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Evidence That Serotonin Has a Role in Brain Function

The finding of serotonin (5-hydroxytryptamine) in brain (*1, 2*) has aroused speculation that it may have a role in brain function (*3*), perhaps as a neurohumoral agent (*4*). Studies from our laboratory that support this view have shown that lysergic acid diethylamide (LSD), a hallucinogenic agent, suppresses an action of serotonin on the central nervous system (*5*), while reserpine, a drug used in treatment of mental disorders, liberates serotonin from body depots, including intestines (*6*) and platelets (*7*).

The development of a specific fluorometric assay for serotonin in brain (*2*) has now made it possible to test our hypothesis that reserpine action may be mediated through the liberation of serotonin in brain. The serotonin content of brain, normally about 0.55 μg/g, declined rapidly after the intravenous injection of 5 mg/kg of reserpine. Within 30 minutes the total brain serotonin declined by about 75 percent and within 4 hours it declined by about 90 percent. The low level persisted for about 24 hours and then increased slowly, attaining the normal value after about 7 days. The brain is particularly sensitive to the serotonin-releasing properties of reserpine—doses as low as 0.1 mg/kg appreciably lowered the serotonin content.

Reserpine measured fluorometrically was no longer detectable in brain 12 hours after administration (*8*), whereas sedative effects and changes in brain serotonin persisted longer than 48 hours. The sedative effects thus seem to be related to the change in brain serotonin rather than to the concentration of reserpine. This may be interpreted as further evidence that reserpine acts through liberation of serotonin. Throughout the period of low serotonin content in brain, 5-hydroxyindoleacetic acid, the metabolic product of serotonin, appeared in urine in appreciable amount. Presumably, therefore, serotonin was still being formed in the body but was not accumulating in brain tissue. Thus it seems that reserpine causes an alteration in the serotonin-binding capacity of brain cells that persists long after reserpine can no longer be detected.

Our present concept, on the basis of the available facts, is as follows: serotonin in brain is normally present mainly in a bound form, thus being protected from the highly active enzyme, monoamine oxidase (*9*). After reserpine administration, brain cells lose, in part, their capacity to retain serotonin. As a result, serotonin is liberated and metabolized by the action of monoamine oxidase. Although reserpine rapidly disappears from the brain, the effect on the capacity of cells to retain serotonin persists. Since serotonin is still being formed during this period, much of it is presumably present in a free or physiologically active form. This free form of serotonin is considered as the mediator of the prolonged reserpine action. Since free serotonin is rapidly metabolized, the total serotonin remains at a low level.

The data described in this communication are in accord with the view that serotonin has an important role in brain function, possibly as a neurohumoral agent.

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Systematic Effect of C^{14}-Labeling on Ion-Exchange Chromatography of Amino Acids

In the course of studies on amino acid metabolism in mammalian cells in tissue culture (*1*), protein hydrolysates from cells fed C^{14}-labeled glucose or glutamine were chromatographed on an ion exchange column. It was observed that the peaks of radioactivity in the effluent did not precisely coincide with amino acid peaks located by the ninhydrin color reaction, but in every case followed them closely. The two curves were identically shaped, and activity did not occur elsewhere in the effluent. It seemed likely that the heterogeneity was the result of the partial resolution of labeled from unlabeled amino acid. This was shown to be the case by chromatography of known C^{14}-labeled amino acids. Some preliminary observations concerning the nature of the effect are reported in this paper.

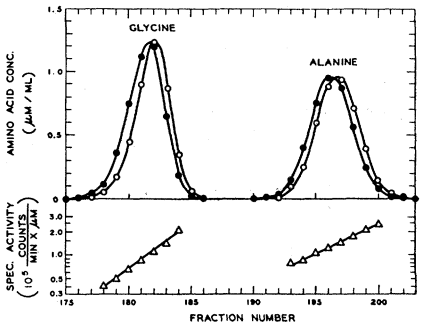

Fig. 1. Portion of the effluent curve from the chromatography of C^{14}-labeled amino acids on a 100- by 0.9-cm column of Dowex 50: ●, amino acid concentration as determined by ninhydrin; ○, amino acid concentration as determined by C^{14} count assuming 100 percent recovery; and △, calculated specific activities.

The ion-exchange separation used was a modification of the methods of Moore and Stein (*2, 3*). A 100- by 0.9 cm column of Dowex 50 (*4*), operated at 50°C and 6 ml/hr throughout the run, was employed. Elution was started from a reservoir containing 250 ml of a 0.37*M* citrate buffer (0.25*N* in sodium citrate) of *p*H 3.10. A continuous *p*H gradient was produced by the addition of 0.25*N* sodium hydroxide to the buffer to give a ratio of flow rates into and out of the reservoir of 1 to 5.7. After about 170 ml of effluent had been collected, the ratio of flow rates was changed to 1 to 1. The degree of resolution provided was significantly greater than it was in the original Moore and Stein procedure (*2*) with a 100 cm column and in some respects was equal to that of their newer method (*3*) employing a 150 cm column.

Approximately 4 μmoles each of L-aspartic, L-threonine, L-serine, L-proline, glycine, L-alanine, and L-valine (*5*), all uniformly labeled with C^{14} at a level of about 0.1 μc/μmole, were chromatographed. One-milliliter fractions were collected and 3 ml of water were added to each. One milliliter was taken for determination of amino acid concentration with ninhydrin (*6*). Carbon-14 was determined on a 0.5-ml portion of the remainder after diluting 1 to 50, except at the leading and trailing edges of the peaks, where 0.5 ml of undiluted sample