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SUPERNATANT–PARTICULATE DISTRIBUTION OF EXOGENOUS SEROTONIN IN RAT BRAIN HOMOGENATES

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Abstract—The distribution of exogenously added serotonin between the residue and high-speed supernatant of brain homogenates prepared from normal and drug-pretreated rats was investigated at 0°, 20°, and 37°. The results indicate that the accumulation of exogenous serotonin by particulate fractions of rat brain homogenates was neither temperature dependent nor affected by previous pretreatment of the animals with either reserpine or tranylcypromine. Addition of serotonin up to a thousand times the normal endogenous levels resulted in no apparent saturation of the binding sites.

The results strongly suggest that accumulation of exogenous serotonin by residues of brain homogenates is a passive phenomenon. The absence of a saturable component in the residues indicates that uptake of exogenous serotonin at high external concentrations is the result of nonspecific binding.

WORKING with brain homogenates, previous investigators have obtained evidence that a dynamic steady-state relationship exists between serotonin in stored reserves and that in a free form.[1, 2] Drugs known to alter serotonin levels *in vivo* were found to influence this relationship.[2, 3] However, few data are available on the effect of exogenous serotonin on this relationship. We have therefore investigated the fate of exogenously added serotonin in rat brain homogenates with respect to temperature, concentration, and drug pretreatment.

MATERIALS AND METHODS

Adult female rats (Wistar strain) ranging in weight from 220–250 g were used throughout. The animals were stunned, decapitated, and the brains rapidly extirpated. For each experiment, the brains, less cerebellums of two rats, were homogenized in unbuffered 0·3 M sucrose containing tranylcypromine (1 μM). The preparation of the homogenates was carried out in a cold room (4°) with a Kontes 7-ml all-glass homogenizer and a standard homogenizing time of 1 min. The final homogenate consisted of two brains dispersed in sufficient 0·3 M sucrose to produce a final volume of 16 ml. Inasmuch as the brain weights varied only between 2·95 and 3·20 g, the final dilution factor was 20% $\pm$ 2% (w/v). An aliquot volume was placed in 25 ml Erlenmeyer flasks and exogenous serotonin added as the creatinine sulfate. Thus, the amount added was constant when expressed as micrograms per millimeter of homogenate and approximately constant when calculated as micrograms added per gram of wet weight of tissue. Subsequently the flasks were shaken for 15 min in a Dubnoff incubator at

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0°, 20°, or 37°. After the incubation period, aliquots were placed in 6·5-ml Spinco cellulose tubes and centrifuged for 30 min at 37,000 rev/min. The serotonin contained in the residue (<1·0 ml) and supernatant (>5·5 ml) was extracted separately as described by Mead and Finger[4] and assayed fluorometrically in concentrated hydrochloric acid as described by Bogdanski *et al.*[5] In each series of experiments, homogenates from rats which had received no drug pretreatment were prepared in the same manner as the experimental samples except that exogenous serotonin was not added. Two duplicate samples were run from each pooled homogenate, and the values presented in the tables represent the average of at least six such determinations. In view of the internal consistency and essentially negative findings of the results they are presented without statistical evaluation.

Animals were injected i.p. with either tranylcypromine (5 mg/kg) or reserpine (5 mg/kg) on each of two days and killed on the third day.

It was of interest to follow the distribution between the particulate residue and the supernatant of only the added serotonin. In order to do this the fluorometric reading for the control samples was subtracted from the experimental samples. The difference was taken to represent exogenous serotonin added as the creatinine sulfate. For purposes of calculation the additions are expressed on the basis of 1 g original wet weight of tissue and all experimental values calculated on this basis; hence all figures in Tables 2, 3, and 4 refer only to the exogenously added serotonin and are not totals of exogenous and endogenous amine. The distribution of serotonin is expressed by two types of concentration ratios: CR_I is the amount of exogenous serotonin found in the residue, divided by the amount of endogenous serotonin present in control residues; CR_{II} is a classic concentration ratio expressing the concentration of serotonin in the tissue particulate fraction relative to the concentration of serotonin in the medium. In this case the tissue concentration is taken as the level of exogenous serotonin in the residue, and the medium concentration is taken as the amount initially added in micrograms per milliliter of homogenate. CR_{II} was uncorrected for the small amounts of supernatant trapped in the residue, which in any case would be only a small fraction of the total volume.

RESULTS

The distribution of endogenous serotonin between the supernatant and high-speed residue in the normal and drug-treated animals after incubation at 0°, 20°, and 37° is given in Table 1. These data are the base line controls for the material presented in the succeeding tables.

Incubation of homogenates obtained from the control animals at 0° for 15 min gave a subcellular distribution similar to that obtained by others for unincubated homogenates.[1] Seventy-five per cent of the serotonin was found in the residue. This may be lower than that actually occurring *in vivo*, since the distributional values obtained vary with the techniques employed[6] and, according to Zieher and De Robertis,[7] the true *in vivo* value may exceed 90%. Higher incubation temperatures resulted in a loss of serotonin from the residue to the supernatant. At 37°, only 52% of the serotonin was found in the residue. Increasing the incubation time from 15 min to 1 hr did not result in a further loss of endogenous serotonin from the residue.

Although administration of tranylcypromine, a monoamine oxidase inhibitor, elevated the total serotonin content of the brain homogenates threefold, we were unable

to find any alteration in the distribution between residue and supernatant at any of the three incubation temperatures. Green and Erickson[8] found an increase of serotonin in the supernatant after administration of tranylcypromine, but the effect diminished with increasing times after injection. As our observations were made three days after the initial injection and one day after the final injection, it appears that tranylcypromine alters the distribution only temporarily.

TABLE 1. BASELINE VALUES OF THE DISTRIBUTION OF ENDOGENOUS SEROTONIN IN RAT BRAIN HOMOGENATES

Drug treatment	Temperature of incubation (°C, 15 min)	5-HT in Residue (μg/g wet wt. tissue)	5-HT in Supernatant (μg/g wet wt. tissue)	Total 5-HT (μg/g wet wt. tissue)	5-HT in Residue (% of total)	5-HT in Supernatant (% of total)
None	0	0·48	0·16	0·64	74·5	25·5
	20	0·47	0·20	0 67	69·1	30·1
	37	0·36	0 32	0 68	53	47
	37*	0·36*	0·32	0·68	52·6	47·4
Tranylcypromine (SKF 385)	0	1·49	0·50	2·00	74·8	25·2
	20	1·43	0·70	2·14	66·8	33·2
	37	0·94	0·80	1·74	53·9	46·1
Reserpine	0	0·115	0 078	0·193	59·5	40·5
	37	0·091	0·083	0·17	52·2	47·8

* Incubation period 1 hr.

Changes in the distribution of endogenous serotonin were, however, observed after administration of reserpine. The serotonin content of the homogenates fell to 25% of control values, and only 60% of this was found in the residue at 0° and only 53% at 37°. Similar findings were reported by others for unincubated homogenates.[2] It is unlikely that values obtained by these techniques can represent the true extent of release of serotonin from its storage sites since, as demonstrated below, the particulate fractions of brain tissue possess a considerable capacity to take up serotonin in a nonspecific manner.

When exogenous serotonin was added to brain homogenates obtained from normal animals, a constant fraction was found associated with the residue after incubation and high-speed centrifugation (Table 2). This is true even though the added serotonin attained a total equivalent to 2 mg/g wet weight tissue. Concentration ratio I shows that the residue picks up a quantity of serotonin proportional to the amount added, and concentration ratio II shows that the ratio between the amine bound by the residues and that added to the homogenate is approximately a constant (2·5). Small losses of exogenous serotonin occurred at 37° or if the homogenates were incubated for 1 hr instead of 15 min.

Essentially similar results were obtained when the animals were pretreated with either tranylcypromine (Table 3) or reserpine (Table 4). Neither elevation nor depletion of pre-existing endogenous levels of serotonin *in vivo* had any appreciable effect on the uptake of exogenous serotonin by the particulate fraction. There was a tendency for the percentage of exogenous serotonin recovered in the residue to be slightly

TABLE 2. NO DRUG PRETREATMENT; BASE-LINE VALUES OF THE DISTRIBUTION OF EXOGENOUS SEROTONIN IN RAT BRAIN HOMOGENATES

Temperature of incubation (°C, 15 min)	Exog. 5-HT added (μg/ml homog.)	Exog. 5-HT in residue (R) (μg/g wet wt. tissue)	Exog. 5-HT in residue as % of total exog. in residue and supernatant (S)	Concentration ratio: Recovery, exog. 5-HT in R+S μg/g wet wt. (exog. added, μg/g wet wt. tissue)	Concentration ratio: CR_I exog. 5-HT in residue (endog. 5-HT in residue)	Concentration ratio: CR_{II} exog. 5-HT in residue (μg/g wet wt.) (exog. 5-HT concentration (μg/ml homog.)
0	0·94	2·37	45·2	103	4·96	2·53
	4·7	11·9	45·2	97	24·8	2·53
	9·4	22·9	45·7	98	47·8	2·44
	93·8	232	41·6	103	484·0	2·47
20	0·94	2·69	51·2	99	5·69	2·87
	4·7	11·8	47·2	93	24·8	2·51
	9·4	22·4	45	95	47·4	2·4
	93·8	199	43·4	89	421	2·2
37	0·94	2·48, 2·62*	51·2, 54*·	93, 95*	6·94, 7·32*	2·64, 2·79*
	4·7	11·8, 12·2*	51·2, 52·4*	89, 90*	33, 33·9*	2·52, 2·6*
	9·4	24·3	49·8	88	68·4	2·61

* Homogenates incubated for 1 hr.

TABLE 3. EFFECT OF PARNATE PRETREATMENT ON THE DISTRIBUTION OF EXOGENOUSLY ADDED SEROTONIN TO BRAIN HOMOGENATES

Temperature of incubation (°C)	Exog. 5-HT added (μg/ml homog.)	Exog. 5-HT in residue (R) (μg/g wet wt. tissue	Exog. 5-HT in residue as % of total exog. in residue and supernatant (S)	Concentration ratio: Recovery, exog. 5-HT in R+S μg/g wet wt. (exog. added, μg/g wet wt. tissue)	Concentration ratio: CR_I exog. 5-HT in residue (endog. 5-HT in residue)	Concentration ratio: CR_{II} exog. 5-HT in residue (μg/g wet wt.) (exog. 5-HT concentration (μg/ml homog.)
0	0·94	2·8	47	112	1·88	2·98
	4·68	11·0	46	93	7·43	2·36
	9·4	23·8	46	92	16·0	2·54
	93·8	198	40	95	133·0	2·11
20	0·94	2·32	47·4	95	1·64	2·48
	4·68	12·5	46·8	98	8·72	2·66
	9·4	24·7	46·5	93	19·2	2·64
37	0·94	2·6	50	98·2	2·76	2·77
	4·68	13·0	53·4	94	13·8	2·77
	9·4	23·0	51·7	86	24·3	2·44

higher at 37° than at 20° or 0°. This was observed in homogenates obtained from both normal and drug-treated animals (Tables 2, 3, and 4). The effect was, however, small in magnitude and indicates that the added serotonin is easily taken up by the residue during the 15-min incubation period.

TABLE 4. EFFECTS OF RESERPINE PRETREATMENT ON THE DISTRIBUTION OF EXOGENOUSLY ADDED SEROTONIN TO RAT BRAIN HOMOGENATES

Temperature of incubation (°C)	Exog. 5-HT added (μg/ml homog.)	Exog. 5-HT in residue (R) (μg/g wet wt. tissue)	Exog. 5-HT in residue as % of total exog. in residue and supernatant (S)	Concentration ratio: Recovery, exog. 5-HT in R+S μg/g wet wt. — exog. added, μg/g wet wt. tissue	Concentration ratio: CR_I exog. 5-HT in residue — endog. 5-HT in residue	Concentration ratio: CR_{II} exog. 5-HT in residue (μg/g wet wt.) — exog. 5-HT concentration (μg/ml homog.)
0	0·94	2·41	50	98	21·0	2·57
	4·7	11·8	47	93·7	103	2·51
	9·4	22·6	46	93	196	2·41
	93·8	237	43·9	98·3	2060	2·53
37	0·47	1·45	52·5	106	15·9	3·09
	0·94	2·56	53	89	28·1	2·73
	4·7	12·2	52·6	90·4	134	2·61

DISCUSSION

We wish to emphasize that the techniques employed in this study would not reveal the presence of a specific accumulation mechanism for serotonin in brain homogenates which was either labile or easily saturable, and that the results apply only to the fate of amounts of exogenous amine in excess of that normally present in brain tissue. However, it does seem clear that in these experiments we are dealing with a nonspecific association of the added serotonin with the particulate phase of brain homogenates. In fact our results are essentially equivalent to those that would be expected if one considered serotonin to behave as a solute distributing itself between an organic and an aqueous phase. The concentration ratios merely express the distribution coefficient or serotonin. Viewed in this manner it is not surprising that the small variations in initial serotonin concentrations induced by reserpine and tranylcypromine had no significant effect on the distribution of serotonin between the residue and the supernatant. The fact that the temperature of incubation had little effect on the percentage recoveries and concentration ratios of exogenous serotonin also is consistent with a nonspecific mechanism.

However, a specific accumulating mechanism does exist in the brain, which can account for the high percentage of serotonin, that can be obtained in the residue of normal brain homogenates. A subcellular system consisting of vesicles derived from disrupted nerve-ending particles, which is labile and easily saturable at low serotonin concentrations, has been described by Maynert *et al.*[9] Some indication of the labile nature of the normal accummulating mechanism is indicated by our finding that there

was a progressive fall in the percentage of endogenous serotonin in the residue of normal brain homogenates as the incubation temperature was increased from 0° to 37° (Table 1). The capacity of the specific association system to store serotonin is severely limited, as indicated by the fact that, after addition of 2·5 μg serotonin/g tissue to the brain homogenates, the percentage of added amine in the residue was no greater than that observed after the addition of 100 times this amount.

In our hands the residue accumulated more exogenously added serotonin (50%) than was found by other workers (Giarman and Schanberg,[1] 23%; Green and Sawyer,[3] 38%). This may be due mainly to differences in overall technique or more specifically to the use of different dilutions of the brain homogenate. Interestingly enough, however, brain slices exhibit the same properties as homogenates with respect to uptake of added serotonin. Schanberg[10] demonstrated that there is no effect of temperature on the accumulation of serotonin by brain slices and, if his data are recalculated to yield a concentration ratio equivalent to our CR_{II}, they agree with our value of 2·5 for the ratio of serotonin in the medium to that in the particulate matter of the slice. In contrast to 5-HT, Schanberg did demonstrate an active transport mechanism for the precursor of serotonin, 5-HTP.

Our homogenates were also incubated in the presence of small amounts of the monoamine oxidase inhibitor, tranylcypromine. This was found necessary to prevent appreciable losses of added serotonin, as suggested by Giarman.[1]

In conclusion, it appears that specific binding of serotonin by subcellular particulate matter of brain tissue cannot easily be studied in crude homogenates, owing to the large capacity of this material to accumulate the amine in a wholly nonspecific manner. In particular, release of serotonin from its selective storage sites during homogenization and its reassociation with the particulate components will serve to complicate any studies in an unpurified system.

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