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Spatial Distribution of Dopamine, Methotrexate and Antipyrine During Continuous Intracerebral Microperfusion

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The spatial distributions of 3 model drugs, [14C]dopamine hydrochloride (DA), [3H]sodium methotrexate (MTX) and [14C]antipyrine (AP), were determined after 6 days continuous microperfusion of the diencephalon of rabbits. The basic pattern of distribution was the same for each drug: the tissue concentration, expressed as a function of the perfusate concentration, was maximal at the cannula tip, and declined sharply with radial distance from the tip. However, at any given distance, concentrations of radioisotopes derived from DA and MTX, both ionized, lipid-insoluble drugs, were one to two orders of magnitude higher than those derived from AP, a lipid-soluble drug. Although concentrations of isotopes derived from DA and MTX were in the same range, their distribution patterns were consistently different. The results demonstrate that intracerebrally microperfused drugs may have quantitatively different spatial distributions, related to their physicochemical characteristics and/or their binding and metabolism in brain tissue.

INTRODUCTION

Compounds fall into two classes with respect to their movement through cerebral capillary endothelium. Those that have high lipid/water partition coefficients move freely across the capillaries. Polar, lipid-insoluble compounds do not pass the cerebral capillaries readily unless aided by carrier-mediated processes, and are said to be excluded by the blood–brain barrier[22]. When drugs of this type are administered systemically, the blood–brain barrier effectively prevents attainment of therapeutic levels in brain or brain tumors. Various means of elevating brain tissue concentrations of such drugs are being investigated, i.e., administration of the drug in modified form[3,5], intraventricular administration of drug[1,2,6,24,27] and osmotic modification of the barrier[18,22,28].

Another method entails direct microperfusion of brain or tumor tissue with drug solutions. Its first research use was norepinephrine microperfusion of feline visual cortex in studies of visual cortical neuronal plasticity[14]; the first suggestion that microperfusion might have therapeutic value came from Kroin and Penn[15]. This same group has recently demonstrated[19] antitumor efficacy of cisplatin and 5-fluorouracil microperfused directly into experimental intracranial neoplasms. The volume of tissue that can be microperfused is considered to be a sphere of tissue, the radius (r) of which is the distance from the cannula tip to the point of minimum effective concentration of drug in the tissue. Tissue microperfusion is practical only if: (1) r is much larger than the cannula radius; and (2) r is sufficiently large to reach a clinically significant volume of tissue. The focus of this study was on measuring microperfusion volumes for substances of potential therapeutic interest under conditions which would simulate a regimen of microperfusion over a period of days. Two model drugs were selected: methotrexate (MTX) for its possible use as an antitumor agent in the brain, and dopamine (DA) for its potential in treating Parkinson's disease. Local delivery of MTX to a cerebral neoplasm or of DA to the substantia nigra would not only improve access of drug to the target tissue but would limit the side effects due to elevated levels of the drug in other areas of the brain, or elsewhere in the body.

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All the model drugs were microperfused in rabbit diencephalon for 6 days to simulate a chronic regimen. This perfusion period was long enough to assure that the animals fully recovered from the effects of surgery, and that steady-state conditions prevailed at the microperfusion site.

DA and MTX are highly ionized at physiological pH and are representative of drugs that do not cross the blood–brain barrier readily[10]. When they diffuse through brain tissue, they are removed slowly if at all by the capillary circulation, and are essentially trapped in the tissue. Specific characteristics of these drugs might influence their distribution. DA is taken up by catecholamine-containing nerve terminals[25] and is metabolized locally[13]. MTX equilibrates rapidly between intracellular and extracellular space but is not metabolized[1,12]. MTX binding to brain cells has been suggested[16,23] but not demonstrated.

It was of interest to compare the microperfusion volumes of these drugs with the distribution of a substance that crosses the blood–brain barrier easily. Antipyrine was selected to represent this type of compound on the basis that it has a high lipid/water coefficient, and has been shown to move rapidly through cerebral capillaries[21]. It is metabolized primarily by oxidative processes in the liver[4,7]. Metabolism in brain tissue has not been demonstrated.

MATERIALS AND METHODS

Infusion system

The infusion system consisted of an L-shaped 30-gauge (i.d. 0.15 mm, o.d. 0.30 mm) stainless steel cannula connected by vinyl tubing to an ALZET mini-osmotic pump (Model 2001, ALZA Corporation, nominal pumping rate 1 μl/h for 7 days) filled with the drug solution. Drugs were dissolved in artificial cerebrospinal fluid (CSF) (in mmol/l: Na 150, K 3.0, Ca 1.4, Mg 0.8, P 1.0, Cl 155) containing sodium fluorescein (0.001–0.01%) to mark the infusion site in the brain. The solution was sterilized by passage through a 0.22 μm filter (Millipore). Antipyrine [N-methyl-^{14}C] (58 mCi/mmol) was prepared at a concentration of 50 μCi/ml. Dopamine [7-^{14}C] hydrochloride (57 mCi/mmol and sodium methotrexate [3′,5′,7-^{3}H] (297 mCi/mmol) were combined in solution at concentrations of 44 and 85 μCi/ml, respectively. The solution pH was adjusted to 6.2. All ra-

diolabeled compounds were obtained from Amersham. Mini-osmotic pumps were filled with the appropriate solution and incubated in normal saline at 37 °C for at least 16 h to establish steady-state pumping rates prior to implantation.

Determination of stability of radiolabeled compounds

[^{14}C]AP was assumed to be stable in artificial CSF at pH 7, 37 °C. Because DA and MTX are easily oxidized, they were prepared in deoxygenated artificial CSF. Radiochemical purity of [^{14}C]DA was determined by paper chromatography in n-butanol–water–acetic acid (12:5:3). Purity of the material was checked as received from Amersham, and again after it was lyophilized, redissolved in artificial CSF, and stored in the freezer. To test stability under experimental conditions, a single mini-osmotic pump was filled with a solution (pH 6.2) containing [^{14}C]DA and unlabeled MTX. Radiochemical purity of the [^{14}C]DA was determined before and after incubation for 7 days under nitrogen at 37 °C. [^{3}H]MTX was analyzed by paper chromatography in n-butanol: pyridine: water (1:1:1). The material was dissolved in artificial CSF and the radiochemical purity checked immediately, and again after 7 days storage in the freezer. A single mini-osmotic pump was filled with a solution (pH 6.2) containing [^{3}H]MTX and unlabeled DA. Radiochemical purity of the [^{3}H]MTX was determined before and after incubation for 7 days under nitrogen at 37 °C.

Surgical procedures

Female New Zealand white rabbits weighing 3.0–3.5 kg were sedated with xylazine (Rompun; Haver-Lockhart), 8 mg/kg i.m., then anesthetized with ketamine hydrochloride (Ketaset; Bristol), 44 mg/kg i.m. Each rabbit was placed in a standard Horsley–Clarke stereotaxic apparatus with a rabbit head-holder (David Kopf Instruments). Using standard procedures, the stainless steel cannula was placed in the brain 6.5 mm posterior and 2.0 mm lateral to bregma, at a depth of 14.5 mm. This placement put the cannula in the mid-thalamic region of diencephalon, the center of a block of tissue about 20×20×10 mm, comprising diencephalon and midbrain areas[17]. The cannula was secured to the skull with dental cement and the attached mini-osmotic pump was placed in the subcutaneous tissue behind

the ears. The skin was sutured over the cannula-pump assembly.

Determination of drug distribution following infusion in the diencephalon

Two rabbits were infused with [14C]AP, and 3 with the combination of [14C]DA and [3H]MTX. After implantation of the infusion system, each rabbit was returned to its cage, where it was maintained under standard laboratory conditions, without restraint, for 6 days. The animals were then killed by i.v. injection of a euthanasia solution (T61; Hoechst). The brain was removed immediately and placed on a chilled plate. The diencephalon and midbrain were dissected free as a unit and the tissue frozen on dry ice. A portion of the cortex was also frozen. The above procedures were accomplished as quickly as possible (within 10 min) to minimize any change in drug distribution after death.

The diencephalon-midbrain unit was cut through the perfused area using the cannula as a guide. The exact site was visualized under UV light as a fluorescent area about 2 mm in diameter, produced by the fluorescein in the perfusate. In all 5 animals the infusion site was confirmed to be near the center of the diencephalon-midbrain unit. A 5×5 mm column of tissue was cut in a lateral or anterior direction extending from the cannula tip outward approximately 10 mm to the ependymal surface of the diencephalon-midbrain unit. Using the procedure described by Kasamatsu et al.[14] and Kroin and Penn[15], the column was sectioned at 1 mm intervals, yielding slices 5×5×1 mm. In a similar manner, 3 control slices were made from the cortex. All slices were placed in tared vials and weighed, dissolved in tissue solubilizer (NCS; Amersham), and combined with fluor (Liquifluor; New England Nuclear). Samples were counted in a liquid scintillation counter (Nuclear Chicago Mark II). The radioactivity in each slice was expressed as a percent of the concentration in the perfusate. For purposes of data presentation, each of the 1 mm thick sections was designated by the distance from the cannula to the midpoint of the 1 mm tissue section. Thus, data from the tissue section nearest the cannula are designated in the figures as being 0.5 mm from the cannula. Note that the sampling of 5×5×1 mm tissue slabs tends to underestimate the concentration of drug in tissue lying within the first few millimeters of the cannula.
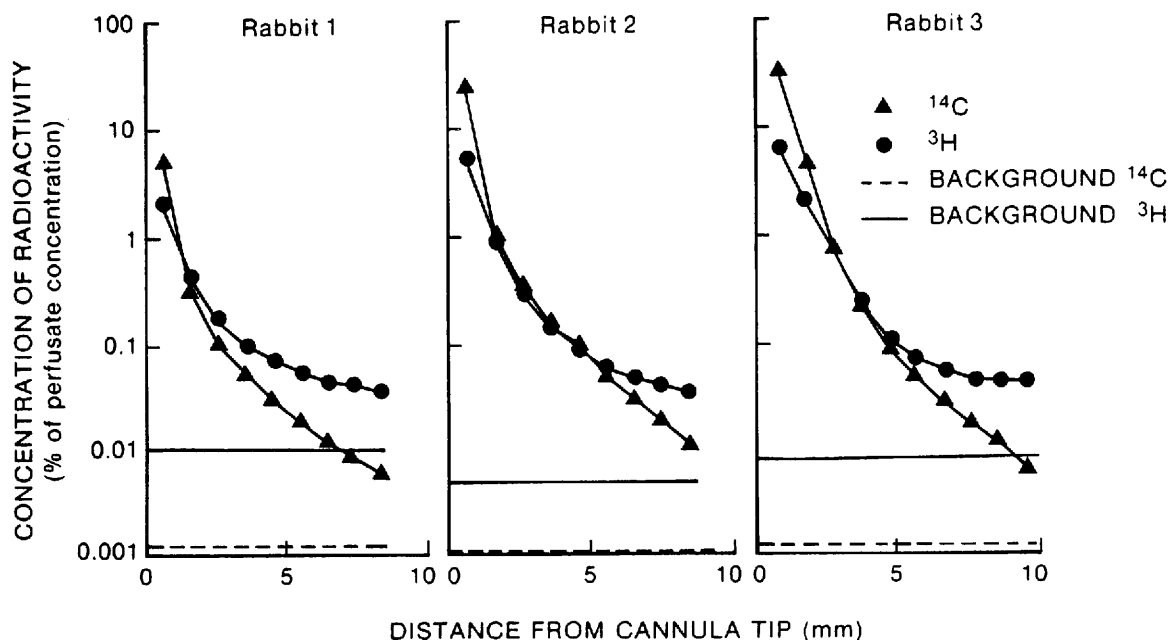

Fig. 1. The spatial distribution of carbon-14 and tritium after 6 days of microperfusion of [14C]DA and [3H]MTX in the diencephalon of 3 rabbits. Background carbon-14 and tritium concentrations are averages of 3 measurements in the cortex of each animal. Note that 'distance from the cannula tip' represents the distance from the cannula tip to the midpoint of 1 mm thick tissue sections.

RESULTS

Stability of [14C]DA and [3H]MTX

Radiochromatographic analysis of [14C]DA showed that the DA peak comprised 96% of the radioactivity initially and 90% after handling and storage. However, it decreased to 56% after incubation of the solution in the mini-osmotic pump at 37 °C, pH 6.2, for 1 week. Thus, it is inappropriate to interpret tissue carbon-14 levels as signifying the presence of DA. Radiochemical purity of [3H]MTX was 91% when received and also after storage, and 89% after incubation in the mini-osmotic pump.

Distribution of [14C]DA, [3H]MTX and [14C]AP from the cannula tip in the diencephalon

Fig. 1 shows distribution profiles of carbon-14 and tritium in the individual rabbits microperfused simultaneously with [14C]DA and [3H]MTX. Radioactivity in cortex, representing background due to recirculated isotopes, is plotted as a reference.

Concentrations of both [3H]MTX and DA-derived carbon-14 in the diencephalon declined with distance from the cannula tip. [3H]MTX levels, however, were always lower at the site of infusion but declined more gradually than DA-derived carbon-14. In every rabbit, tritium leveled off at a concentration equivalent to 10^{-7} M MTX (0.04% of the microperfusate concentration), whereas DA-derived carbon-14 continued to decline to the edge of the diencephalon. Neither isotope reached the background levels measured in the cortex.

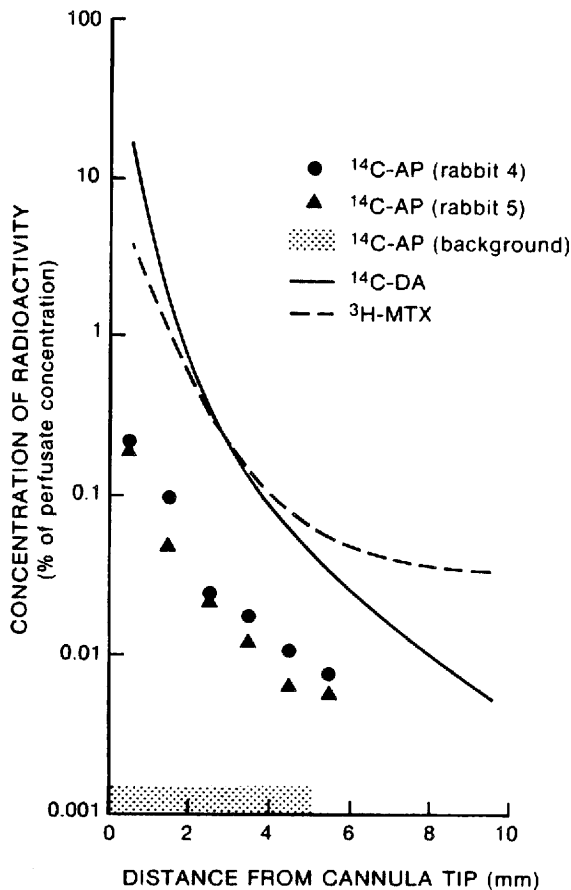The basic pattern of distribution of [14C]AP was similar to those of [3H]MTX and DA-derived carbon-

Fig. 2. The spatial distribution of carbon-14 in rabbits 4 and 5 after 6 days' microperfusion of [14C]AP in the diencephalon. The solid bar shows the range of 3 background carbon-14 measurements in the cortex of each rabbit. The spatial distributions of [3H]MTX and DA-derived carbon-14 are the averages from rabbits 1, 2 and 3 (Fig. 1), plotted here for reference. The abscissal scale is as described for Fig. 1.
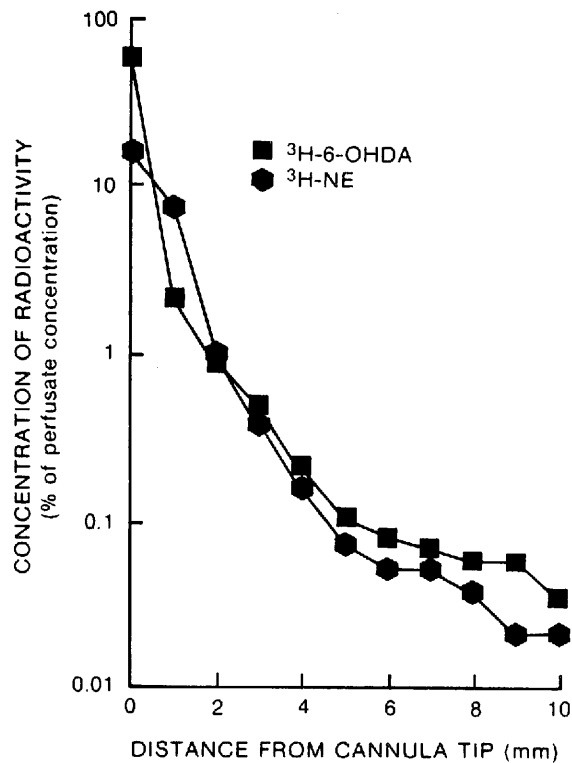

Fig. 3. The spatial distribution of tritium after intracortical microperfusion of [3H]6-OHDA or [3H]NE for 7 days. Each curve represents the average of data from two cats. Reprinted by permission from ref. 14.
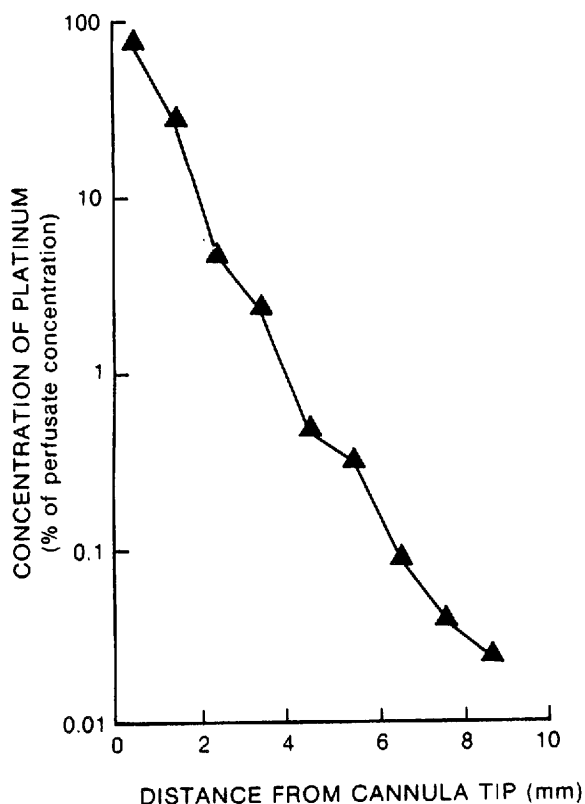
Fig. 4. The spatial distribution of platinum after intracortical microperfusion of cisplatin for 7 days in the rat. Reprinted by permission from ref. 15.

14 (Fig. 2), but the absolute concentrations of [14C]AP in tissue were one to two orders of magnitude lower. For technical reasons, [14C]AP concentrations were not sampled beyond 5 mm from the cannula tip.

DISCUSSION

Simultaneous infusion of [14C]DA and [3H]MTX made it possible to determine their concentrations in the same tissue samples, thus eliminating sampling variables and allowing direct comparison of their distributions. A disadvantage of the two-agent microperfusion, however, was that the optimum pH for stability differs for MTX and DA. The pH of the solution was set near neutral (6.2) to minimize tissue irritation and to assure stability of the MTX. Catecholamines, however, are more stable at acid pH. In vitro incubation of [14C]DA in the mini-osmotic pump showed that as much as 50% of the carbon-14 infused

on the 6th day may have been degradation products. Furthermore, once in the brain, DA is subject to local metabolism[13]. Thus, the distribution shown for DA-derived carbon-14 must be regarded as a composite of the distributions of DA and of its products of degradation and metabolism. The microperfused [3H]MTX was only 90% pure, initially. It can be assumed, however, that MTX was not further degraded or metabolized: our data show that [3H]-MTX was radio-chemically stable in the mini-osmotic pump for 1 week in vitro, and its stability under similar conditions in vivo was demonstrated by Dakhil et al.[6]. Metabolic studies with [3H]MTX in a number of species have shown that virtually 100% of the tritium is excreted in urine and bile as intact MTX[11,12].

The spatial distributions of all 3 drugs could have been altered by post-mortem diffusion during the 10 min before the brain was frozen. The relative contribution of post-mortem diffusion might differ for each drug, depending on binding or other factors. Indeed, the possibility of immediate post-mortem artifacts cannot be completely excluded from this study, which suggests that in vivo imaging techniques ought to be applied to measure microperfusion volumes.

In the animals microperfused with [14C]DA and [3H]MTX, the concentration of DA-derived carbon-14 was high near the cannula tip, then declined steeply. While the degradation of [14C]DA precludes definitive interpretation of these data, the results do set an upper limit on the distribution space of DA. The distribution pattern is consistent with the fact that DA is taken up and metabolized in the nerve terminals, thus limiting further diffusion. The flatter MTX curve suggests that MTX may be bound by a high affinity, low capacity tissue component. Such binding, in effect, could facilitate diffusion of the drug through the tissues in a manner analogous to the facilitation of oxygen transport by hemoglobin[26]. With any drug, the relative contribution of binding to the distribution pattern would diminish with increasing concentration of drug in the perfusate.

The highest concentrations of [14C]AP measured in the immediately pericannular 1 mm thick tissue sections were only about 0.2% of the [14C]AP concentration in the microperfusate. In contrast, the immediately pericannular tissue concentrations of [3H]-MTX and DA-derived carbon-14 were of the order, respectively, of 4 and 20% of their corresponding mi-

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croperfusate concentrations. It would appear that there was an exceptionally steep decline in [^{14}C]AP levels within the first fractions of a millimeter of peri-cannular tissue. These results are consistent with the ability of [^{14}C]AP to move very readily into cerebral capillaries and thence into the systemic circulation[21], and suggest that drugs with an AP-like lipophilicity will have negligibly small microperfusion volumes. The gradually declining [^{14}C]AP concentrations some millimeters from the cannula (Fig. 2) are in contrast to the steep decline in the immediately peri-cannular tissue, and probably represent the more gradual disposition of a 0.5–1% radiochemical con-taminant.

Fenstermacher's group has done extensive studies of the penetration of various substances into brain parenchyma following experimental ventriculo-cisternal perfusion[1,2,8,9]. They demonstrated that substances that cross cerebral capillaries readily, such as thiotepa, 1,3-bis(2-chloroethyl)-1-nitrosurea (BCNU) and water, were removed rapidly into the systemic circulation. These substances did not reach significant concentrations in brain parenchyma be-yond 1 or 2 mm of the ependymal surface. Substances with low rates of transcapillary exchange, such as creatinine, MTX and hydroxyurea, penetrated more deeply than the above substances, and the depth of penetration increased with time over a 4 h period of study. These substances had larger tissue spaces than extracellular markers such as sucrose or inulin, indi-cating that they penetrated or were bound to brain cells.

Because Fenstermacher's technique involved dif-fusion from two opposing plates, while ours utilized diffusion from a single point source, our data on dis-tribution patterns cannot be compared directly. However, the same basic conclusion can be reached with both types of administration, i.e., that sub-stances which do not exchange rapidly across cere-bral capillaries achieve a wider distribution in brain parenchyma than substances which do. Blasberg et al.[2] have suggested that chemotherapeutic agents with low rates of transcapillary exchange might be administered intraventricularly according to a mul-tiple dose regimen to increase further the levels of these drugs reaching cerebral neoplasms. We suggest that long-term continuous microperfusion of the tu-mor be considered an alternate means of achieving adequate concentrations with less toxicity to normal tissue.

Kasamatsu et al.[14] and Kroin and Penn[15], using techniques similar to ours, have described the spatial distribution of intracerebrally microperfused drugs. Note that this technique, which involves sampling of approximately 5×5 mm slabs of tissue at 1 mm inter-vals from the cannula tip, underestimates the concen-tration of drug in tissue lying within the first few mm of the cannula. Useful comparisons of the distribu-tion patterns derived from these data can be made, however, as long as all the results are expressed in the same terms. The published results have been re-calculated in terms of the microperfusate concentra-tion and plotted on the same scale as Fig. 2. Fig. 3 shows the distribution of [^{3}H]6-hydroxydopamine (6-

TABLE I

Tissue microperfusion volumes of drugs infused intracerebrally at rates or 0.5 of 1.0 μl/h

Radius is the distance from the cannula tip to the point where the tissue concentration of radioisotope or platinum is 1 or 0.1% of the perfusate concentration. Values were taken from Figs. 2–4, and represent the distribution of the unaltered drugs plus their products of degradation and/or metabolism. Because [^{14}C]dopamine in the microperfusate was degraded extensively during the 6 day study, these estimates represent only an upper limit on the microperfusion volumes of dopamine.

Material	Species and tissue	Radius (mm)		Estimated tissue microperfusion volume (cm³)	
		1%	0.1%	1%	0.1%
[^{3}H]Methotrexate	Rabbit diencephalon	1.6	4.2	0.02	0.31
[^{14}C]Dopamine	Rabbit diencephalon	1.8	3.9	0.02	0.25
[^{3}H]6-Hydroxydopamine	Cat cortex	1.8	5.3	0.02	0.62
[^{3}H]Norepinephrine	Cat cortex	2.0	4.6	0.03	0.41
Cisplatin	Rat cortex	4.0	6.4	0.27	1.10

OHDA) and [³H]norepinephrine (NE) in the cortex of kittens[14]. Fig. 4 shows the distribution of cisplatin (Pt) in the cortex of rats[15]. The distribution patterns of 6-OHDA, NE and Pt are remarkably similar to that of DA-derived carbon-14 (Fig. 2).

The volume of the sphere of tissue that can be microperfused with safe, effective levels of each drug was estimated. For this purpose, we made two alternative arbitrary assumptions: that a 100-fold or a 1000-fold range exists between toxic and minimally effective concentrations of drug. Then:

$$\text{tissue perfusion volume} = 4/3\pi r^3$$

where r = radial distance from the cannula tip to the point in the tissue where the concentration of radioisotope or platinum is 1 or 0.1% of that in the perfusate. The results are presented in Table 1. The data for AP were too limited to estimate microperfusion volume.

The microperfusion volumes of the catecholamines, MTX an Pt ranged from 0.02 to 0.27 cm³ when r = 1%, and 0.25–1.1 cm³ when r = 0.1%. Differences among these compounds are probably not important, considering that different species and brain regions were studied and that the number of animals microperfused with each compound was small. Also, the measured distributions were subject to large experimental errors because the compounds could not be distinguished from their degradation/metabolic products in the tissue. We have discussed this problem in relation to DA and MTX, but it also pertains to NE, 6-OHDA and Pt in previously published studies by others. For example, Kasamatsu et al.[14] demonstrated that 66% of the [³H]NE remaining in the cortex at the end of a 7 day microperfusion was unchanged NE. The data of Kroin and Penn[15] in Fig. 4 are based on the distribution of platinum, but the amount present as unaltered cisplatin is unknown. In addition, the microperfusion volumes of chemotherapeutic agents such as Pt or MTX in a brain tumor might be smaller than in normal tissue if the blood–brain barrier in the tumor were partially disrupted[16,29].

The effective microperfusion volume of an intracerebrally infused drug depends on the drug's therapeutic concentration range as well as its spatial distribution. The actual therapeutic concentration ranges

TABLE II

Number of cannulae as a function of microperfusion volume

Sphere radius (mm)	Microperfusion volume (cm³)	Number of cannulae per g
1	0.004	240
2	0.03	30
3	0.11	9
4	0.27	4
5	0.52	2
6	0.90	1.1
7	1.44	0.7
8	2.14	0.5
9	3.05	0.3
10	4.19	0.2

are unknown and are probably different for each drug. To estimate perfusion volume, we assumed either a 100- or 1000-fold range between a toxic and a minimally effective concentration. Table II shows the number of cannulae required to perfuse a gram of tissue as a function of the perfusion volume. If the assumption of a 1000-fold range were correct, 1–4 cannulae would be required to treat 1 g of tissue with the agents listed in Table I. If only 100-fold range is tolerable, then 4 to over 30 cannulae would be required. We believe these results support the view that intracerebral microperfusion is a useful research tool, and one that should be further studied as regards any potential therapeutic value it may have, especially for intractable diseases that pass through a stage during which the future course of the disease is governed by a pea-sized lesion.

It is apparent that many factors affect a drug's spatial distribution during microperfusion of cerebral tissue. Until these have been well-defined, it will be necessary to consider each drug as a special case, determining spatial distribution as well as therapeutic limits on tissue concentration in appropriate animal models.

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