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SHORT COMMUNICATION

The uptake of 5-hydroxytryptamine-^{3}H from the cerebral ventricles: autoradiographic localization*

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THE ABILITY of rat brain to take up norepinephrine injected into the lateral cerebral ventricles has recently been reported.[1–3] Glowinski has indicated that 5-hydroxytryptamine (serotonin) injected in this manner is also taken up by brain.[4] By injecting directly into the ventricle, the problems arising from the brain permeability barrier to parenterally administered amines are avoided. However, the specificity of this uptake in terms of cellular elements within the brain has not been established. The choroid plexus, for example, has been shown to possess an active transport system for 5-hydroxytryptamine[5] and represents one of the possible non-neuronal sites of uptake from the ventricular system. The purpose of the present study is to ascertain the localization of tritiated 5-hydroxytryptamine in rat brain after intraventricular injection by means of combined autoradiographic and biochemical assay procedures.

Rats, lightly anesthetized with chloral hydrate, were mounted in a stereotactic headholder, and 10 μl 5-hydroxytryptamine (3 μc, sp. act. 4·3 c/m-mole)† was injected into a lateral ventricle. At various times after the injection, animals were sacrificed and the brains quickly excised and quartered in saline by sagittal and coronal transection. The resulting four pieces of brain were rinsed in a second container of saline and then homogenized in 0·1 N HCl. Aliquots of the brain homogenate, the saline washes, and a serotonin extract of the brain homogenate[6] were counted in a Packard Tri-Carb liquid scintillation spectrometer. For the autoradiographic studies, the rats were taken 4 hr after an intraventricular injection of 20 μc 5-hydroxytryptamine-^{3}H. At this time the animals were fully awake and appeared normal. Brains were fixed by perfusion through the heart with 5% buffered glutaraldehyde.[7] Various paraventricular regions of the brain were dissected into small blocks and refixed in osmium tetroxide, dehydrated in graded ethanols, and embedded in Maraglas-DER 732.[8] Approximately 45 per cent of the total fresh brain radioactivity remains after the processing. Thus, this residual, more firmly bound activity is what is being determined by the autoradiographs described below. Sections of these blocks, 2 μ in thickness, were cut with glass knives, mounted on microscope slides, and coated with Ilford L-4 nuclear emulsion. The slides were then allowed to expose for 2-4 weeks at 4°.

Spectrophotofluorometric assays[6] showed that neither the anesthesia used nor the injection of the tritiated amine produced a detectable alteration in the endogenous brain level of 5-hydroxytryptamine. Within 15 min of the injection, about 2/3 of the original label has left the brain, possibly because of an active transport system in the choroid plexus.[5] At the end of 4 hr 16 per cent of the originally injected 5-hydroxytryptamine has survived and comprised more than 75 per cent of the total brain radioactivity at that time (as determined by the solvent extraction method). The saline washes, which are likely to include the ventricular fluid, contain less than 10 per cent of the total activity at 4 hr. We estimate a 4- to 5-hr half-life of survival of the intraventricularly injected amine. This figure is in line with the longer half-life estimate of Gal *et al.*, obtained by a different technique.[9] Other experiments tend to indicate a much shorter half-life for brain 5-hydroxytryptamine.[9–11] At any rate, the present results indicate that 5-hydroxytryptamine injected into the ventricles is retained and presumably bound in a protected fashion and suggest that autoradiographic localization of the amine may be feasible.

The results of the autoradiographic studies indicate that the isotope retained after intraventricular

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injection is localized in a specific pattern within the parenchyma of the brain. Four hours after the injection, all brain areas examined (amygdala, septum, caudate nucleus, hypothalamus, and the central gray region of the brain stem) clearly exhibit activity in the neuropil adjacent to the ventricles. The penetration of the labeled amine into the hypothalamus and septum are illustrated in Fig. 1. The intensity of the activity is attenuated with increasing distance from the ventricular surface. At 4 hr, some autoradiographic grains still overlie the ependymal cells which line the ventricles. Within the neuropil, the arrangement of grains as seen by phase microscopy indicates a selectivity of distribution: virtually the only sites showing intense activity (i.e. grain clusters) are peridendritic and perineuronal in location. Few grains are actually seen *within* either glial or neuronal cell bodies, myelinated axons, or blood vessels.

Preliminary experiments on the relative pharmacological responsiveness of labeled and endogenous amine indicate that specific activity can be doubled by pretreatment of the animals with 20–30 mg harmine/kg. In addition, reserpine (2 mg/kg i.v.) given 1 hr after the ventricular injection produces approximately a 30 per cent reduction of labeled 5-hydroxytryptamine retained at the 4-hr point. In agreement with similar experiments on intraventricularly injected norepinephrine,[3] the reserpine depletion results in increased specific activity because there is a two- to threefold greater depletion of endogenous amine than labeled amine. It is also of interest that animals deeply anesthetized during the intraventricular injection with combinations of pentobarbital and chloral hydrate retain approximately 20 per cent less radioactive amine.

The results of the present experiments show that intraventricularly injected 5-hydroxytryptamine-H has actually penetrated into the parenchyma of the brain rather than merely being "adsorbed" upon a non-neuronal element such as the ependymal lining. In addition, the intense peridendritic autoradiographic activity is strongly suggestive of localization to nerve terminals. Preliminary electron microscopic autoradiography tends to confirm this. Light and electron microscopic autoradiography of intraventricularly injected norepinephrine[12] also shows the sites of amine binding to be mainly in relation to nerve endings and unmyelinated axons. The question therefore arises whether the uptake of intraventricularly injected amines is specific in terms of those cellular sites that store the different endogenous amines. The observation that neither the labeled norepinephrine nor the labeled 5-hydroxytryptamine is depleted to as great an extent as the endogenous amines suggests that, whatever the cellular specificity of the uptake, thorough mixing of the isotope with the reserpine-sensitive portion of endogenous amine is not complete by the end of 4 hr. In any event, the relationship between the cellular sites that bind the intraventricularly injected 5-hydroxytryptamine and the various hypothesized pools of endogenous amine must now be investigated.

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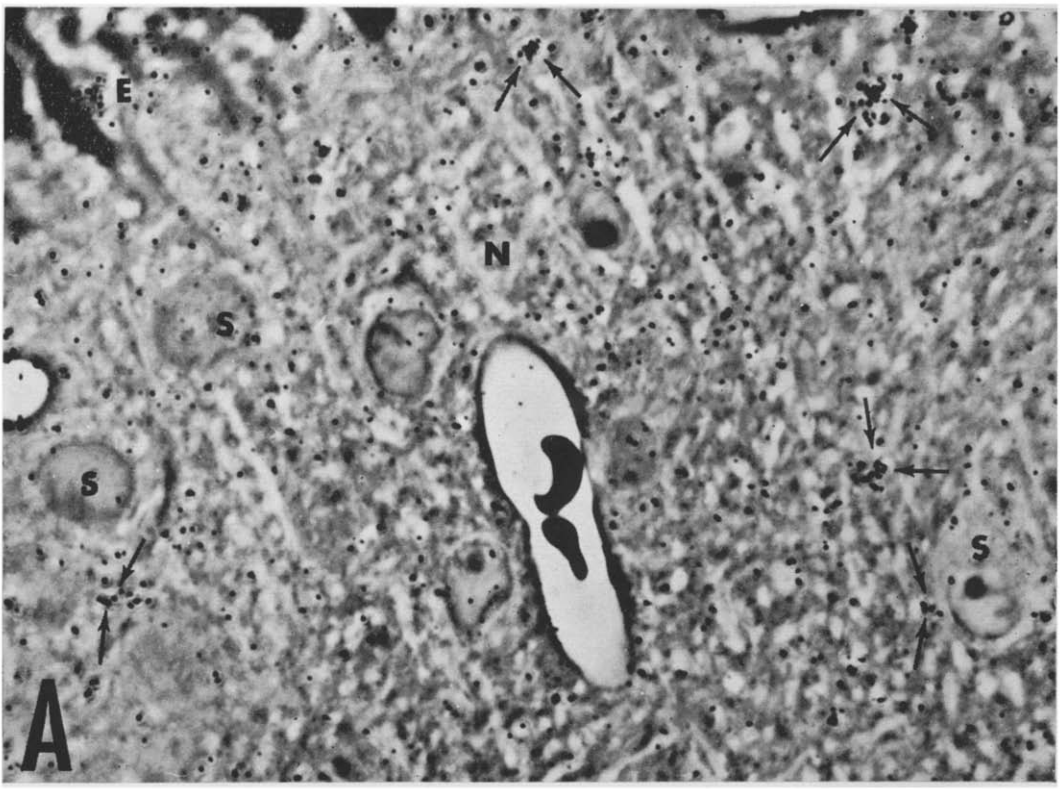

FIG. 1A.

FIG. 1. Autoradiography of anterior hypothalamus and septum after injection of 5-hydroxytryptamine-^{3}H into a lateral ventricle. Sections were developed after a month of exposure and visualized by phase microscopy.

A. Hypothalamus. Autoradiographic grains (black dots) are found some distance away from the ependymal cell layer (E), indicating a considerable penetration of brain parenchyma from the ventricle. Dendrites can be recognized as small, light streaks or patches scattered throughout the neuropil (N). There are some peridendritic sites exhibiting extremely intense activity (arrows). Neuron somata (S), in contrast, do not contain such concentrations of activity.

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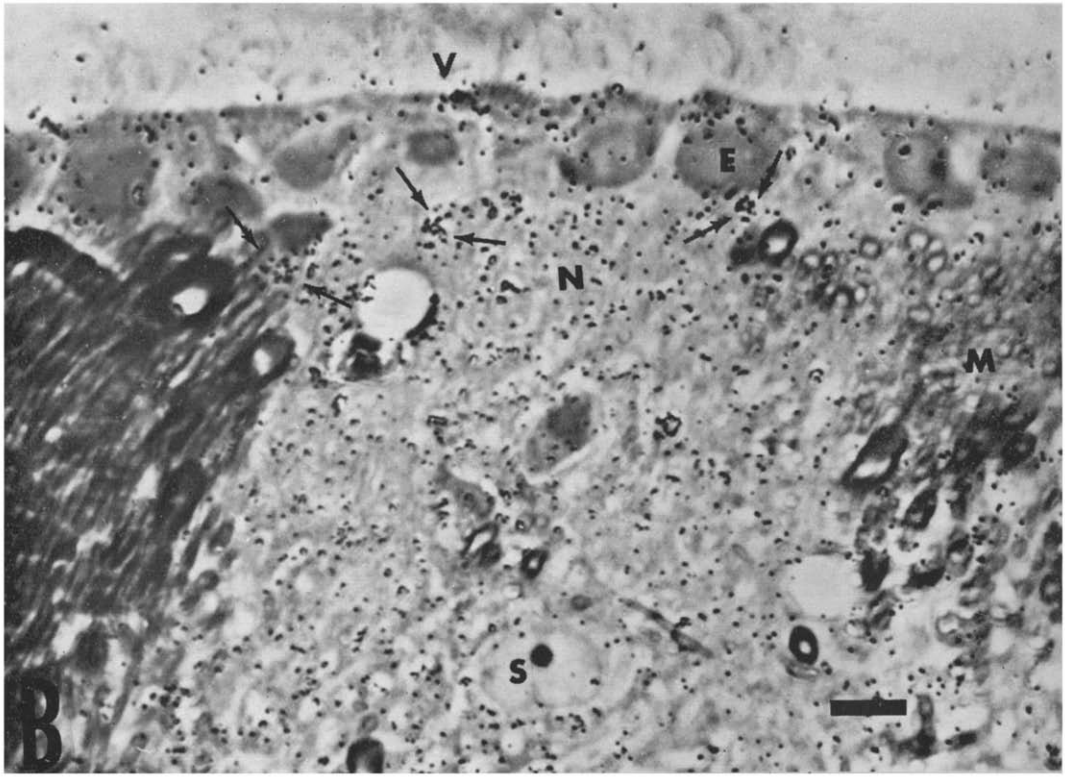

FIG. 1B.

B. Septum. Although the neuropil (N) near the ventricular surface (V) is intensely labeled (arrows), adjacent myelinated fiber tracts (M) and ependymal nuclei (E) have little activity. A large neuron soma (S)bordered by grains is seen in this field. Scale 5 μ.

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