Inhibition of [H³]Serotonin Uptake by the Mouse Spleen. A Structure–Activity Study

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A rapid and simple method for determining the effect a given compound may have on the uptake and/or storage of [H³]serotonin in spleen has been developed. Several drugs known to exert a depleting effect on brain serotonin were found to block the uptake of [H³]serotonin in the spleen. Mice were pretreated with drugs 1 hr before the administration of 3.0 μCi of [H³]serotonin. Spleens from two vehicle pretreated animals contain approximately 0.64 μCi of [H³]serotonin after 3 hr, while spleens of mice treated with active drugs contain lesser amounts. The effect of a variety of indoles and CNS agents has been examined and their structure–activity relationship discussed.

Serotonin has been implicated in the action of a variety of centrally acting drugs, such as reserpine,[1] MAO inhibitors,[2] imipramine,[3] and certain hallucinogens.[4] It has also been suggested that serotonin, or possible metabolites of serotonin, may be the causative factor in certain mental diseases.[5] Existing methods for the determination of serotonin are laborious[6] and do not permit large scale screening. The measurement of the steady-state levels would not detect compounds which cause a more rapid turnover of serotonin nor those which would block its uptake. Therefore, an attempt has been made to develop a relatively simple method for assessing interactions of compounds with uptake and storage of serotonin. Such a method would be of value in the search for centrally active agents.

Studies with [H³]norepinephrine have demonstrated the value of using radioactive materials in such screening procedures.[7] Serotonin, like norepinephrine, does not readily enter the brain following systemic administration. Therefore, it was necessary to select a peripheral organ which normally contains serotonin, and where a given compound's ability to interfere with binding of serotonin could be easily measured. The serotonin in such an organ should be affected by those drugs which are known to exert an effect on brain serotonin and especially those drugs which are believed to interfere with the binding of serotonin since this would indicate a storage mechanism comparable to that found in the brain. If this were so, then the method should have some application in screening for compounds which might alter serotonin levels or turnover in brain. One of the obvious difficulties in attempting to use a peripheral organ in such a study is that compounds which are active in this test may not be centrally active due to their inability to cross the blood–brain barrier.

The method employed here assesses a compound's ability to block the uptake and/or storage of [H³]-serotonin in the spleen. The tested compound is given prior to [H³]serotonin since this will permit the detection of drugs which are depletors of serotonin as well as those which block its uptake. Similarly, in studying the effect of drugs on [H³]norepinephrine in the mouse heart, one can detect the effects of the tricyclic antidepressants, reserpine-like compounds, and a variety of phenethylamine derivatives (*i.e.*, amphetamine, methylphenidate, and phenmetrazine) when the drugs are given before the label. However, when the drugs are given after the [H³]norepinephrine many of these compounds have no observable effect or, if so, only at high doses.[8]

A particulate-soluble distribution study of [H³]-serotonin was carried out by homogenizing the spleens

Table I
Time Study of [H³]Serotonin in Mouse Spleen

Time (min)	cpm/1.5 ml
30	10994
60	14196
120	12787
180	9969

Table II
The Effect of Various Agents on the Uptake of [H³]Serotonin by the Mouse Spleen[a]

Compound	Dose, mg/kg	[H³]-Serotonin in spleen as % of control
Chlorpromazine·HCl	5	96
Imipramine·HCl	10	62
Imipramine·HCl	25	43
Amitriptyline·HCl	25	68
Protriptyline·HCl	10	96
Desipramine·HCl	25	90
Nortriptyline·HCl	25	82
Chlordiazepoxide·HCl	10	101
Haloperidol	10	110
d-Amphetamine sulfate	5	93
p-Chloroamphetamine	10	51
p-Chloro-*N*-methylamphetamine	10	48
Metaraminol bitartrate	10	95
Reserpine	2.5	20
RO 4-1284[b]	10	57
2-Bromo-D-lysergic acid diethylamide	10	96
Methsergide maleate	25	97

[a] Method as described in text. [b] RO 4-1284 is 2-ethyl-1,3,4,6,7-11b-hexahydro-3-isobutyl-9,10-dimethoxy-2*H*-benzo[*a*]quinolizin-2-ol·HCl. A change of 17% from control is significant at $P = 0.05$.

(1) A. Pletscher, P. A. Shore, and B. B. Brodie, *J. Pharmacol. Exp. Ther.*, **116**, 84 (1956).
(2) P. O. Ganrot, E. Rosengren, and C. G. Gottfries, *Experientia*, **18**, 260 (1962).
(3) A. Carlson, K. Fuxe, and U. Ungerstedt, *J. Pharm. Pharmacol.*, **20**, 151 (1968).
(4) D. X. Freedman and N. J. Giarman, *Ann N.Y. Acad. Sci.*, **96**, 98 (1962).
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(6) N. E. Anden and T. Magnusson, *Acta. Physiol. Scand.*, **69**, 87 (1967).
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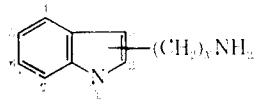
(8) R. A. Lahti, unpublished observations.

TABLE III

THE EFFECT OF VARIOUS INDOLES ON THE UPTAKE OF [H³]SEROTONIN BY MOUSE SPLEEN[a]



Compound	Substitution: Ring	Substitution: Side Chain α	Substitution: Side Chain N	Dose mg/kg	[H³]-Serotonin in spleen as % of control
Tryptamine·HCl				40	59
Tryptamine·HCl				80	33
5-Methyltryptamine	5-CH_3			40	83
dl-α-Methyltryptamine acetate		CH_3		10	39
d-α-Methyltryptamine acetate		CH_3		10	30
l-α-Methyltryptamine acetate		CH_3		10	61
dl-1-Methyl-α-methyltryptamine·HCl	1-CH_3	CH_3		10	54
dl-α-Methyl-*N*,*N*-dimethyltryptamine acetate		CH_3	$(CH_3)_2$	10	81
dl-5-Methoxy-α-methyltryptamine acetate	5-OCH_3	CH_3		10	91
dl-α-Ethyltryptamine acetate		C_2H_5		10	54
d-α-Ethyltryptamine acetate		C_2H_5		10	55
l-α-Ethyltryptamine acetate		C_2H_5		10	62
dl-1-Methyl-α-ethyltryptamine·HCl	1-CH_3	C_2H_5		10	76
dl-2-Methyl-α-ethyltryptamine maleate	2-CH_3	C_2H_5		10	96
dl-1,2-Dimethyl-α-ethyltryptamine·HCl	1,2-diCH_3	C_2H_5		10	100
dl-6-Fluoro-α-ethyltryptamine·HCl	6-F	C_2H_5		10	51
dl-6-Methoxy-α-ethyltryptamine acetate	6-OCH_3	C_2H_5		10	71
dl-7-Methyl-α-ethyltryptamine·HCl	7-CH_3	C_2H_5		10	49
d-7-Methyl-α-ethyltryptamine·HCl	7-CH_3	C_2H_5		10	55
l-7-Methyl-α-ethyltryptamine·HCl	7-CH_3	C_2H_5		10	63
d-Hydroxytryptamine creatinine sulfate	5-OH			10	24
dl-5-Hydroxy-α-methyltryptamine creatinine sulfate	5-OH	CH_3		10	41
5-[(1-Methyl-2-piperidyl)methyl]indol-5-ol	5-OH	3-[(1-Methyl-2-piperidyl)-methyl]		10	43
3-Aminomethyl indole acetate				40	99
1-(2-Aminoethyl)indole maleate				40	89
3-(3-Aminopropyl)indole·HCl				40	82
3-(3-Aminobutyl)indole		CH_3		10	71

[a] A change of 17% from control is significant at $P = 0.05$

of [H³]serotonin-treated mice in 0.32 *M* sucrose containing pheniprazine. The resulting homogenate was centrifuged at 30,000*g* for 30 min at 4° and [H³]serotonin and endogenous serotonin were isolated and determined by previously described methods.[9] The particulate-soluble distribution of [H³]serotonin was comparable to endogenous serotonin in the spleen, 3.0 *vs.* 3.4, respectively, suggesting that [H³]serotonin should behave similarly to endogenous serotonin. Further investigation revealed that the majority of the tritium in the spleen after 3 hr was serotonin. Comparable results were obtained whether [H³]serotonin was isolated by ion-exchange chromatography[9] and counted, or by simply counting an aliquot of the supernatant from the centrifuged homogenate.

Experimental Section

Materials.—Generally labeled [H³]serotonin was obtained from Nuclear Chicago (specific activity 5.28 Ci/mmole). Compounds were obtained from The Upjohn Company Screening Office, commercial sources, or as acknowledged. Male Carworth Farm mice weighing 18–20 g were used in all studies.

Method.—Mice were administered the drug or vehicle (0.25% aq methyl cellulose) subcutaneously in a total vol of 0.2 ml. One hour later 3.0 μCi of [H³]serotonin, in Merlis solution, was injected iv into the tail vein (0.1 ml). Mice were sacrificed 3 hr after the administration of the label. The spleens were removed from two mice, rinsed in cold saline, frozen, homogenized in 3.0 ml of 0.4 *N* $HClO_4$ using a ground glass homogenizer, and the resulting homogenate was centrifuged at 10,000*g* for 10 min. A 1.5-ml aliquot of the supernatant was placed in a scintillation vial containing 15 ml of toluene–Triton X-100 (3:2) scintillation fluid[10] and counted. Control samples were found to contain approximately 10,000 cpm/1.5 ml, while samples from mice treated with active compounds contained lesser amounts (see Table II).

All determinations were made in duplicate and two spleens were used per determination. Results are expressed as per cent of control values, and a change of 17% from control is significant at the $P = 0.05$ level.

Results and Discussion

Diverse Agents.—A variety of agents commonly used in treating mental diseases or which have been found to exert an effect on brain serotonin were examined in this study (Table II).

Chlorpromazine, haloperidol, chlordiazepoxide, and certain phenethylamines were inactive in this test and these results seemed to be suggestive of a selective uptake mechanism. An exception among the phenethylamines is *p*-chloro-*N*-methylamphetamine which has been shown to exert an effect on brain serotonin.[11]

(9) R. A. Lahti, P. A. Platz, and R. V. Heinzelman, *Biochem. Pharmacol.*, **18**, 1601 (1969).

(10) M. S. Patterson and R. C. Greene, *Anal. Chem.*, **37**, 854 (1965).

(11) A. Pletscher, W. P. Burkand, H. Brudener, and K. F. Gey, *Life Sci.*, **2**, 828 (1963).

Reserpine and a potent tetrabenazine-like compound[12b] (see also Table II) are active, and they also exert an effect on brain serotonin.[12]

Among the tricyclic antidepressants, weak activity is demonstrated by imipramine and amitriptyline while protriptyline and desipramine are inactive. These results are similar to the effects of these agents on the uptake of serotonin into serotonergic neurons in the central nervous system.[13]

The results obtained with reserpine, RO 4-1284 (Table II), *p*-chloro-*N*-methylamphetamine and imipramine strongly support the idea that the uptake and storage mechanism of serotonin in the spleen is comparable to that found in the brain.

Indoles. The examination of a number of indoles, Table III, further suggests a very selective uptake or binding of serotonin in the spleen. Tryptamine effectively blocked the uptake of [H³]serotonin, whereas placement of the ethylamine side chain in the 1 position produced an inactive compound as did the substitution of a methylamine group in the 3 position. Lengthening of the tryptamine side chain to a propylamine reduced the activity considerably, compared to tryptamine.

Substitution of an α-Me or α-Et group on the tryptamine or 3-propylaminoindole molecule enhanced the activity. This is undoubtedly due to the rapid metabolism of tryptamine by monoamine oxidase and lack of metabolism of α-substituted amines by this enzyme. This is supported by the demonstration that tryptamine is more effective in blocking the uptake of [H³]serotonin when pheniprazine, an MAO inhibitor, is given 1 hr before the tryptamine to prevent its metabolism (Table IV). It is worthwhile to note that serotonin is more active than α-methylserotonin, in contrast to tryptamine where the α-substituted derivatives are more active.

The terminal amine of tryptamine can be cyclized into a pyrrolidine ring, and the terminal amine and α-C of serotonin can be cyclized into a piperidine ring, and both compounds still maintain activity. The *N*,*N*′-Me_2 analog of α-methyltryptamine is less active than its parent compound.

(12) (a) A. Bertler, *Acta Physiol. Scand.*, **51**, 75 (1961). (b) This compound has been reported as RO 4-1284. K. F. Gey and A. Pletscher, *Brit. J. Pharmacol.*, **19**, 161 (1962).

(13) K. Fuxe and U. Ungerstedt, *Eur. J. Pharmacol.*, **4**, 135 (1968).

TABLE IV

EFFECT OF PRETREATMENT WITH PHENIPRAZINE ON THE ACTIVITY OF TRYPTAMINE IN BLOCKING THE UPTAKE OF [H³]SEROTONIN

Treatment[a]	Injection route	Dose, mg/kg	[H³]-Serotonin in spleen as % of control
Tryptamine	I.P.	80	69
Tryptamine	S.C.	80	33
Pheniprazine + tryptamine	I.P.-S.C.	5–20	39
Pheniprazine + tryptamine	I.P.-I.P.	5–20	46
Pheniprazine	I.P.	5	88

[a] Pheniprazine (5 mg/kg) was administered 1 hr prior to tryptamine, where designated. Remainder of the procedure was as described in text.

Several other substitutions reduce the activity considerably when compared to their parent compounds. A 2-Me substitution reduces activity more than 1-Me. The substitution of MeO in the 5 or 6 position produces a less active compound. A 6-F or 7-Me substitution did not alter the activity of α-ethyltryptamine.

In certain cases it was found that differences in activity could be obtained among optical isomers. The *d* isomer of α-methyltryptamine is much more active than the *l* isomer. The *d* and *l* isomers of α-ethyltryptamine and 7-methyl-α-ethyltryptamine are almost equally active.

The results presented here indicate that interference with the uptake and/or binding of [H³]serotonin in spleen is a highly selective process. In a number of instances, the results correlate well with data obtained in the central nervous system. The technique should provide a simple method to screen for compounds that may interfere with the uptake and binding of serotonin.

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