The Effect of NSD-1055 on Tissue Levels of Histamine and Spermidine in Rat (35334)

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The biochemical and pharmacological significance of histamine formed in the body is largely unknown. Furthermore, the physiological role of this potent monoamine is poorly understood. The origin of histamine in mammalian tissue is, like many of the other monoamines, the result of the intracellular decarboxylation of an amino acid, in this case histidine (1–3). The metabolic conversion of histidine to histamine is catalyzed by at least two distinct enzymes: (i) a specific L-histidine decarboxylase (EC 4.1.1.22) found only in some tissue and (ii) the ubiquitous nonspecific aromatic L-amino acid decarboxylase [also referred to as 3,4-dihydroxyphenylalanine (DOPA) decarboxylase (EC 4.1.1.26)] (4, 5).

Pyridoxal-5-phosphate is the essential enzyme cofactor for the activity of various L-amino acid decarboxylases (6), including histidine decarboxylase, in mammalian tissue (2, 7, 8). Bromo-3-hydroxybenzyloxyamine (NSD-1055) inhibits histidine decarboxylase (EC 4.1.1.22) *in vitro* (9) and blocks the formation of histamine *in vivo;* its administration leads to depletion of histamine from tissues and its decreased excretion in the urine of the rat (10). The development of an enzyme inhibitor affords a means of testing for the physiological role of histamine. It has been suggested that NSD-1055 inhibits enzyme activity by inactivating the essential cofactor pyridoxal-5-phosphate (9).

On the other hand, kinetic studies (28) of inhibition by NSD-1055 and several other O-substituted hydroxylamines suggest that these agents are competitive inhibitors with respect to the substrate (histidine), the oxyamine group of NSD-1055 competing with the amino group of the substrate for a carbonyl site on the holoenzyme (28).

There is non-mast cell histamine (11) in mammalian brain associated with isolated nerve endings (12–14). An inhibition of the histamine-forming enzymes and the resulting depletion of tissue histamine would be useful in testing for some of the functions that have been ascribed to histamine in the body.

The primary purpose of these experiments was to explore a method to lower the brain histamine concentration. The use of NSD-1055 was predicated on its reported ability to lower the concentration of tissue histamine by inhibiting decarboxylation of histidine (10). The influence of NSD-1055 on the histamine and spermidine concentration of brain and peripheral tissues was also examined.

Chemicals. NSD-1055 (Cl 54,998; 4-Bromo-3-hydroxybenzyloxyamine, Brocresine) was a gift from Lederle Laboratories, Pearl River, New York. The intermediate phosphonic acid cation-exchange resin (Bio-Rex-63) was obtained from Bio-Rad Laboratories, Richmond, California. *o*-Phthaldialdehyde (Mann Research Laboratories, New York, New York) was purified by recrystalization from ligroin (bp 30–60°). Methanol was redistilled before use. Histamine dihydrochloride and spermidine phosphate, hexahydrate were obtained from Nutritional Biochemicals, Cleveland, Ohio. Standards of histamine and spermidine were prepared in 0.1 *N* and 0.2 *N* HNO_3 and stored frozen (—20°) until used.

Animal care. Male Sprague-Dawley rats (Carworth Farms, Portage, Michigan), weighing 180-200 g, when received were housed in metal cages with open-grid floor in

[1] Supported by Research Grant U.S. Public Health Service NB-06053.

groups of 5 animals to a cage, with water and Purina rat food *ad libitum*. Natural illumination of animal quarters commenced with arrival of daybreak and ceased at the end of daytime light. For the time of year these studies were conducted the cages were illuminated by daylight for about 14 hr, during 10 of which overhead fluorescent lighting was also present. Room temperature was maintained at 24 ± 1°.

Drug administration. NSD-1055 (100 mg/kg: 50 mg/ml in 0.9% saline) was injected intraperitoneally for 7 days. Control animals received 7 comparable injections of saline. The animals were killed 4 hr after the last injection.

Preparation and analysis of tissue. Each rat was decapitated, and, when necessary, the organs were pooled to give about 1 g of each tissue (midbrain, cerebral cortex, cerebellum, and heart). Acid extraction (15), cation-exchange separation (15, 16), and fluorometric estimation of histamine (17) and spermidine (18, 19) were essentially as described earlier (15).

Results and Discussion. In the 7 days of drug treatment the animals displayed no abnormal activities that could be ascribed to drug effects. They behaved like the control group, *e.g.*, in avoiding being caught for weighing and injection. At the time of the last injection the control group weighed 248 ± 11.7(5) g whereas those receiving NSD-1055 weighed 220 ± 16.7 (5) g.

Tables I and II list the concentration of histamine and spermidine in tissues of control and NSD-1055-treated male rats as determined in this study and those of other laboratories. Some investigators use female rats (10, 20) because the female rat excretes large quantities of free histamine in urine (10), but we, and others (21), have used male rats. It would appear that the tissues of both sexes have about the same histamine content but that the turnover of histamine is probably faster in the female. The present findings and those of others (10) demonstrate the variability in the degree of effectiveness of NSD-1055 as an inhibitor of histidine decarboxylase *in vivo* as reflected in diminution of histamine levels of the tissues examined. Sex, dosage schedules, strain, and source of animals (24) may contribute to variability in results from one laboratory to another, but one sees that where this appears to be standardized in part, there is unanimity in conclusions. Both this study and that of Green and Erickson (21) use male rats, the dosage of NSD-1055 being 100 mg/kg intraperitoneally. Whereas in the present study,

TABLE I. Rat Tissue Concentration of Histamine (μg/g).[a]

	Sex	Normal untreated	NSD-1055	%Δ	Ref.	*p* value	Method
Lung	M	4.0 ± 1.23 (5)	2.8 ± 0.37 (5)	−30	—[b]	.05	A
	F	4.0	5.0	+25	(20)		B
Liver	M	0.4 ± 0.1 (5)	0.32 ± 0.07 (5)	−20	—[b]	NS	A
Kidney	M	0.1 ± 0.03 (5)	0.05 ± 0.01 (5)	−50	—[b]	.01	A
Testis	M	0.06 ± 0.02 (5)	0.05 ± 0.02 (5)	−17	—[b]	NS	A
Spleen	M	1.02 ± 0.42 (5)	0.67 ± 0.07 (5)	−34	—[b]	.01	A
Heart	M	1.28 ± 0.18 (5)	1.27 ± 0.3 (5)	± 0	—[b]	NS	A
	M	2.251 ± 0.126 (6)	2.865 ± 0.748 (6)	+27.3	(21)		A
	F	2.9 ± 0.5 (12)	3.8 ± 0.7	+31	(20)		B
	F	4.3 ± 0.4 (35)	2.9 ± 0.4 (12)	−32.5	(10)		B
Midbrain	M	0.074	0.044	−41	—[b]	NA	A
Brain cortex	M	0.024	0.018	−15	—[b]	NA	A
	M	0.021	0.016	−14	—[b]		A
Cerebellum	M	0.068	0.011	−84	—[b]	NA	A

[a] A, ion exchange purification and fluorometric assay; B, solvent extraction and fluorometric assay; [b] Present study. NS = *p* value not significant; and NA = not applicable.

TABLE II. Concentration of Spermidine ($\mu g/g$) in Tissues of Male Rats.

	Sex	Normal	NSD-1055	%	p value[a]
Lung	M	61.52 ± 10.9 (5)	54.38 ± 3.2 (5)	−11	NS
Liver	M	66.82 ± 5.1 (5)	75.31 ± 5.4 (5)	+11	.02
Kidney	M	34.5 ± 1.0 (5)	30.63 ± 3.5 (5)	−11	.02
Testis	M	20.88 ± 3.0 (5)	21.32 ± 2.3 (5)	+5	NS
Spleen	M	109.6 ± 6.1 (5)	109.5 ± 4.6 (5)	±0	NS
Heart	M	22.83 ± 1.8 (5)	19.14 ± 0.9 (5)	−16	.01
Midbrain	M	43.3	33.3	−23	NA
Brain cortex	M	29.5	18.45	−38	NA
		25.8	20.8	−20	NA
Cerebellum	M	46.3	31.7	−32	NA

[a] NS = p value not significant; NA = not applicable.

animals received 7 doses over a period of 7 days, being killed 4 hr after the last dose, Green and Erickson (21) used 1 dose and the animals were killed 6 hr later. They reported no significant change in the histamine concentration of the rat *heart* after NSD-1055 and this is consistent with the present finding despite our use of more intensified drug treatment. Furthermore, Johnson (20) used female rats treated with NSD-1055 (100 mg/kg i.p.) 12 and 2 hr before his experiment and concluded that the effects of NSD-1055 on endogenous histamine levels in *heart* tissue from control rats [2.9 ± 0.5 (12) $\mu g/g$] as compared to treated animals [3.8 ± 0.7 (12)] was not significant.

According to Schayer (22) mast cell histamine has a half-life of approximately 50 days. Multiple metabolic pools with different turnover rates for mast cell and non-mast cell histamine might explain the magnitude of the drug effect as seen *in vivo* in peripheral tissue (*e.g.*, heart) as compared to brain. In addition, Maudsley and Kobayashi, (23) found that NSD-1055 produces a transient 40% drop in histidine decarboxylase activity within 30 min, the enzyme recovering to normal levels of activity within a few hours.

The pattern of changes in tissue concentration of the polyamine, spermidine, under the influence of NSD-1055 is not unlike that of histamine. Putrescine is the direct precursor of spermidine synthesis (25) and is formed by the catalytic decarboxylation of ornithine via L-ornithine decarboxylase (EC 4.1.1.17), which is a pyridoxal phosphate-dependent enzyme system (26). If, indeed, NSD-1055 interacts with the cofactor pyridoxalphosphate (9) or competes with amino acids for reactive sites on appropriate decarboxylating enzymes (28), then one would expect ornithine decarboxylase activity to be affected by this drug. The present study shows that the effects of NSD-1055 on spermidine *in vivo* is no greater than that of histamine. The ratio of spermidine to histamine in all tissues is heavily in favor of spermidine which has a half-life of 5 days in both liver and brain in the adult rat (27). Furthermore, the transient effect by NSD-1055 on decarboxylating enzymes *in vitro* (23) and its rapid disappearance from the body with a half-life of less than 105 min, depending on the dose (29), negates the opportunity of sustained inhibition of the decarboxylating enzymes resulting in significant depletion of amines within the tissue. A similar suggestion has been made by other investigators (30).

Furthermore, it has been found that the disappearance of NSD-1055 from the plasma of rat is more rapid than that observed for man (31). Considering the dosage schedule employed in this study and the rapid clearance of the drug from the body (29–31) it may very well be that we effectively produced an acute intermittent inhibition of histidine decarboxylase activity over a period of 7 days. Therefore, the histamine and spermidine tissue concentrations as depicted in Tables I and II could actually be the effects of the drug following the final dose 4 hr after its last administration.

These preliminary data were subjected to Student's *t* test statistical analysis and any significance to the magnitude of the changes in histamine and spermidine is indicated in Table I. Furthermore, we compare these results to those of other workers (20, 21). It would be grossly premature at this time to relate lack of obvious behavioral changes as seen in five treated rats whose pooled midbrains showed a 41% depression in histamine content relative to that of nontreated animals. Any attempt to relate non-mast cell histamine in brain to a particular physiological role in the central nervous system will undoubtedly require agents more selective than general amino acid decarboxylase enzyme inhibitors.

I am grateful to Dr. Robert J. Levine for his suggestions and willingness to share his unpublished data.

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Received Oct. 19, 1970. P.S.E.B.M., 1971, Vol. 136.