lindqvist, M. and Waldeck, B.: Mechanism of release of α-methylated noradrenaline analogues by monoamine oxidase inhibitors. Eur. J. Pharmacol. 3: 34–39, 1968.
14. Costa, E.: Simple neuronal models to estimate turnover rate of noradrenergic transmitters *in vivo*. *In* Biochemistry of simple neuronal models, ed. by E. Costa and E. Giacobini, Advan. Biochem. Psychopharmacol. 2: 169–204, 1970.

15. Ichiyama, A., Nakamura, S., Nishizuka, Y. and Hayaishi, O.: Tryptophan-5-hydroxylase in mammalian brain. Advan. Pharmacol. 6A: 5–17, 1968.
16. Ikeda, M., Fahien, L. A. and Udenfriend, S.: A kinetic study of bovine adrenal tyrosine hydroxylase. J. Biol. Chem. 241: 4452–4456, 1966.
17. Kehr, W., Carlsson, A. and Lindqvist, M.: To be published.
18. Kumar, M. J., Roth, R. H. and Aghajanian, G. K.: Selective reduction of tryptophan hydroxylase activity in rat forebrain after midbrain raphe lesions. Brain Res. 35: 167–176, 1971.
19. Lindqvist, M.: Quantitative estimation of 5-hydroxy-3-indole acetic acid and 5-hydroxytryptophan in the brain following isolation by means of a strong cation exchange column. Acta Pharmacol. Toxicol. 29: 303–313, 1971.
20. Lovenberg, W., Jequier, E. and Sjoerdsma, A.: Tryptophan hydroxylation in mammalian systems. Advan. Pharmacol. 6A: 21–36, 1968.
21. Magnusson, T.: To be published.
22. Udenfriend, S.: Tyrosine hydroxylase. Pharmacol. Rev. 18: 43–51, 1966.