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INTERACTION OF SEROTONIN WITH THE CATECHOLAMINES—II

ACTIVATION AND INHIBITION OF ADRENOCHROME FORMATION

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Abstract—Adrenochrome formation from epinephrine is either accelerated or inhibited by serotonin, the effect being dependent on the relative concentrations of the indoleamine compared to the catecholamine. The possibility that serotonin can regulate adrenochrome formation is discussed with respect to the postulations that these compounds may be involved in mental disease.

IN THE preceding paper it was reported that serotonin could effectively inhibit the oxidation of dopamine and norepinephrine by forming an inhibitor–substrate complex.[1] It was proposed that complex formation could represent a purely molecular mechanism by which serotonin would modulate the activities of dopamine and/or norepinephrine *in vivo*. From further studies on the structural requirements for the catechol–indoleamine interaction, it became apparent that serotonin would also complex with epinephrine, and, therefore, could modulate its activity. One might have assumed from this, that serotonin would also inhibit the oxidation of epinephrine. However, this assumption did not fit with some of our earlier studies with brain preparations. Our initial studies indicated that serotonin increased epinephrine oxidation at least with these brain preparations. Subsequent studies with other oxidative systems now have shown that generally when the concentration of serotonin is lower than that of epinephrine, adrenochrome formation is accelerated; and when the serotonin concentration is greater than that of epinephrine, oxidation is inhibited. This paper reports these findings.

EXPERIMENTAL

Materials. Epinephrine HCl was obtained from Mann Research Labs. Serotonin creatinine sulfate and tyrosinase were obtained from Sigma Chemical Co. All solutions were prepared in glass distilled water to eliminate metal ion contamination.

Methods. Male albino rats obtained from K-G Farms were used as the source for brain tissue. Brain enzyme preparations were made according to a previously described method.[2] In experiments using brain preparations, the adrenochrome was trapped as the semicarbazide, and the rate of formation was followed by measuring absorbancy at 345 mμ at various time intervals of incubation. The reaction was stopped by heating the flasks for 2 min in a boiling water bath, and the precipitate was removed by centrifugation. Samples were incubated at 37° in a Dubnoff metabolic shaker.

Adrenochrome formation catalyzed by tyrosinase was assayed as previously described.[3]

Base- and copper-catalyzed oxidations were measured spectrophotometrically at 310 mμ in a Beckman DK-2 recording spectrophotometer at room temperature. In the base-catalyzed reaction, the oxidation was initiated by the addition of 0·01 N NaOH, while in the copper-catalyzed reaction, 0·1 M $CuSO_4$ replaced the NaOH.

RESULTS

Brain preparations. Figure 1 presents the results of a typical experiment which demonstrates the activating effect of serotonin on adrenochrome formation by brain preparations. In a series of four different experiments, the range of activation was 36 to 70 per cent under the conditions of this assay. Incubation of serotonin with the brain preparations in the absence of epinephrine did not contribute to absorption at this wavelength.

Tyrosinase. When the serotonin concentration was held below that of epinephrine, activation of adrenochrome formation was also observed with the tyrosinase reaction

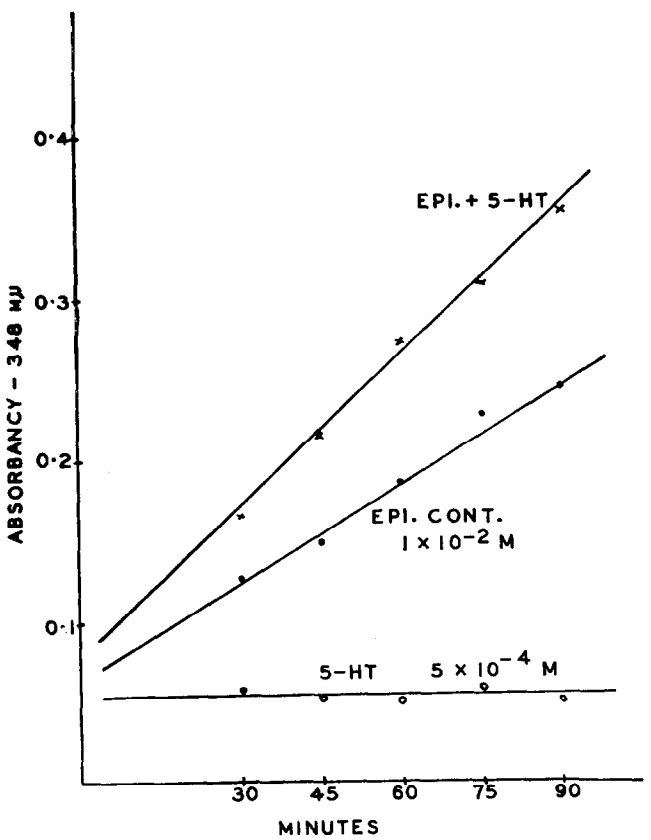

FIG. 1. Effect of serotonin on epinephrine oxidation by rat brain. The assay system contained: 0·2 ml brain preparation; 0·01 M semicarbazide HCl; 0·01 M epinephrine HCl; 0·1 M $NaPO_4$ buffer, pH 6·8; 5×10^{-4} M serotonin in a total volume of 2·0 ml.

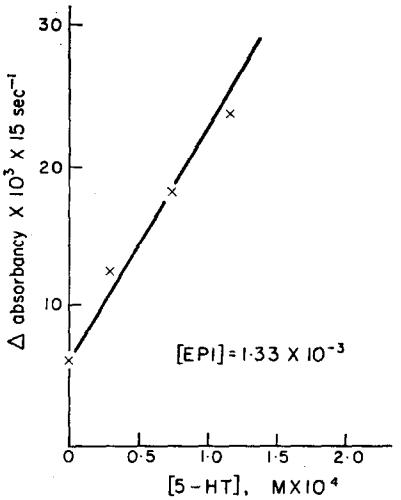

FIG. 2. Effect of low concentrations of serotonin on epinephrine oxidation by tyrosinase. The assay system contained: 0·1 mg tyrosinase; $1{\cdot}33 \times 10^{-3}$ epinephrine HCl; 0·1 M $NaPO_4$ buffer, pH 6·8, in a total volume of 3·0 ml.

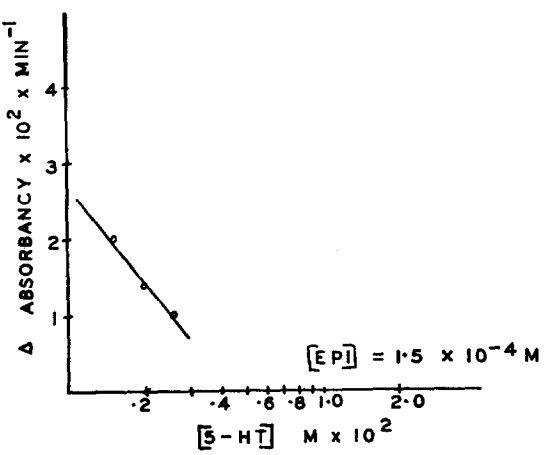

FIG. 3. Effect of high concentrations of serotonin on epinephrine oxidation by tyrosinase. The assay system was the same as seen in Fig. 2, except the concentration of epinephrine was $1{\cdot}5 \times 10^{-4}$ M.

(Fig. 2). The activation proved to be a logarithmic function of serotonin concentration. No changes in absorption occurred when serotonin alone was incubated with the enzyme or when incubated with epinephrine in the absence of the enzyme.

In contrast to the above results, when the concentration of serotonin exceeded that of epinephrine, inhibition resulted (Fig. 3). In Fig. 4, a Lineweaver–Burk plot shows the inhibition to be noncompetitive. With very high concentrations of serotonin, the inhibition was complete.

Copper- and base-catalyzed oxidation. To determine whether the activation phenomenon was independent of the enzyme protein, adrenochrome formation from base-

and copper-catalyzed oxidation of epinephrine was examined. It was found that activation would also occur under these conditions, indicating that enzyme protein was not required. Figures 5 and 6 show these results and demonstrate that the activation is a function of serotonin concentration.

Again, as shown previously, when the serotonin concentration was elevated,

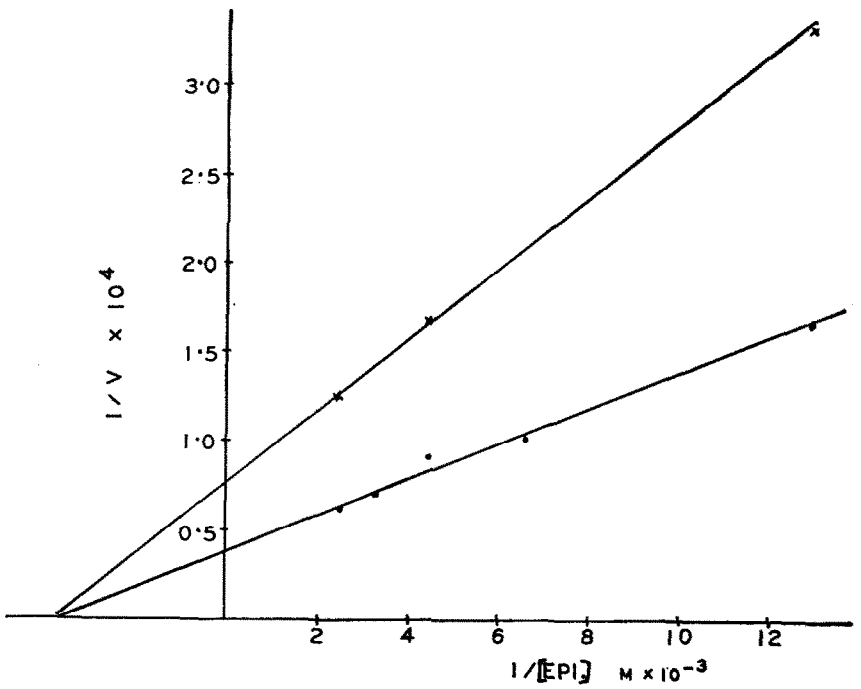

FIG. 4. Lineweaver–Burk double reciprocal plot of the inhibitory effect of serotonin. See Fig. 2 for assay system. V is the change in absorbancy per minute.

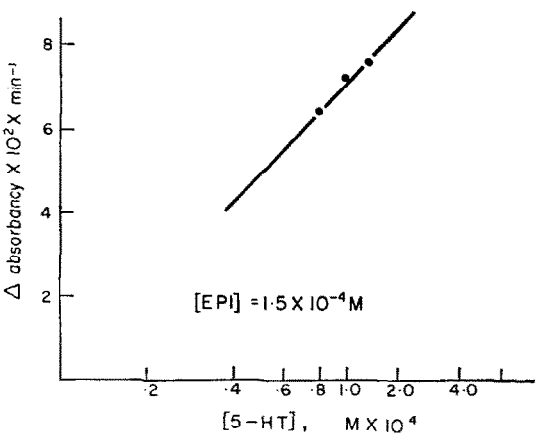

FIG. 5. Effect of low concentrations of serotonin on base-catalyzed oxidation of epinephrine. The reaction was carried out in a total of 3 ml of glass distilled water at room temperature. To initiate the reaction, 0·1 ml of 0·01 N NaOH was added.

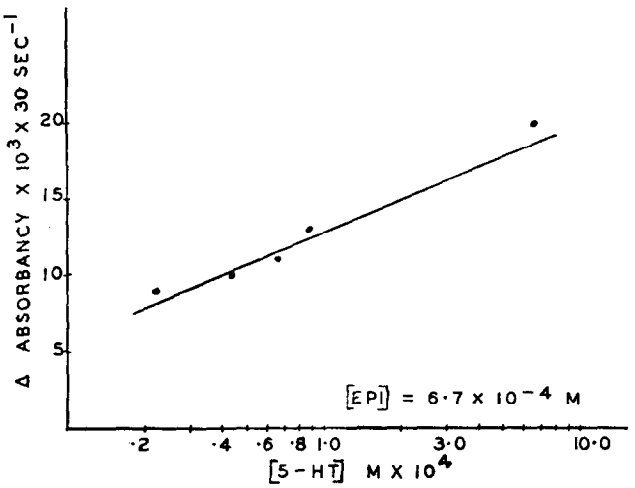

FIG. 6. Effect of low concentrations of serotonin on copper-catalyzed oxidation of epinephrine. The method was the same as in Fig. 5, except 0·1 M $CuSO_4$ replaced the NaOH.

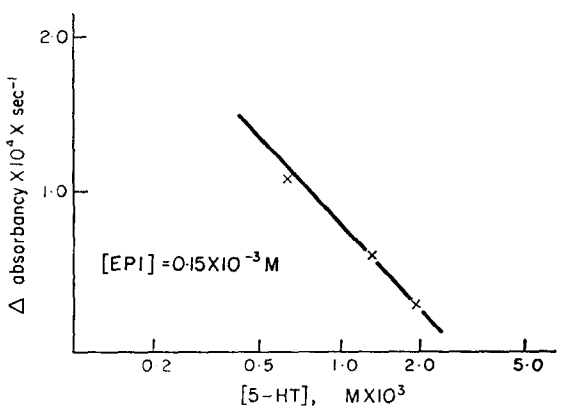

FIG. 7. Effect of high concentrations of serotonin on base-catalyzed oxidation of epinephrine. See Fig. 5 for method.

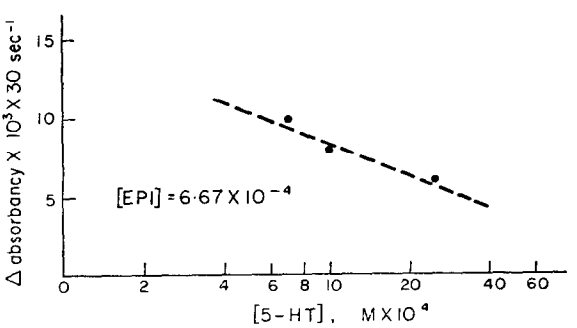

FIG. 8. Effect of high concentrations of serotonin on copper-catalyzed oxidation of epinephrine. See Fig. 6 for method.

TABLE 1. COMPARISON OF THE RELATIVE CONCENTRATIONS OF EPINEPHRINE AND SEROTONIN FOR 50 PER CENT INHIBITION OR ACTIVATION

Catalyzed reaction	Epinephrine concn (M)	Serotonin concn for 50% effect (M)	Ratio
Activation			
Tyrosinase	$1{\cdot}33 \times 10^{-3}$	$1{\cdot}50 \times 10^{-5}$	87·00
Copper	$6{\cdot}67 \times 10^{-4}$	$1{\cdot}60 \times 10^{-5}$	42·00
NaOH	$1{\cdot}50 \times 10^{-4}$	$1{\cdot}55 \times 10^{-4}$	1·00
Inhibition			
Tyrosinase	$1{\cdot}50 \times 10^{-4}$	$2{\cdot}40 \times 10^{-3}$	0·06
Copper	$6{\cdot}67 \times 10^{-4}$	$1{\cdot}40 \times 10^{-4}$	4·80
NaOH	$1{\cdot}50 \times 10^{-4}$	$1{\cdot}10 \times 10^{-3}$	0·14

inhibition resulted. This effect is seen in Figs. 7 and 8. The inhibitory activity also was a function of serotonin concentration within a given range.

Table 1 compares the relative concentrations of epinephrine and of serotonin which were required to produce either 50 per cent activation or 50 per cent inhibition. Only in the case of the copper-catalyzed oxidation was the required concentration of serotonin to give 50 per cent inhibition not greater than that of epinephrine.

DISCUSSION

The exact mechanisms of activation and inhibition of adrenochrome formation by serotonin are presently not understood. However, there is little question that serotonin can modify this reaction.

Hoffer and Osmond[4] proposed that the oxidation of epinephrine to adrenochrome may play a role in the etiology of schizophrenia, but this concept never gained favor with other investigators. Instead, attempts have been made to relate norepinephrine and/or serotonin to the control of behavior and, therefore, to the disease. It has been difficult, however, to identify the specific roles of any of these compounds from the conflicting literature. It is our belief that this difficulty lies in the fact that the catecholamines and serotonin are probably more intimately related than may be apparent at the levels of biological organization thus far studied. The results reported here and in our preceding paper[1] would support this argument. These results provide a basis for establishing a more unified picture of the role of these substances in the control of both normal and abnormal behavior. Serotonin would be modulating the activities of the catecholamines on a purely molecular level and, consequently, any alteration of the brain or systemic levels of the indoleamine would necessarily alter the activities of the catecholamines. The reverse situation could also apply.

There is no reason to believe that what we see *in vitro* does not apply to the environment *in vivo*. It is interesting that the ratio of epinephrine concentration to serotonin concentration is very critical and actually determines the effect which results. If the proper ratio is maintained, adrenochrome formation would be supressed or even absent completely. However, if the serotonin concentration is lowered to trip the balance in favor of epinephrine, adrenochrome formation would be accelerated. In spite of the fact that formation *in vivo* of adrenochrome has never been accepted, it would seem significant to reconsider the question, taking into account the possibility that serotonin may be modulating its formation.

Greiner[5] recently reopened the question of the abnormal pigment formation in schizophrenic patients and concluded that there is an increase of melanin formation either from enzymatic or auto-oxidative mechanisms. Our postulation would provide a common basis for Greiner's observation[5] and the suggestion by Wooley[6] that the disease results from a deficiency of serotonin. Not only would adrenochrome formation be accelerated at low levels of serotonin and, consequently, melanin formation from epinephrine, but also melanin formation from dopamine; and norepinephrine would be freed from the inhibitory effect of the indoleamine.

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