DRUG-INDUCED ALTERATIONS OF THE SUB-CELLULAR DISTRIBUTION OF 5-HYDROXYTRYPTAMINE (SEROTONIN) IN RAT'S BRAIN*

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STUDIES of drug-induced alterations in the total level of 5-hydroxytryptamine (5-HT) in whole brain or in specific brain regions have failed to show any trend consistent with simultaneous drug-induced behavioral changes. This has led us, along with other investigators in this field, to speculate that the size of the total cerebral store of a neurohumor may not be nearly as important a correlate of drug-induced behavioral change as the sub-cellular status and form of the neurohumor in the brain.

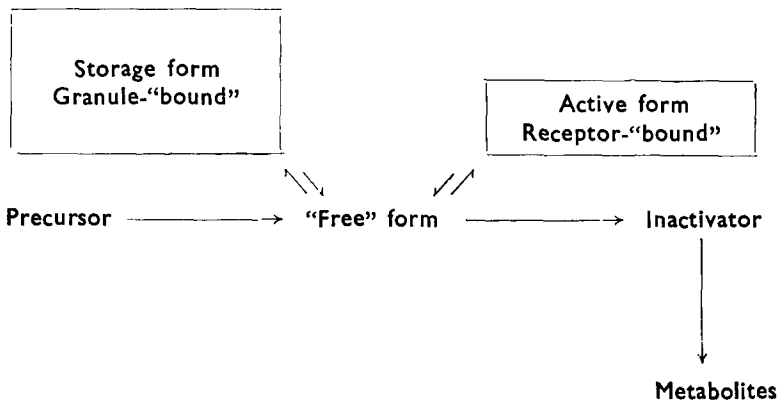

FIG. 1

Fig. 1 shows schematically our current concept of these subcellular forms. We visualize two bound forms of the neurohumor: (1) the inactive storage form, which is granule-bound and inaccessible to the inactivator enzyme; and (2) the active form represented by the complex of the neurohumor with its receptor protein. Schanberg and I[1] have shown that the bulk (70%) of the 5-HT in homogenates of rat's brain is associ-

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ated with sub-cellular particles — mainly, it may be assumed, in the storage form. There is also the so-called "free" form, usually equated with the active material, but, in reality representing a transition form, potentially storable, potentially active, and potentially destructible. A drug, conceivably by a number of different mechanisms, could influence the amount of one or another of these forms, disturb the equilibria governing them and alter responses evoked by the active complex.

The data to be presented will show that by high-speed centrifugation (100,000 *g*) of rat brain homogenates in isotonic sucrose (0·25 M) it is possible to get reliable and reproducible values of "bound" and "free" 5-HT, which have enabled us to establish a working hypothesis based upon responses of animals to the prior administration of certain classes of neuropharmacologic agents.

TABLE I

Influence of a variety of C. N. S. depressants on the subcellular distribution of 5-HT

Treatment	Number of animals	Mean 5-HT (mμg/whole brain) Partic. ("bound")	Supernate ("free")	Total	% "Free"	Partic./ supernate (P/S)
1. None	62	389 ± 89†	158 ± 36†	547	29	2·5
2. Chlorpromazine (25 mg/kg – 75 min)	12	300 ($p < ·05$)*	247 ($p < ·001$)	547	45	1·2
3. Reserpine (5 mg/kg – 4 hr)	14	88 ($p < ·001$)	61 ($p < ·001$)	149	41	1·4
4. Isoreserpine (5 mg/kg – 4 hr)	4	275 ($p < ·01$)	129	404	32	2·1
5. γ-Methyl-DOPA (100 mg/kg – 1 hr)	12	267 ($p < ·001$)	182	449	41	1·5
6. Phenobarbital (100 mg/kg – 2·5 hr)	5	450 ($p < ·01$)	325 ($p < ·01$)	775	42	1·4
7. Methylparafynol (300 mg/kg – 15 min)	7	321	139	460	30	2·3

* Standard deviation

† The p values listed were derived from Student's "t" table.

Table I summarizes results obtained with drugs which represent various sub-classes under the broad heading of C. N. S. depressants. These data are expressed as mμg 5-HT/whole brain and comparisons are made, with pooled control data. It should be emphasized that these experiments were done each with its own control and that the direction of change in any experiment was always the same as that found when compared with the pooled control data.

(1) One sees first the 2 separable and distinct forms of 5-HT in the brain (with 29% free and thus 2·5 times as much bound as free).

(2) Reserpine, it may be seen, does what many have suspected it does in the brain, namely, it causes a greater depletion of stored 5-HT than of the free form. This results in a proportionally greater amount in the free form and a bound/free ratio of 1·4, instead of 2·5.

(3) It may be seen further that some of the other potent depressants, regardless of their effect on the total cerebral level of 5-HT cause a similar proportional increase in free 5-HT and shift of bound/free ratio toward 1. This includes α-methyl DOPA which inhibits synthesis and decreases the total slightly; phenobarbital which raises the total by 42%; and chlorpromazine which does not alter the total. These results with chlorpromazine fit well the recent suggestion of Gey and Pletscher[2] that chloropromazine acts to reduce the permeability of the storage organelles for the monoamines. Such an action might extend to other drugs.

(4) It is interesting that isoreserpine, which is practically devoid of C. N.S. activity, causes little change in either total 5-HT or in its subcellular distribution.

(5) Finally, 2 other depressants, methylparafynol ("Dormison") and hydroxydione ("Viadril") both of which Pepeu and I have shown to be associated with 70–90% elevations in cerebral acetylcholine, when used in anesthetic doses, have no effect upon the sub-cellular distribution of 5-HT.

TABLE II

Influence of anti-depressants; euphoriants, C. N. S. stimulants and a psychotomimetic agent on subcellular distribution of 5-HT

Treatment	Number of animals	Mean 5-HT (mμg/whole brain) Partic. ("bound")	Supernate "free"	Total	% "Free"	Partic/ supernate (P/S)
1. Iproniazid (100 mg/kg - 12 hr)	17	996 ($p < .001$)*	485 ($p < .001$)	1481	33	2·1
2. β-Phenylisopropyl-hydrazine (10 mg/kg - 12 hr)	16	1050 ($p < .001$)	343 ($p < .01$)	1393	25	3·1
3. Imipramine (25 mg/kg - 75 min)	10	506 ($p < .03$)	200	706	29	2·5
4. LSD-25 (520 μg/kg - 20 min)	8	531 ($p < .01$)	156	687	23	3·4
5. None	62	389	158	547	29	2·5

* The p values listed were derived from Student's "t" table

Table II summarizes data obtained with some drugs which produce behavioral effects essentially opposite to those evoked by the agents in Table I. These are anti-depressant, euphoriant, hallucinogenic and general C. N. S. excitants.

(1) Here, one sees in general that all of these agents cause a rise in total 5-HT but simultaneously either have no effect on the sub-cellular distribution or (as in the case of LSD and to a lesser extent, PIH) lead to an absolute and proportional increase in the *bound* fraction of the amine.

(2) This is most strikingly seen with LSD-25. At a time (20 min) when this dose of LSD had exerted a marked effect on the conditioned behavior of rats, the level of total 5-HT in the brain increased by 25%, all of which can be accounted for in the particulate fraction. Freedman[3] has shown that this effect is more marked in reserpinized animals and can be seen with threshold doses for autonomic and behavioral effects of LSD. Thus, LSD appears to cause an increased binding and storage of 5-HT.

(3) One final interesting aspect of these data is that the large increase in level of total 5-HT in the brain following inhibition of MAO by iproniazid was distributed between particulate and non-particulate compartments with the maintenance of a normal bound/free ratio. This suggests the existence of a dynamic steady-state relationship between the concentration of 5-HT in the stored reserves and that free in the cytoplasm. Presumably, this relationship would be maintained normally by a balance between synthesis and storage of the amine and the rates of release, utilization, and metabolism of the free amine.

We have concluded that this direct approach of studying bound and free neurohumors can:

(1) Substantiate some of the hypothesis already proposed for the action of certain neuropharmacologic agents, e. g., the action of reserpine;

(2) Yield data useful in the development of new and testable hypotheses, such as enhancement of binding of 5-HT by LSD;

(3) Allow study of the interaction of certain agents with the changes produced by reserpine in behavior and in sub-cellular distribution of 5-HT, as well as studies of similar drug and behavioral interactions.

REFERENCES

1. N. J. GIARMAN and S. SCHANBERG; *Biochem. Pharmacol.* **1** 301 (1958).
2. K. F. GEY and A. PLETSCHER; *J. Pharmacol. Exp. Therap.* **133** 18 (1961).
3. D. X. Freedman; *J. Pharmacol. Exp. Therap.* **134** 160 (1961).