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Labeled Putrescine as a Probe in Brain Tumors

Abstract. *The polyamine metabolism of transplanted* N-*nitrosomethylurea–derived rat glioma was determined with radiolabeled putrescine used as a marker for malignancy. The uptake of putrescine in vivo was complete within 5 minutes and was specific for tumor tissue. The conversion of putrescine to spermine and other metabolites by the tumor was rapid, in contrast to the case for adjacent normal brain. These results suggest that putrescine labeled with carbon-11 may be used as a positron-emission tomographic tracer for the selective metabolic imaging of brain tumor and may be used in an appropriate model as a marker for tumor growth rate.*

With positron emission tomography (PET) it is now possible to determine the location of brain tumors and to distinguish between the metabolic activities of the neoplasm and the surrounding brain (*1*). Earlier strategies for the diagnosis of brain tumors have used substances that are both readily available and modeled for tomographic reconstruction, such as glucose, oxygen, amino acids, and their analogs (*2*). However, appropriate rate constants and equated steady-state conditions for the quantitation of metabolic and functional pathways remain to be determined. Were a compound to be found that is taken up and metabolized solely by the tumor, this would enhance the selectivity of the technique and facilitate the assessment of the response to treatment. Because adult brain parenchyma does not normally divide, we considered that putrescine, a polyamine and putative marker of cell division and growth, should provide a superior index of neoplastic activity. Numerous studies have confirmed the importance of polyamines in both normal and pathological states (*3*). The metabolism of these substances has been correlated with the potential for cell replication and appears to be coupled to the stages of the cell cycle that precede mitosis (*4*). In brain tumors the concentration of putrescine has been shown to correlate with the degree of malignancy (*5*), whereas in normal brain putrescine metabolism is minimal (*6*).

To test the feasibility of putrescine as a PET tracer for brain tumors, we conducted a series of experiments in which we used a transplanted rat gliosarcoma derived from the *N*-nitrosomethylurea–induced rat glioma (*7*, *8*). Using conventional autoradiographic and densitometric techniques, we found that the in vivo uptake of [^{14}C]putrescine was greater in the tumor than in the surrounding normal brain (Fig. 1). Uptake of the label was evident as early as 1 minute after injection of the putrescine into the femoral vein, and uptake was essentially complete within 5 minutes. The specific activity of the label within the tumor was 35 times greater than in normal brain. No radiodense material was observed in necrotic tissue, and the only brain structure that showed evidence of uptake within the time frame of these experiments was the choroid plexus.

Clearance of putrescine from the circulation was equally rapid (95 percent of the amount injected was cleared in less than 5 minutes). These data are consistent with published reports for both rat and human (*9*). This rapid loss of putrescine from circulation is the result of uptake by tissue and excretion in urine (Table 1). The radioactivity remaining in the tissue 2 hours after an intravenous injection of [^{3}H]putrescine accounts for 30 percent of the injected amount and is confined primarily to skeletal muscle, liver, and the blood compartment (Table 1). Furthermore, the excretion of putrescine accounted for 30 percent of the amount injected and was as rapid as its clearance from circulation. Essentially all the radioactivity recovered in urine at 2 hours occurred within the first 5 minutes after the initial injection (data not shown). The radioactivity that cannot be accounted for (approximately 40 percent) is probably lost through putrescine catabolism, since it has been reported that 43 percent of labeled putrescine injected intraperitoneally into rodents was metabolized and expired as CO_2 within 2 hours (*10*). We also observed the evolution of a tritiated compound in expired air within minutes after the injection (data not shown).

These findings demonstrate the efficacy of putrescine as a PET tracer for brain

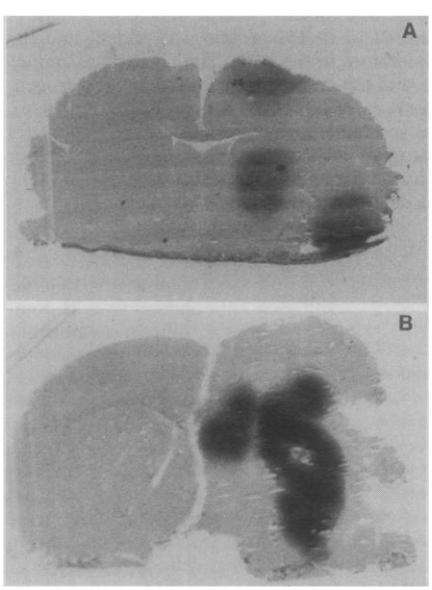

Fig. 1. Autoradiography of [^{14}C]putrescine in transplanted rat gliosarcoma. Representative overlays at (A) 1 minute and (B) 30 minutes of the hematoxylin and eosin–stained brain section on the corresponding autoradiogram. The overlay was necessary because the uptake of label by normal brain was too low for photography. Rats 6 to 8 weeks old (cesarean derived CD Fisher strain) were injected intracerebrally by stereotaxic technique with 10^6 cells as described (*8*). Twelve days after transplantation, they were anesthetized with sodium pentobarbital (25 mg/kg, intraperitoneal), the femoral vein was cannulated, and approximately 50 μCi of [^{14}C]putrescine (New England Nuclear) (107.1 mCi/mmole) was administered in 1 ml of 0.9 percent NaCl. Animals were killed (at 1, 5, 15, or 30 minutes after the administration of isotope) with 1 ml of saturated KCl, intravenous; the brain was removed and frozen in liquid Freon (−20°C). Cryostat sections were prepared for autoradiography as described (*21*), and the tissue radioactivity of the autoradiogram was determined densitometrically (*21*). The unfixed tissue sections used for autoradiography were stained by conventional hematoxylin and eosin. The specific activity of the isotope in tumor at 1 minute was 475 nCi/g (wet weight) versus 15 mCi/g in normal brain. At 5 minutes, the respective specific activities were 750 versus 20; at 15 minutes, 710 versus 26; and at 30 minutes, 800 versus 22.

Table 1. Pharmokinetics of [^{3}H]putrescine in rats. Data in columns 2 and 3 are expressed in microcuries per gram (wet weight) and are given as the averages of two experiments per column. Rats were prepared according to the description in legend to Fig. 1. Two hours after the injection of isotope, the animals were killed and the tissue was removed, blotted, weighed, and digested in Protosol (New England Nuclear). Tissue radioactivity was counted in Econofluor (New England Nuclear).

Tissue	Activity of isotope in tissue after injection of		Percentage of organ weight relative to body weight	Percentage of amount injected remaining in organ after 2 hours
	43 μCi	8 μCi		
Kidney	0.445	0.099	0.43	0.55 ± 0.31
Spleen	0.387	0.092	0.21	0.52 ± 0.08
Heart	0.262	0.062	0.43	0.63 ± 0.06
Liver	0.234	0.093	2.4	4.67 ± 0.91
Adrenal gland	0.188	0.099	0.028	0.17 ± 0.04
Testes	0.072	0.037		
Skeletal muscle	0.070	0.032	43	17.3 ± 3.18
Brain	0.055	0.034	0.5	0.25 ± 0.08
Lung	0.196	0.113	1.4	1.08 ± 0.37
Blood	0.054	0.037	7.0	4.89 ± 1.59
Fat	0.025	0.011		
Urine				30.0 ± 3.56

Table 2. In vivo metabolism of [^{3}H]putrescine in gliosarcoma and normal brain. In experiment A, 11.3 μCi (in experiment B, 5.4 μCi) of [^{3}H]putrescine was administered intravenously to rats anesthetized as described in Fig. 1. Numbers in parentheses (experiment B) refer to the activity separated as the dansylated amines (see below); ND, not determined. For experiment A, tumor was transplanted as described in the text. At 5 or 30 minutes after the injection of [^{3}H]putrescine, the rats were killed; the brain was removed, and the tumor was excised and frozen in liquid nitrogen. The contralateral cerebrum was used as the control. The frozen tissue was weighed and homogenized in ten volumes of cold 0.2*N* $HClO_4$ and kept on ice for 1 hour. After centrifugation at 800*g* for 15 minutes, the supernatant was removed and an equivalent volume of 1*N* NaOH was added. To this solution a mixture of solid anhydrous Na_2SO_4 and Na_3PO_4 (7:1 by weight) was added. The polyamines were extracted into *n*-butanol according to the procedure of Raina (*17*). The combined *n*-butanol extracts were evaporated under a stream of nitrogen, and the residue was dissolved in 0.1*N* HCl. An aliquot was removed for chromatographic separation on silica gel G thin-layer plates according to the method of Shimizu *et al.* (*18*). Authentic polyamine standards (Sigma Chemical) were chromatographed separately. After development, the plate was air-dried, sprayed with 0.4 percent ninhydrin in absolute ethanol, and heated for 10 minutes at 110°C. The observed R_F values for spermine, spermidine, and putrescine were 0.47, 0.66, and 0.78, respectively. The amines were scraped and placed into scintillation vials; 1 ml of a Protosol-ethanol mixture (1:2) was added. (Release of radioactivity from the silica was incomplete in the absence of Protosol-ethanol.) In experiment B, a second aliquot of the *n*-butanol extract was dansylated according to the procedure of Igarashi *et al.* (*19*). The aliquot was added to 400 μl of deionized water and 100 μl of 5*N* Na_2CO_3. Then 500 μl of dansyl chloride in acetone (10 mg/ml) was added. The mixture was incubated overnight at room temperature in the dark. The next morning 50 μl of 1*M* proline was added, and the incubation continued for 30 minutes at room temperature in the dark. The dansylated amines were extracted in toluene, and the toluene phase was evaporated under a stream of nitrogen. The residue was dissolved in toluene and an aliquot removed for silica gel chromatography (*20*). The observed R_F values for authentic dansylated spermine, spermidine, and putrescine were 0.50, 0.42, and 0.32, respectively. After chromatographic resolution, the dansylated amines were scraped and placed into vials; 1 ml of a Protosol-ethanol mixture (1:2) was added, and the radioactivity was counted. Quench correction was determined by the method of channel ratios.

Tissue	Time (min)	Activity (nCi/g, wet weight)				
		Spermine	Spermidine	Putrescine	Unknown metabolites	Volatile ^{3}H components
			Experiment A			
Tumor	5	78	1.7	1.7	38	323
Contralateral cerebrum	5	0.19	4.5	0.16	1.7	28
Tumor	30	88	0.54	6.9	34	300
Contralateral cerebrum	30	1.08	0.44	0.95	0.89	50
			Experiment B			
Tumor	5	21 (22.1)	1.8 (2.2)	ND (1.5)	6.3	129
Contralateral cerebrum	5	0.72 (0.97)	ND (0.03)	ND (0.09)	0.18	8.3
Tumor	30	30 (32.5)	0.73 (0.57)	0.47 (1.0)	3.9	153
Contralateral cerebrum	30	0.65 (1.75)	ND (0.16)	ND (0.06)	ND	22

tumor. However, the selective uptake of putrescine by brain tumor may be attributed to an absent or incomplete blood-brain barrier within the tumor tissue (*11*) and not to putrescine metabolism, since putrescine does not readily permeate the intact blood-brain barrier (*12*). To evaluate the metabolism of putrescine in vivo, we administered [^{3}H]putrescine and measured its metabolic products. As early as 5 minutes after administration, considerable labeling of spermine had occurred, and this labeling was independent of the concentration of the added tracer (Table 2). Spermine synthesis was essentially complete within 5 minutes. In contrast, however, the uptake and metabolism of putrescine in the normal contralateral cerebrum was minimal compared to the uptake and metabolism in the tumor. Curiously, we also found that a significant percentage of the radioactivity in the $HClO_4$ digest could not be extracted into the organic phase from either the gliosarcoma or normal brain tissue. The radioactivity remaining in the aqueous phase was a volatile ^{3}H-labeled compound that could not be resolved or recovered after silica gel chromatography. It seems likely that this volatile component represents water derived from the nonpolyamine metabolism of putrescine (*13*).

These data indicate that putrescine may be valuable as a PET tracer because of its pharmokinetics, nontoxic nature (*14*), and preferential uptake and metabolism by brain tumors. Thus ^{11}C-labeled putrescine, whose synthesis and potential usefulness as a probe for tumor have been described (*15*), may serve as a near-ideal substance with which to characterize brain tumor as a function of polyamine metabolism and the response of brain tumor to therapy. Preliminary studies with a wide variety of human tumors incubated in vitro support this premise (*16*).

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Ventral Posterior Thalamic Neurons Differentially Responsive to Noxious Stimulation of the Awake Monkey

Abstract. *Of 76 cutaneously activated neurons recorded from the ventral posterior thalamus of awake, behaving monkeys, nine were weakly excited by innocuous skin stimulation and responded maximally only when noxious mechanical cutaneous stimuli were delivered within small, contralateral receptive fields. These results show that neurons capable of encoding the spatial and temporal features of noxious stimuli are located in the ventral posterior thalamus of the awake primate.*

Neurons of the ventral posterior lateral (VPL) and ventral posterior medial (VPM) nuclei of the thalamus receive somatic sensory input from the body and face, respectively, and precisely encode the location and timing of cutaneous stimuli. These ventral posterior (VP) neurons are organized somatotopically and respond to innocuous tactile stimuli such as movement of hair or light pressure on the skin (*1–3*). Little information is available, however, about the thalamic mechanisms for encoding the spatial and temporal features of noxious stimuli. Neurons recorded from the posterior group nuclei of the anesthetized cat (*4*) and the posterior ventrobasal complex of the anesthetized rat (*5*) respond exclusively or differentially to noxious stimuli, but these cells differ from the tactile VP neurons in having larger, often bilateral, receptive fields and occasionally responding to sensory stimuli of other modalities. In the primate, there is substantial anatomical and physiological evidence that spinothalamic and trigeminothalamic neurons project to VP and that a significant fraction of these projection neurons either respond exclusively to noxious stimuli (NS neurons) or have a wide dynamic range (WDR) of response that is graded in intensity as stimulus strength increases from innocuous to noxious (*6*). Nonetheless, extensive surveys of single neuron responses in the VP thalamus have failed to reveal NS or WDR neurons in either the anesthetized or unanesthetized intact monkey (*2, 3, 7*). Even when tactile input to the thalamus was markedly reduced by extensive dorsal spinal cord lesions, Perl and Whitlock (*8*) found, in the anesthetized monkey, that 52 VP neurons responded maximally to innocuous mechanical stimuli, whereas only two were exclusively nociceptive. Recently, however, Kenshalo *et al.* (*9*) found 73 NS and WDR neurons among thousands of tactile-responsive cells recorded from the VPL of monkeys anesthetized with pentobarbital and chloralose. The NS and WDR VP neurons recorded from these anesthetized animals had small contralateral receptive fields and typically responded to noxious thermal as well as mechanical cutaneous stimuli. Similar results in the anesthetized cat have been reported recently (*10*).

Although the anesthetics that have been used in studies of VP neurons do not generally depress neuronal responses to tactile stimuli, it is possible that anesthesia reduces the range of cutaneous stimuli exciting some neurons with both tactile and nociceptive inputs so that they appear to respond differentially to noxious stimuli. Small doses of pentobarbital produce this effect on medial thalamic neurons of awake squirrel monkeys (*11*). It is essential, therefore, to determine if there are nociceptive VP neurons in the awake, intact primate. Some neurons responding to pinprick have been recorded from the VPL of awake monkeys, but the proportion of such neurons and their differential responses to noxious stimuli were not documented (*12*). We recorded the differential responses of VP neurons to innocuous and noxious somatic stimuli delivered to awake, behaving squirrel monkeys.

Eight squirrel monkeys had a skull-mounted microdrive (*13*) and electronic headstage implanted during anesthesia under sterile surgical conditions. In some animals, bipolar stimulating electrodes were placed in the midbrain trajectory of the spinothalamic tract (*14*). These stimulating electrodes were used to deliver single pulses (0.2 msec, 0.1 to 1.0 mA at 1.0 Hz) to activate VP neurons not otherwise spontaneously active or not activated by continual testing of the body surface with natural somatic stimuli. The midbrain stimulation produced no obvious behavioral effect. Natural innocuous somatic stimuli included brushing the hair without touching the skin and cutaneous stimuli such as touch, pressure, tapping, and gentle squeezing of the skin. The noxious stimuli included pinching the skin with fingers or forceps, pinprick, and touching with a metal rod that had been heated to approximately 50°C. Stimuli were not sufficiently intense to produce skin damage. Noxious stimuli were used only toward the end of a study of a single neuron, were applied for 5 seconds or less, and were applied repeatedly (typically no more than five times) only when initial testing clearly suggested that the response to noxious stimulation exceeded the response to innocuous stimulation. Whenever possible, innocuous electrical stimulation was