Hydroxyindole-*O*-methyltransferase V: Effects of Substituents on Hydrolysis of *N*-Acyltryptamines in Rats

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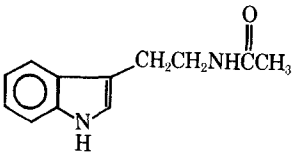
Abstract □ The effects of substituents on the hydrolysis of the amide linkage were studied in rats with five tritium-labeled, substituted *N*-benzoyltryptamines and *N*-phenylacetyltryptamines. In the blood, amides of phenylacetic acid were hydrolyzed at a faster rate than those of benzoic acid. Methylation of the amide nitrogen facilitated hydrolysis rather than retarding it. These five inhibitors of hydroxyindole-*O*-methyltransferase accumulated in pineal glands 15 min. after intravenous injection. Substitution of the chlorine atom on 3- and 4-positions of the phenyl ring decreased the transport into the pineal glands but retarded the metabolism in the organ. Of the compounds studied, *N*-phenylacetyltryptamine demonstrated the longest duration in the rat pineal gland.

Keyphrases □ Hydroxyindole-*O*-methyltransferase—substituted *N*-acyltryptamines, effect on hydrolysis, rats □ *N*-Acyltryptamines, substituted—effect on hydroxyindole-*O*-methyltransferase hydrolysis, rats □ TLC—separation

During studies of hydroxyindole-*O*-methyltransferase, a series of *N*-acyltryptamines was synthesized and tested as inhibitors of the enzyme (1–3). Replacement of the methyl group of *N*-acetyltryptamine (I) by a phenyl



I

or phenylmethylene group gave Compounds II and V which were four and eight times, respectively, better inhibitors than I (1). The activity was increased further by chlorine substitution on the phenyl ring, bromine substitution on the C-5 position of the indole nucleus, or both. The 3,4-dichlorophenyl compound (III) and the 5-bromo-3,4-dichloro compound (IV) were 70 and 280 times, respectively, more active than I (2). Methylation of the amide nitrogen, however, did not cause any change in the activity of V.

To explore the relative stability of these inhibitors *in vivo*, tritium-labeled compounds were synthesized, and the effects of substituents on the hydrolysis of amide linkages were studied in rats.

EXPERIMENTAL

Tritiation of 5-bromotryptamine was performed by a tritium–hydrogen exchange method (4). The 5-bromotryptamine-^{3}H was then acylated as previously described (1, 2). The specific activities (microcuries per milligram) obtained are as follows: II, 166; III, 120; IV, 80; V, 174; and VI, 123. 5-Bromoindole-3-acetic acid was purchased[1] and 5-bromotryptamine (m.p. 77–80°, ether) was prepared as previously described (2). 5-Bromotryptophol was synthesized by the following procedure.

To a stirred suspension of 150 mg. (4 mmoles) of $LiAlH_4$ in 150 ml. of anhydrous ether was added 508 mg. (2 mmoles) of 5-bromoindole-3-acetic acid in 30 ml. of ether. The mixture was refluxed for 3 hr., decomposed with 10% NaOH, and then filtered. The solid on the filter was extracted with hot ether (four 150-ml. portions). The combined ether filtrate and extracts were dried (Na_2SO_4), treated with charcoal, filtered, and evaporated *in vacuo*. The crude product crystallized from $CHCl_3$ upon cooling to give 400 mg. (83%) of yellow-pink solid, m.p. 84–87°. Subsequent recrystallizations from chloroform gave an analytical sample, m.p. 87–88°. $\nu_{max.}$ (KBr): 3400 cm.$^{-1}$ (OH), 60 MHz. NMR (CD_3—$COCD_3$): 2.28 (singlet, H—4); 2.80 (multiplet, H—2, H—5, H—6); 6.20 (triplet, J = 7.0 Hz., CH_2—OH); 7.05 J (triplet, J = 7.0 Hz., CH_2).

Anal.—Calcd. for $C_{10}H_{10}BrNO$: C, 50.02; H, 4.20; N, 5.83. Found: C, 50.06; H, 4.23; N, 5.59.

Male Sprague-Dawley rats, weighing 200–250 g., were injected intravenously with 25 mmoles/kg. of each tritiated compound in 0.1 ml. of propylene glycol. The animals were sacrificed by decapitation at the designated time intervals. Three rats were used for each time interval, except for Compound IV which had four rats per time interval. A 0.1-ml. aliquot of blood was diluted with 3 ml. of methanol and 15 ml. of liquifluor containing phenyl-oxazolyl-phenyl-oxazolyl-phenyl, 2,5-diphenyloxazole, and toluene, and it was assayed for tritium in a Nuclear Chicago Mark I liquid scintillation spectrometer. All values were corrected for 100% efficiency (channel ratio) and recovery. The brains and pineal glands were dissected and homogenized separately in 4 and 200 parts of water, respectively. Aliquots (0.1 ml.) of the homogenates were assayed for tritium.

The blood and tissue homogenates were then extracted with acetone until over 95% of the radioactivity was taken up (2 × 10 ml.). The acetone was evaporated to a small volume, spotted on silica gel TLC plates, and developed in ethyl acetate. From the chromatograms, silica in bands (0.5 cm. wide) was scraped into counting vials, digested with the methanol and liquifluor mixture, and then assayed for tritium. On each plate, the unchanged compound was identified by its R_f value and isotope dilution. Percent unchanged was calculated from: disintegrations per minute of unchanged compound/total disintegrations per minute from TLC plate × 100.

DISCUSSION

Fifteen minutes following the intravenous injections of the five compounds (II–VI), high radioactivity appeared in all pineal glands, indicating the accumulation of the hydroxyindole-*O*-methyltransferase inhibitors at the site of action (Table I). Substitution of the chlorine atom on 3- and 4-positions of the phenyl ring of II decreased the transport of the compound (III) into the brain and pineal gland. This was likely due to a lower level of III in blood as compared to II. The low uptake of III in the brain was improved considerably by placing a bromine atom on the 5-position of the indole nucleus to give IV.

In the blood, amides of phenylacetic acid (V and VI) were hydrolyzed at a faster rate than those of benzoic acid (II–IV), as seen in the presence of lesser amounts of unchanged compounds (Table I). Although the methylation of the amide nitrogen of V resulted in a higher level of VI in the blood, it facilitated hydrolysis rather than retarding it. Concurrently, the unchanged compounds with phenylmethylene linkage (V and VI) were present in lower percentages in the brain than those with a phenyl group (II–IV).

Of the five compounds studied, V demonstrated the longest duration in rat pineal gland. The concentration of unchanged molecules remained highest at all time intervals. No unchanged V was detected in the brain after 2 hr.

The effects of substitution on the rate of metabolism were observed. The metabolism of III and IV appeared to be slow in the brain and pineal gland as a result of introducing 3,4-dichlorine sub-

[1] Aldrich Chemical Co.

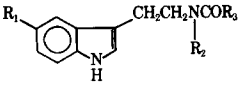
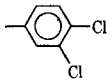
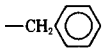
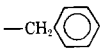


Table I—Distribution of Total and Unchanged Compounds in Rat Tissues[a]

Number	Compound R_1	Compound R_2	Compound R_3	Tissue	Micromoles per Gram Tissue or per Milliliter Blood[b] 15 min. Total	15 min. Unchanged[c]	1 hr. Total	1 hr. Unchanged[c]	2 hr. Total	2 hr. Unchanged[c]	4 hr. Total	4 hr. Unchanged[c]
II	H	H		Brain	30.3	20.3	6.6	4.6	4.7	3.9	7.7	2.6
				Pineal	100.4	74.3	30.5	9.8	21.2	6.6	1.1	0
				Blood	33.6	24.5	14.8	1.8	5.2	0.6	12.2	0.2
III	H	H		Brain	8.5	7.1	2.1	1.8	2.4	1.2	1.6	1.3
				Pineal	59.2	48.5	25.3	16.4	16.6	10.1	0.6	0.4
				Blood	5.6	4.5	1.4	1.2	1.8	1.7	1.3	1.1
IV	Br	H		Brain	30.1	26.2	14.7	12.8	7.5	4.7	3.6	2.0
				Pineal	60.8	31.0	33.6	14.4	16.2	3.9	10.2	1.6
				Blood	11.2	9.9	10.0	3.5	10.3	2.4	5.3	0.9
V	H	H		Brain	29.5	13.0	13.2	11.6	7.4	7.1	8.2	8.1
				Pineal	101.4	75.0	5.5	1.5	6.6	2.9	5.3	1.9
				Blood	11.2	4.4	8.2	3.8	8.3	1.5	5.4	0.3
VI	H	CH_3		Brain	33.4	12.0	17.5	4.0	13.9	0	9.4	0
				Pineal	147.2	83.9	56.2	17.4	62.3	16.2	64.3	4.5
				Blood	21.1	3.8	12.6	3.2	6.6	1.3	10.0	0.2

[a] Each compound (25 mmoles/kg.) was administered intravenously. [b] Each value represents the mean of three rats (four in IV); each determination was done in duplicate. [c] Silica gel TLC in ethyl acetate. R_f values were II, 0.85; III, 0.91; IV, 0.85; V, 0.85; and VI, 0.75.

Table II—Distribution of Metabolites of 5-Bromo-3,4-dichlorobenzoyltryptamine in Rat Tissue

Tissue	Time	Unchanged Compound %	Unchanged Compound μmole[e]	5-BrT[a, d] %	5-BrT[a, d] μmole[e]	5-BrIAA[b, d] %	5-BrIAA[b, d] μmole[e]	5-BrTOL plus Unknown[c, d] %	5-BrTOL plus Unknown[c, d] μmole[e]
Brain	15 min.	87	26.0	3	0.9	8	2.4	2	0.6
	1 hr.	87	12.8	3	0.4	9	1.3	1	0.2
	2 hr.	63	4.7	7	0.5	11	0.8	19	1.4
	4 hr.	55	2.0	17	0.6	16	0.6	12	0.4
Pineal	15 min.	51	31.0	11	6.7	15	9.1	13	7.9
	1 hr.	43	14.4	16	5.4	18	6.0	23	7.7
	2 hr.	24	3.9	22	3.6	28	4.5	26	4.2
	4 hr.	16	1.6	24	2.4	22	2.2	48	4.9
Blood	15 min.	88	9.9	2	0.2	1	0.1	9	1.0
	1 hr.	35	3.5	16	1.6	19	1.9	28	2.8
	2 hr.	23	2.4	24	2.5	21	2.2	22	2.3
	4 hr.	17	0.9	32	1.7	28	1.5	23	1.2

[a] 5-BrT = 5-bromotryptamine. [b] 5-BrIAA = 5-bromoindole-3-acetic acid. [c] 5-BrTOL = 5-bromotryptophol. [d] Silica gel TLC in ethyl acetate. R_f values were 5-BrIAA, 0.58; 5-BrTOL, 0.26; and 5-BrT, 0.15. [e] Each value represents the mean of four rats; each determination was done in duplicate.

stitution to II. Placement of the methyl group on V resulted in a decrease in the metabolism of VI in the pineal but not in the brain.

Since IV was the best *in vitro* hydroxyindole-*O*-methyltransferase inhibitor, its metabolism in rats was studied in a more detailed fashion. Hydrolysis of the amide linkage *in vivo* gave 5-bromotryptamine, which was then subjected to the attack of monoamine oxidase to form 5-bromoindole-3-acetaldehyde. The aldehyde was either oxidized by aldehyde dehydrogenase to 5-bromoindole-3-acetic acid or reduced by alcohol dehydrogenase to 5-bromotryptophol. In rat brain, the metabolism of IV was slower than in the pineal or blood (Table II). In most of the later time intervals, the 5-bromotryptamine, 5-bromoindole-3-acetic acid, and possibly the 5-bromotryptophol were present in approximately the same amounts in the three tissues studied.

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