The uptake of hydrocortisone in mouse brain and ependymoblastoma

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At 2, 10, and 60 min after intravenous injection of tritiated hydrocortisone into tumor-bearing mice, samples of brain and tumor were taken for autoradiography. Within 2 min of injection, large amounts of the steroid had left the bloodstream and had penetrated normal brain. By 60 min virtually all the drug had left the brain. The most radioactive structure was the choroid plexus. Within the normal and edematous brain, hydrocortisone was not found in cells alone but was spread randomly throughout the tissue. Edematous brain adjacent to implanted tumor contained much more steroid than normal brain. This difference was maximal at 10 min after injection. Edematous white matter adjacent to tumor was usually as radioactive as tumor. In the ependymoblastoma at 2 min after injection, neoplastic cells and interstitial tissue adjacent to blood vessels contained much hydrocortisone. At 10 min the drug was uniformly spread through the tumor tissue and by 60 min was largely gone. The uptake of the drug by the edematous brain suggests a direct local action. The high choroid plexus concentration may indicate a direct action there, perhaps to reduce cerebrospinal fluid production.

KEY WORDS · hydrocortisone · mouse brain · cerebral edema · experimental brain tumor

Over the last 10 years, glucocorticosteroids have proved effective in the treatment of cerebral edema due to brain tumors and other causes, but their mechanism of action is still obscure. In an attempt to elucidate the way in which this class of drugs works, we have studied by autoradiography the pattern of uptake of radioactive hydrocortisone in normal mouse brain, subcutaneously implanted mouse ependymoblastoma, and swollen brain bearing implants of the same tumor. We felt that a knowledge of where injected glucocorticoids lodge might provide information about how they prevent or reduce cerebral edema.

Microscopic autoradiography involves first the making of a histological section of the tissue containing radioactivity and then the mounting of the section on photographic film sensitive to radioactivity. After a storage period the film is developed and the tissue stained. Grains of silver are viewed microscopically superimposed upon the tissue section. The grains indicate the sites of concentration of radioactive substances in the tissue. Until quite recently, autoradiography was only useful for demonstrating the location of radioactive-labeled substances that had been fixed in tissue, since the standard methods of histological tissue preparation require the use of solvents that leach soluble substances, such as drugs, from their in vivo
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sites of concentration. The present study uses a new method of immediate fixation and autoradiography developed by Stumpf and Roth\textsuperscript{12} that permits localization of labeled drugs in their natural sites rather than in the sites to which they may have migrated after tissue removal but before completion of fixation.

**Materials and Methods**

Female mice of strain C57BL/6J weighing between 17 and 20 gm were used for all experiments. An ependymoblastoma of the type induced by Zimmerman and Arnold\textsuperscript{17} maintained by serial subcutaneous implantation in our laboratory for 6 years was implanted subcutaneously for one series of experiments and intracerebrally for another. For the subcutaneous implants the tumor was cut into fragments small enough to be drawn into a 16-gauge trocar, and four to five fragments were implanted subcutaneously into the lower abdominal region of mice stunned with ether. The tumors grew in about 80% of the animals and were used for experiments about 3 weeks after implantation. For the intracerebral implants, the tumor was removed from the donor mouse with aseptic technique, added to a small amount of physiological saline, and ground to the consistency of broth with a mortar and pestle. The resulting suspension was drawn into a 1 ml syringe on which was mounted a 22-gauge needle. Under sterile conditions the needle was forced through the right parietal region of the skull of a mouse stunned with ether, and approximately 0.01 ml of broth injected. Tumors grew in all of these mice and were used for experiments about 3 weeks after implantation.

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Tritiated hydrocortisone* was supplied in a benzene-ethanol solution. For each experiment, an appropriate volume of the hydrocortisone solution was allowed to dry in a Petri dish at room temperature and then was redissolved in a 0.9% solution of sodium citrate for injection. All mice were weighed and received an intravenous injection via the tail vein of 75 $\mu$g/20 gm body weight in a volume of approximately 0.3 ml. This dose corresponds to 3 mg hydrocortisone in a 70 kg man.

At 2, 10, or 60 min after injection, samples of brain and tumor were taken for autoradiography from mice bearing subcutaneous or intracerebral tumor implants. Four animals were studied at each time. The tissues were quenched in isopentane cooled in liquid nitrogen within 15 to 60 sec of interruption of their blood supply. Sections 2 $\mu$m thick were cut from the frozen specimens in a cryostat at $-30^\circ$ to $-50^\circ$C\textsuperscript{13} and freeze-dried in a Stumpf/Roth cryosorption apparatus at $-68^\circ$176 without thawing. Once dry, the tissue sections were mounted dry on desiccated microscopic slides previously dipped in Kodak NTB-3 emulsion diluted 50:50 with distilled water according to the method of Stumpf and Roth.\textsuperscript{13} At no time was the tissue allowed to come in contact with water or any other solvent that might dislodge the tritiated hydrocortisone from its in vivo localization. After an exposure period of 2 to 5 weeks, the autoradiographs were developed and stained with methyl green pyronin.

**Results**

**Uptake in Normal Brain**

The autoradiographs taken 2 min after injection of tritiated hydrocortisone showed that a large amount of the tracer had left the bloodstream and penetrated the normal brain. However, radioactivity in the lumina of the blood vessels was greater than the radioactivity in adjacent brain. At 10 min (Fig. 1), much more radioactivity had penetrated cerebral tissue but the number of grains over the blood vessels was still greater than over adjacent brain. By 1 hour only a small amount of radioactivity remained in the brain and blood vessels.

At all times the uptake in the cortex was approximately twice as high as in white matter. In normal brain the most radioactive structure was the choroid plexus and this was true at all times after injection.

Within the brain, radioactivity was not limited to any particular type of cell. The number of grains over the perikarya of neurons and the cell bodies of glial cells was approximately the same as over the adjacent areas free of cell bodies.

\* Hydrocortisone-1,2-\textsuperscript{3}H, specific activity 88 mCi/mg in benzene-ethanol solution, supplied by Amersham/Searle Corporation, Chicago, Illinois.
Uptake in Edematous Brain

The accumulation of radioactivity in the brain adjacent to intracerebrally-implanted ependymoblastoma was studied at 2, 10, and 60 min after injection of the tracer. At 2 min this edematous brain tissue, which was vacuolated, lighter staining, and distorted in architecture due to compression, was much more radioactive than more remote areas of the brain. Between 2 and 10 min this edematous brain gained much more radioactivity than did remote brain tissue, and at 10 min the contrast between normal and edematous brain was maximal. By 60 min there was no recognizable difference between edematous and nonedematous brain.

The cerebral cortex in edematous brain was more radioactive than the white matter, except for areas of white matter immediately adjacent to the ependymoblastoma. This white matter was more radioactive than the cortex adjacent to the tumor; indeed, it was usually as radioactive as the tumor itself. As with normal brain, there was no selective uptake of tracer by any particular cell type. There were as many grains over the perikarya of neurons and the cell bodies of glial

Fig. 1. Normal mouse cerebral cortex remote from an intracerebral ependymoblastoma 15 days after implantation. Autoradiograph 10 min after injection of hydrocortisone. A capillary and several neurons are seen. Exposure time, 19 days. ×1700.

Uptake in Ependymoblastoma

There was a rapid spread of radioactivity from the blood vessels into the tumor tissue. At 2 min, neoplastic cells adjacent to the blood vessels were already markedly radioactive with some radioactivity spreading farther out into the tumor tissue (Fig. 3). By 10 min the radioactivity had spread evenly throughout the tumor (Fig. 4), and at 60 min only a small amount of radioactivity remained in the tumor. There were no detectable differences in the distribution of the radioactivity between intracerebral and subcutaneous tumors. At all times, the subcutaneous tumors contained much more radioactivity than did normal gray and white matter

Fig. 2. Choroid plexus of mouse bearing intracerebral ependymoblastoma 21 days after implantation. Autoradiograph 2 min after injection of hydrocortisone. Exposure time, 19 days. ×1150.

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Fig. 3. Blood vessel in intracerebral ependymoblastoma 22 days after implantation. Autoradiograph 2 min after injection of hydrocortisone. Exposure time, 19 days. ×1150.

from the same animals. Likewise, brain-implanted tumors contained much more radioactivity than areas of brain remote from the tumor, and, as mentioned above, white matter adjacent to the tumor usually became as radioactive as tumor.

Within the tumors, grains were spread evenly between neoplastic cells and interstitial tissue, and this distribution did not alter with time. Similar to brain, endothelial cells were not selectively labeled. When the thin connective tissue capsule of the subcutaneous tumors was included in the section, it was slightly more radioactive than the neoplastic cells.

Discussion

One of the striking findings in this study was the speed of penetration of hydrocortisone into normal mouse brain, edematous brain, and ependymoblastoma. In normal brain, a large amount of extravascular radioactivity was present as early as 2 min after injection, indicating the permeability of the normal brain capillary to this steroid. At 2 and 10 min edematous brain contained approximately twice as much radioactivity as normal brain, indicating either a breakdown in the blood-brain barrier for hydrocortisone in edematous brain or seepage of hydrocortisone from the adjacent tumor. Perhaps autoradiographs performed at less than 2 min after injection could have determined the route by which hydrocortisone entered the edematous brain. At 60 min in both normal and edematous brain there was almost no radioactivity remaining. Using C57BL mice, previous investigators have shown in inflamed and normal non-neural tissue (connective tissue) that hydrocortisone and its metabolites, principally tetrahydrocortisone, diffuse back into the bloodstream and are recovered quantitatively in the urine. There is no degradation of the steroid nucleus. It seems likely, therefore, that the rapid disappearance of the autoradiographic grains in the present study was due to the passage of labeled steroids back into the bloodstream rather than to degradation of the steroid nucleus in tumor or brain.

The uptake of tritiated hydrocortisone by the ependymoblastomas was also very swift. At 2 and 10 min there were numerous neoplastic cells that appeared to be highly labeled, although many grains were also present

Fig. 4. Blood vessel in intracerebral ependymoblastoma 15 days after implantation. Autoradiograph 10 min after injection of hydrocortisone. Exposure time, 19 days. ×1700.
over the interstitial space of the tumor. There was also a rapid loss of radioactivity from the tumor, which occurred between 10 and 60 min.

These remarkable permeability characteristics of tritiated hydrocortisone in brain and tumor contrast markedly with other tracers that we have studied by autoradiography in our laboratory. For example, radioiodinated human serum albumin (\(^{125}\)I-HSA) did not penetrate the capillary wall in normal brain, even up to 24 hours after injection.\(^{14}\) In the ependymoblastoma, \(^{125}\)I-HSA was still mainly in the blood vessels 10 min after injection, and then over the next 24 hours it penetrated slowly into the interstitial space and into the neoplastic cells. The ability of hydrocortisone to permeate normal brain, edematous brain, and tumor may point to the mechanism of the therapeutic action of steroids in patients with brain tumors.

Many theories exist to explain the efficacy of steroids in the treatment of patients with intracranial tumors. It has been postulated that steroids reduce cerebral edema,\(^{2-5,7}\) or shrink the size of tumors possibly by retarding the growth of neoplastic cells,\(^{1,6,16}\) or reduce the production of cerebrospinal fluid (CSF).\(^{15}\) It has also been suggested that steroids exert their influence on some extracerebral site, for example, the kidneys.\(^{9}\)

Hypotheses about the mechanism of action of a drug derived from examination of its distribution in tissue are predicated upon the assumption that the presence of the drug in sufficient quantity to be detected is necessary for its action. This study has examined the distribution of hydrocortisone in normal and edematous brain, choroid plexus, and ependymoblastoma. The presence of hydrocortisone or its metabolites in considerable quantity in tumor and both normal and edematous brain supports the hypothesis of local action in these tissues. This is similar to the studies of the mechanism of action of hydrocortisone in inflammation in non-neutral tissues which find a local action.\(^{2}\) It is interesting to note that there is no selective concentration of the drug within cell bodies of the neural tissue or the tumor.

The heavy uptake of the tracer by choroid plexus far in excess of extracerebral connective tissue suggests that this may be an important site of action of steroids. Unfortunately, it could not be determined whether this heavy uptake was due to tracer lodged in the lumina of the blood vessels or in the endothelial or epithelial cells.

Although there are some species differences in the metabolism of steroids,\(^{8}\) the rapid uptake and disappearance of the tritiated hydrocortisone in the mouse tissues suggest that, when this drug is being used therapeutically in patients with brain tumors, it should be given frequently to maintain high blood levels.

**Summary**

The uptake of tritiated hydrocortisone was studied by autoradiography in normal mouse brain, subcutaneously implanted mouse ependymoblastoma, and swollen brain bearing implants of the same tumor.

In normal brain, within 2 min of injection the steroid from the bloodstream had penetrated into cerebral tissue. The most radioactive structure was the choroid plexus. The tracer was not found in cells alone but was distributed randomly throughout the tissue. By 60 min virtually all the drug had left the brain.

Edematous brain adjacent to implanted tumor contained much more steroid than normal brain. This difference was maximal at 10 min after injection. Edematous white matter adjacent to tumor was usually as radioactive as the tumor. Within edematous white or gray matter, the tracer was distributed randomly in cells and neuropil.

In the ependymoblastoma at 2 min after injection, neoplastic cells and interstitial tissue adjacent to blood vessels contained much hydrocortisone. At 10 min the drug was uniformly spread through the tumor tissue and by 60 min was largely gone.

The uptake of the drug by the edematous brain suggests a direct action here. A direct action on the tumor has not been excluded. The high choroid plexus concentration may indicate a direct action there, perhaps to reduce CSF production. Because of the rapid uptake and clearance of the drug by all tissues, frequent or continuous administration of steroids in cerebral edema may be more beneficial than dosages at longer intervals.
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Acknowledgments

The authors gratefully acknowledge the technical assistance of Mrs. L. Marmash and Mrs. F. Crawford and the encouragement and direction of Dr. T. P. Morley.

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Received for publication June 11, 1971.
Supported by Ontario Cancer Treatment and Research Foundation Grant No. 247, by Sunnybrook Hospital, University of Toronto Clinic Grant No. 69–35, and by Defense Research Board Grant No. 9020–04.

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