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PHENELZINE-INDUCED CONVULSIONS AND ALTERATIONS IN THE CONVERSION OF 5-HTP-^{14}C TO SEROTONIN-^{14}C *IN VIVO*

AARON FELDSTEIN and CAROL M. SIDEL

The Worcester Foundation for Experimental Biology, Shrewsbury, Mass, U.S.A.

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Abstract—A method was developed for the analyses of 5-hydroxytrptophan (5-HTP), serotonin, 5-hydroxyindoleacetic acid, and neutrals in rat brain after intraperitoneal injection of 5-HTP-^{14}C. Phenelzine altered the metabolism of 5-HTP-^{14}C by inhibition of monoamine oxidase and decarboxylase. The occurrence of convulsions as the dose of phenelzine was increased did not correlate with decarboxylase inhibition.

THE convulsive hydrazides (e.g. semicarbazid, thiosemicarbazid, isoniazid) have been shown to lower brain levels of pyridoxal phosphate,[1] the coenzyme of a number of decarboxylases and transaminases as well as other enzymes.[2, 3] The decreased activity of these enzymes, owing to pyridoxal phosphate depletion and the concomitant alteration of their respective substrates, may increase neuronal excitability, thereby facilitating convulsions.[4]

It has been suggested that γ-aminobutyric acid (GABA) is an inhibitor of neuronal excitability.[5] Further, it has been suggested that the hydrazides induced convulsions by inhibiting the pyridoxal phosphate-linked glutamic acid decarboxylase involved in the synthesis of GABA, thereby lowering its concentration at the functional sites in the brain.[6, 7] There is a possibilty that serotonin, like GABA, functions as an inhibitor of neuronal excitability[8] and that the hydrazides and hydrazines induced convulsions by inhibiting the pyridoxal phosphate-linked 5-hydroxytryptophan (5-HTP) decarboxylase, there by lowering the concentrations of serotonin at the functional sites.

In this report we examine the effect of convulsive doses of phenelzine on the conversion of tracer amounts of DL-5-hydroxytryptophan-^{14}C (5-HTP-^{14}C) to serotonin-^{14}C and 5-hydroxyindoleacetic acid-^{14}C (5-HIAA) and 5-HTP decarboxylase as well as monoamine oxidase (MAO) *in vivo* in rat brain.

EXPERIMENTAL

Materials. Male rats, Sprague–Dawley descendants, 140 to 165 g, obtained from Gofmoor Farms, were used. DL-5-HTP-3-^{14},C specific activity 2·50 mc/mmole, was purchased from New England Nuclear Corp. 5-Hydroxytryptamine-3′-^{14}C creatinine sulfate, specific activity 11·4 mc/mmole, was purchased from Nuclear-Chicago Corp. Phenelzine sulfate (Nardil) was generously donated by Dr. E. A. DeFelice of Warner-Lambert Research Institute; reported dosages are for the sulfate salt. Pyridoxine

hydrochloride was purchased from Calbiochem Inc.; reported dosages are for the salt.

Methods. For biochemical experiments, the rat was injected intraperitoneally with phenelzine sulfate dissolved in 1·75–2·05 ml of physiological saline. Thirty min after the phenelzine, the rat was injected with 25 μ (2·2 mg) of DL-5-HTP-^{14}C. Thirty min after the 5-HTP-^{14}C injection, the rat was sacrificed by decapitation. The brain was removed, rinsed in cold saline, blotted dry, and weighed. A 20% homogenate was prepared in cold 0·3 N hydrochloric acid. A Vir-Tis model 23, with a 30-ml flask immersed in an ice-salt bath operated for 2 min at rheostat setting 50, was used for homogenization. An aliquot of the acidified homogenate was taken for analysis of 5-HTP-^{14}C, serotonin-^{14}C, neutral-^{14}C, and 5-HIAA-^{14}C. The extraction procedure was basically the same as that previously described[9] for liver homogenates but modified for brain tissue. The results are summarized in Table 1.

For the convulsion study, the rat was injected intraperitoneally with phenelzine sulfate and observed for changes in behavior over a 2-hr period from the time of injection. Pyridoxine hydrochloride, 50 or 100 mg/kg, dissolved in 1·75–2·05 ml of physiological saline, was administered intraperitoneally 15 min prior to phenelzine and the rats observed for 2 hr after the phenelzine injection.

RESULTS

Ten rats received an intraperitoneal injection of 25 μ (2·20 mg) of DL-5-HTP-^{14}C and sacrificed 30 min later; this group served as control (Tables 1, 3). Uptake of ^{14}C by brain was quite low, only 0·038% (less than 1 μg) of the administered 5-HTP-^{14}C. The levels of 5-HTP-^{14}C and its metabolites were reported as per cent of ^{14}C found in the brain. 5-HTP-^{14}C unmetabolized constituted 37% of the brain radioactivity. Only 5% of the ^{14}C consisted of serotonin-^{14}C, which indicated a fairly rapid rate of metabolism by MAO. The neutral fraction, 1%, was almost negligible, which indicated that 5-hydroxindoleacetaldehyde was efficiently metabolized by aldehyde dehydrogenase. The major product of 5-HTP-^{14}C metabolism was 5-HIAA-^{14}C, constituting 57% of the ^{14}C in the brain.

The metabolism of 5-HTP-^{14}C was altered by varying doses of phenelzine injection 30 min prior to 5-HTP-^{14}C. The results are shown in Table 1. The uptake of total ^{14}C by brain progressively increased to twice the control value as the dose of phenelzine increased from 0 to 200 mg/kg. The correlation coefficients between increased uptake and MAO inhibition or decarboxylase inhibition were low and not significant. The 5-HTP-^{14}C levels showed no significant change from 0 to 50 mg/kg and then sharply rose between 50 and 200 mg phenelzine/kg indicating decarboxylase inhbition, 92% (range, 76–98%) at 200 mg/kg. Serotonin-^{14}C levels rose sharply from 5% to 54% as the dose of phenelzine was increased from 0 to 50 mg/kg, a rise which corresponded to increased MAO inhibition, 86% (range, 81–89%) at the 50 mg/kg dose. The serotonin-^{14}C then progressively declined from 54% to 8% as the dose of phenelzine increased from 50 to 200 mg/kg; the decline in serotonin-^{14}C levels in the presence of a MAO block corresponded to the increased intensity of decarboxylase inhibition. The 5-HIAA-^{14}C levels showed a continuous decline from 57% to 0% over the entire phenelzine dose range of 0 to 200 mg/kg. This was mainly due to inhibition which reached a high of 95% (range, 92–97%) at 100 mg/kg; decarboxylase inhibition may

TABLE 1. THE METABOLISM OF 5-HTP-^{14}C IN RAT BRAIN AS A FUNCTION OF PHENELZINE DOSE

Brain metabolism†	Phenelzine sulfate (mg/kg)						
	0	10	25	50	100	150	200
	Per cent of total ^{14}C						
^{14}C Levels	$0{\cdot}038_a$	$0{\cdot}045_{ab}$	$0{\cdot}039_{ab}$	$0{\cdot}048_{ab}$	$0{\cdot}059_{bc}$	$0{\cdot}078_c$	$0{\cdot}071_c$
5-HTP Levels	37_a	35_a	39_a	40_a	77_b	93_c	95_c
Serotonin levels	5_a	34_b	54_c	54_c	27_d	12_e	8_{ae}
Neutral levels	1_a	1_a	1_a	1_a	0_a	0_a	0_a
5-HIAA Formed	57_a	34_b	13_c	7_d	1_e	2_e	0_e
	Per cent enzyme inhibition						
Decarboxylase	0_a	-3_a	3_a	5_a	64_b	89_c	92_c
MAO	0_a	44_b	80_c	86_d	95_e	*	*
Aldehyde dehydrogenase	0_a	3_a	*	*	*	*	*

† Mean values in the same row with a subscript letter in common do not differ significantly ($P > 0{\cdot}05$) as determined by analysis of variance and Duncan's multiple range test.[33]
* MAO and decarboxylase inhibition too intense for an accurate determination.

have contributed in a small degree to the 5-HIAA-^{14}C decline at the higher phenelzine doses.

Convulsions were not observed at 150 mg phenelzine/kg (Table 2). A third of the animals convulsed at 200 mg/kg and 100% at 250 or 300 mg/kg. The time of onset of convulsions showed a distinct trend toward a shorter time as the dose was increased.

Deaths did not occur at 150 mg phenelzine/kg during the 120-min observation period (Table 2). At 200 mg/kg only a sixth of the rats died; but at 250 and 300 mg/kg, 100% died. Again there was a significant trend toward death at shorter periods of time as the dose of phenelzine increased. A 120-min period was selected as the cutoff point for observations of convulsions and deaths.

TABLE 2. CONVULSIONS AND DEATH INDUCED BY PHENELZINE AS A FUNCTION OF DOSE*

Drug	Phenelzine sulfate dose (mg/kg)							
	150		200		250		300	
Toxicity	Time (min)	No. of rats	Time (min)	No. of rats	Time (min)	No. of rats)	Time (min)	No. of rats
Onset of convulsions	120_a	0/6	92_b	2/6	33_b	6/6	25_c	6/6
Death	120_a	0/6	109_{ab}	1/6	88_b	6/6	53_c	6/6

* The cutoff time for observations of onset of convulsions and death after phenelzine administration was 120 min. Mean values in the same row containing a subscript letter in common do not differ significantly ($P > 0{\cdot}05$) as determined by analysis of variance and Duncan's multiple comparisons of means.[33] Pyridoxine hydrochloride blocked convulsions and death during the 120 minutes.

TABLE 3. THE METABOLISM OF 5-HTP-^{14}C, AND CONVULSIONS AND DEATH IN RATS AS A FUNCTION OF PHENELZINE DOSE; ANALYSIS OF VARIANCE*

Statistics	Source of variance: Between	Within	Total
	^{14}C Levels		
Degrees of freedom	6	27	33
Mean square	0·00119550	0·00016937	
F	7·05 (P<0·01)		
	5-HTP-^{14}C (decarboxylase inhibition)		
Degrees of freedom	6	27	33
Mean square	3,489·31	23·07	653·30
F	151·00 (P<0·001)		
	Serotonin-^{14}C levels		
Degrees of freedom	6	27	33
Mean square	2,122·64	15·80	398·86
F	134·34 (P<0·001)		
	Neutral-^{14}C and 5-HIAA-^{14}C		
Degrees of freedom	6	27	33
Mean Square	3,324·32	9·76	612·41
F	340·60 (P< 0·001)		
MAO Inhibition (conversion of serotonin-^{14}C to neutral-^{14}C and 5-HIAA-^{14}C)			
Degrees of Freedom	4	21	25
Mean square	8,762·77	8·61	1,409·28
F	1,017·70 (P<0·001)		
Aldehyde dehydrogenase inhibition (conversion of neutral-^{14}C to 5-HIAA-^{14}C			
Degrees of freedom	12		
t	1·88		
P	not signif.		
	Onset time of convulsions		
Degrees of freedom	3	20	23
Mean square	12,625·26	487·25	2,070·47
F	25·91 (P<0·01)		
	Time of death		
Degrees of freedom	3	20	23
Mean Square	5,117·70	433·34	10,44·34
F	11·80 (P< 0·01)		

* See Tables 1 and 2.

DISCUSSION

A method was developed for the analysis of 5-HTP-^{14}C, serotonin-^{14}C, and 5-HIAA-^{14}C in a single sample with sufficient sensitivity to measure accurately about 25 ng/g rat brain tissue for each metabolite. This enabled us to study the metabolism of 5-HTP-^{14}C in brain after intraperitoneal injection and to quantitate alterations *in vivo* of decarboxylase, MAO, and aldehyde dehydrogenous activites by phenelzine also injected intraperitoneally. The comparison of enzyme activities shown in Table 2 are based on disappearance of substrate in 30 min and are not necessarily linear. The brain concentrations of serotonin-^{14}C were close to physiological, and there were no observable pharmacological effects.

At doses up to 50 mg/kg, phenelzine markedly inhibited MAO, but not decarboxylase; between 50 and 200 mg/kg, phenelzine markedly inhibited decarboxylase

as well. At 50, 100, 150, and 200 mg phenelzine/kg, decarboxylase inhibition was 5, 64, 89, and 92% respectively. In another experiment, four rats were decapitated 30 min after injection of 100 mg phenelzine/kg and 15 min after 5-HTP-^{14}C injection; decarboxylase inhibition in this case was 99% (range, 88–104%).

5-HTP Decarboxylase has been shown to require pyridoxal phosphate;[10] and the hydrazides (e.g. semicarbazide) and hydrazines (e.g. phenelzine) have been shown to inactivate the enzyme .[10, 11] The evidence [11] indicated that hydrazone formation and inhibition of pyridoxal phosphokinase with a concomitant decrease in pyridoxal phosphate were the probable mechanisms by which phenelzine inhibited the decarboxylation of 5-HTP-^{14}C.

There were no recorded convulsions at 100 and 150 mg phenelzine/kg, and only a third of the rats convulsed at 200 mg/kg. At 250 and 300 mg/kg, all rats convulsed. Pyridoxal phosphate was implicated since pyridoxine hydrochloride, 50 and 100 mg/kg, protected against the convulsions.

Although pyridoxal phosphate depletion appeared to be a common factor in 5-HTP decarboxylase inhibition and convulsions induced by phenelzine, there was no correlation with respect to dose. Thus we obtained strong enzymatic inhibition, as high as 100%, at a dose of 100 mg phenelzine/kg, but no convulsions; decarboxylase inhibition was also high at 200 and 300 mg/kg where the rats were excited and convulsing. However, the measurements reported here were on whole brain. A correlation of 5-HTP decarboxylase inhibition with convulsions may become evident only in studies of discrete areas of the brain and at the subcellular level or, in other words, in studies of the functional compartment.

We must therefore still consider the possibility that a physiological function of serotonin is to inhibit neuronal excitability and that phenelzine, by blocking 5-HTP decarboxylase, lowers endogenous brain concentrations of serotonin at the functional sites, thereby inducing convulsions. An attempt to detect lower brain serotonin levels as a consequence of decarboxylase inhibition by using the convulsant semicarbazide[10] failed as did an attempt to alter the phenelzine-induced increase in brain serotonin with pyridoxine.[12] Since total serotonin was measured, it may be that serotonin was decreased at the functional sites, but the change could not be detected in the presence of the stored nonfunctional concentrations of serotonin. Weissbach *et al.*[10] found that a dietary pyridoxine deficiency in chicks produced markedly lower brain serotonin levels, and it is well known that such pyridoxine deficient animals are more susceptible to electroshock and pentylenetetrazole (Metrazol) convulsions. The latter finding is consistent with the hypothesis of a relationship between decreased serotonin and increased neuronal excitability.

On the other hand, if serotonin is an inhibitor of neuronal excitability, then increased brain levels of serotonin may be expected to further decrease excitability and thereby function as an anticonvulsant. Bonnycastle and co-workers[13] were the first to suggest an anticonvulsant role for serotonin based on their finding that anticonvulsants such as diphenylhydantoin raised brain serotonin levels; whether diphenylhydantoin actually raises brain serotonin is a matter of controversy.[13,14, 19] Kobinger[15] found that the combination of iproniazid and 5-HTP elevated the threshhold of the convulsive doses of Metrazol. Lessin *et al.*[16] reported that iproniazid and 5-HTP prolonged the survival time of mice infused with Metrazol. Consistent with these results, Prockup *et al.*[14] reported that iproniazid and 5-HTP protected rats

against electroshock seizures. Grey *et al.*[17] found this combination reversed the reserpine antagonism of the anticonvulsant action of methazolamide. Hehman *et al.*[18] showed that intraperitoneal injection of serotonin in chicks produced an increase of threshold to Metrazol-induced convulsions. Thus the evidence with respect to serotonin supports the conclusion that serotonin has an anticonvulsant role.

The MAO inhibitors have been reported to possess anticonvulsant properties ranging from little or no potency to a high degree of effectiveness;[14–17, 19–25] the MAO inhibitors also reversed the reserpine-induced facilitation of convulsions.[14,23,24,26] Prockop *et al.*[14] offered evidence of a direct correlation between the anticonvulsant activity of the MAO inhibitors and increased brain serotonin, but neither P'an *et al.*[19] nor Anderson *et al.*[20] could confirm the relationship. It is interesting to note that nialamide, iproniazid, and isocarboxazid decreased electroencephalographic abnormalities in epileptic patients and reduced the frequency of grand mal seizures;[27–31] phenelzine was reported[32] to be of questionable benefit in the control of seizures in children. The evidence with respect to MAO inhibitors, despite some apparent contradictions and considering the total picture, suggests that serotonin may have an anticonvulsant role, although other biogenic amines should also be considered.[14, 17]

The weakest link in the chain of evidence is the inability to obtain reproducible biochemical correlates of the anticonvulsants action of serotonin and MAO inhibitors. Stronger and more direct proof of the anticonvulsant action of serotonin and MAO inhibitors would also be highly desirable. We plan therefore to continue the work on the relationship of serotonin to neuronal excitability and anticonvulsant action with particular reference to discrete areas of the brain and subcellular distribution of serotonin and the enzymes controlling brain serotonin concentrations.

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