New Insights on Flow-Independent Mechanisms of ^{99m}Tc-HMPAO Retention in Nervous Tissue: In Vitro Study

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SPECT using ^{99m}Tc-hexamethyl propyleneamine oxime (HMPAO) mainly reflects regional cerebral blood flow, however metabolic abnormalities also affect the retention of ^{99m}Tc-HMPAO. **Methods:** To rule out any flow factor, a test-tube model was used to evaluate the effects of metabolic alterations both on intracellular trapping of ^{99m}Tc-HMPAO and on extracellular glutamate and lactate dehydrogenase (LDH) outflow from rat brain slices. **Results:** Under control conditions, slices took up 7.0% ± 1.4% of ^{99m}Tc-HMPAO contained in the medium, whereas prelabeled slices released 10.8% ± 2.6% of their radioactive content; glutamate and LDH outflow were 49.1 ± 21.6 pmol/mg protein/min and 4.8 ± 0.9 U/L/mg protein/min, respectively. The control medium was altered by adding a metabolic poison (5 mmol/L azide), removing glucose and replacing O_2 with N_2 to mimic ischemia (in vitro ischemia) and replacing Krebs solution with hypotonic medium to evoke cell lysis. Both azide and in vitro ischemia induced a significant increase in ^{99m}Tc-HMPAO release (15.8% ± 3.3% and 18.3% ± 6.2%, respectively), without any modification in LDH efflux. However, only azide reduced the uptake of the tracer. Conversely, glutamate outflow was massive during in vitro ischemia and was far lower during azide treatment. Under hypotonic medium conditions, the release of ^{99m}Tc-HMPAO, glutamate and LDH were dramatically increased. Surprisingly, a two-fold increase of ^{99m}Tc-HMPAO uptake was also found. When 1 mmol/L glutathione was added to the medium, to convert native lipophilic ^{99m}Tc-HMPAO into hydrophilic derivatives, tracer uptake was inhibited both under control and hypotonic medium conditions. **Conclusion:** This study provides evidence that not only poisoning of the tissue but also in vitro ischemia induced a reduction of ^{99m}Tc-HMPAO retention. Moreover, we demonstrated that injuries causing cell membrane disruption led to hyperfixation of ^{99m}Tc-HMPAO.

Key Words: cerebral cortex slices; ^{99m}Tc-labeled hexamethyl propyleneamine oxime; brain metabolism; hyperfixation; in vitro ischemia; cell membrane damage

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Brain SPECT imaging with ^{99m}Tc-labeled hexamethyl propyleneamine oxime (HMPAO) reflects the regional cerebral blood flow (rCBF) over a wide range of flow (*1*). At the cellular level, the net retention of the tracer depends on a dynamic process consisting of three phases: inflow, trapping and backflow. This process has been extensively studied, both in vivo (*2*) and in vitro (*3–6*). The inflow depends mainly on rCBF and on the lipophilic characteristics of the compound, which allow the molecule to easily cross the blood-brain barrier (BBB) and cell membranes. The intracellular trapping of ^{99m}Tc-HMPAO depends on the reduction to more hydrophilic forms by a glutathione-dependent mechanism (*2,4*) and on the binding to nondiffusible cell components (*3,5,6*). The backflow (negligible for the 6 h after tracer injection) originates from the radiopharmaceutical escaping intracellular trapping processes (*7*).

SPECT imaging of cerebral perfusion with ^{99m}Tc-HMPAO is useful in neurologic and psychiatric diseases for detecting possible related flow disturbances (*8*). However, some SPECT images seem to reflect neuronal metabolic abnormalities in addition to rCBF (*9–11*). This seems especially true in the subacute phase of stroke, when ^{99m}Tc-HMPAO hyperfixation (counting rates exceeding the actual rCBF measured by ^{133}Xe [*12*]) has been reported frequently (*13,14*). Unfortunately, the meaning and prognostic value of this phenomenon are not yet understood.

The existence of flow-independent factors affecting ^{99m}Tc-HMPAO distribution in the nervous tissue is further corroborated by some in vitro findings. In fact, it was demonstrated that metabolic poisoning or changes in the oxido-reductive state of the tissue decreased ^{99m}Tc-HMPAO retention in brain slices (*15*) or neuronal cell cultures (*16*), respectively. In both cases, an impairment of the ^{99m}Tc-HMPAO trapping mechanism was evident, leading to a modification of the ratio between lipophilic and hydrophilic forms of the tracer. Therefore, brain ischemia could also evoke flow-independent changes in the cellular net retention of ^{99m}Tc-HMPAO, by modifying either intracellular glutathione levels or extracellular redox states.

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To investigate the flow-independent effects of metabolic alterations on the distribution of ^{99m}Tc-HMPAO, we studied tracer retention in a test-tube model using rat brain slices. Our choice of the in vitro model allowed direct access (lack of BBB) and complete control of the extracellular environment (ions, pH, hormones, gases and temperature), along with a prompt availability of the tissue for functional, biochemical and morphological analysis. We also studied changes in glutamate extracellular levels under the same experimental conditions, because the extracellular concentrations of this neurotransmitter are the crucial effect triggered by an ischemic event (*17*). In fact high extracellular glutamate concentrations during ischemia overstimulate glutamate receptors (mainly of the N-methyl-D-aspartate type) (*18*), promoting calcium overload and nitric oxide production (*19*). Other events may contribute to the development of postischemic neuronal death (mainly free-radical production [*20*] and acidosis [*21*]), but glutamate-triggered neurotoxicity appears to be the foremost event linking brain ischemia to neuronal death (*22*). Therefore, we used extracellular glutamate levels as the hallmark of an effectively performed treatment of in vitro ischemia.

MATERIALS AND METHODS

Preparation of Cerebral Cortex Slices

Male Sprague-Dawley rats (280–300 g) were kept under standard conditions (12-h dark-light cycle, free access to food and water), according to the policy issues approved by the Society of Neuroscience. The rats were decapitated under light ether anesthesia, and the temporoparietal cortex was quickly transferred into a beaker containing oxygenated (95% O_2, 5% CO_2) Krebs solution (in mmol/L: NaCl 118.5, $NaHCO_3$ 25, KCl 4.7, $MgSO_4$ 1.2, KH_2PO_4 1.2, $CaCl_2$ 1.2 and glucose 10) at room temperature. Brain slices (~450 μm thick, 10 mg each) were cut from the temporoparietal cortex using a McIlwain tissue chopper. Then slices were transferred to another beaker containing oxygenated (95% O_2, 5% CO_2) Krebs solution for a 20-min period of recovery from cutting stress.

Preparation, Concentration and Incubation Time of ^{99m}Tc-HMPAO

We prepared ^{99m}Tc-HMPAO by injecting approximately 370 MBq ^{99m}Tc-pertechnetate (eluted from a Sorin Biomedica generator; Sorin, Saluggia, Italy) in 5 mL saline solution into a vial containing HMPAO (Ceretec; Amersham International plc, Buckinghamshire, UK). The solution containing ^{99m}Tc-HMPAO was then diluted with Krebs solution to reach the appropriate final specific activity required for the different tests. The radiochemical purity (RP) of ^{99m}Tc-HMPAO in the kit and in each incubation medium was assessed by the three-strip method (according to the manufacturer's instructions) and by paper chromatography (*23*), and it was expressed as percent decrease of the lipophilic fraction of the tracer. Sample radioactivity was determined by a Cobra autogamma-counter (Packard Instruments Co., Downers Grove, IL).

To evaluate the optimal radioactivity concentration for the detection of a clear uptake signal, we tested four different concentrations of ^{99m}Tc-HMPAO using a 20-min incubation period (Table 1). The concentration of 518 KBq/mL was chosen for subsequent experiments, because it reconciled a high uptake signal with a good RP of ^{99m}Tc-HMPAO. Then, 40-min time-course experiments were performed to evaluate the time-dependent decline in the RP of ^{99m}Tc-HMPAO and the nonspecific cellular incorporation of $Na^{99m}TcO_4$ (Table 2). Although $Na^{99m}TcO_4$ uptake remained stable up to 40 min, ^{99m}Tc-HMPAO uptake increased over the time course of the experiments at the expense of the RP of the tracer. Hence, a 20-min incubation period was chosen for subsequent experiments.

Experimental Conditions Mimicking Metabolic Alterations

Twelve test tubes, each with 1 mL medium, were gently shaken in a Dubnoff thermoregulated bath (Officine Meccaniche KW, Siena, Italy) set at 37°C. The test tubes were divided into four treatment groups, and one slice per tube was put in the solution. The control group contained standard Krebs solution bubbled with 95% O_2 and 5% CO_2. The azide group contained 5 mmol/L azide in standard Krebs solution bubbled with 95% O_2 and 5% CO_2. This compound induces depletion of intracellular adenosine triphosphate (ATP) by inhibiting cytochrome *c* oxidase of the mitochondrial respiratory chain (*24*). The in vitro ischemia group contained a Krebs solution lacking glucose and bubbled with 95% N_2 and 5% CO_2. The cell lysis group contained a solution of 25 mmol/L $NaHCO_3$ bubbled with 95% O_2 and 5% CO_2. The latter experimental condition caused osmotic lysis of the cells, mimicking cell membrane disruption due to tissue necrosis in the core of a stroke.

TABLE 1
^{99m}Tc-HMPAO Uptake at Different Radioactivity Concentrations After 20 Minutes of Incubation

Variables	KBq/mL			
	5.18	51.8	518	7400
^{99m}Tc-HMPAO uptake	5.2% ± 1.3%	6.1% ± 1.0%	7.2% ± 1.5%	8.3% ± 1.6%
RP (t = 0)	50.9% ± 1.3%	58.8% ± 3.1%	88.8% ± 2.0%	90.5% ± 0.7%
RP (t = 20)	41.9% ± 1.7%	52.6% ± 1.7%	81.2% ± 2.0%	81.5% ± 3.4%

HMPAO = hexamethyl propyleneamine oxime.
Radiochemical purity (RP) of lipophilic ^{99m}Tc-HMPAO was determined in incubation medium; t = 0 is beginning of experiment and t = 20 is end of experiment.
Values are mean ± SD of four experiments.

TABLE 2
^{99m}Tc-HMPAO and $Na^{99m}TcO_4$ Uptake as a Function of Incubation Time

Variables	Time (min)				
	0	10	20	30	40
^{99m}Tc-HMPAO uptake	—	5.2% ± 1.0%	7.0% ± 1.7%	12.0% ± 1.4%	20.1% ± 2.4%
$Na^{99m}TcO_4$ uptake	—	1.0% ± 0.1%	1.1% ± 0.1%	1.6% ± 0.2%	1.5% ± 0.3%
RP*	91.4% ± 0.7%	87.4% ± 2.3%	82.1% ± 2.7%	75.6% ± 4.0%	61.6% ± 5.3%

*Radiochemical purity (RP) of lipophilic ^{99m}Tc-HMPAO was determined in incubation medium.
HMPAO = hexamethyl propyleneamine oxime.
Values are mean ± 1 SD of four experiments.

Determination of ^{99m}Tc-HMPAO Uptake and Release by Brain Slices

For uptake studies, ^{99m}Tc-HMPAO was added to each test tube. After 20 min of incubation, slices were carefully washed (three cycles of washing with Krebs solution in separate beakers) and were transferred into counting tubes containing 1 mL 2 N NaOH solution to dissolve the tissue. At the same time, a 0.2-mL aliquot of the incubation medium was picked up from each test tube and was added to 0.8 mL 2 N NaOH solution in counting tubes. The net content of protein of each slice was calculated by the Bradford method (Coomassie Brilliant Blue; Biorad, Munich, Germany) using human serum albumin as a standard (Kabi-vitrum, Stockholm, Sweden) (*25*). Radioactivity taken up by the slices was expressed as percentage of counts per minute (cpm) added to the medium at the beginning of the uptake period (cpm slice plus cpm medium at the end of the uptake period). A correction factor (cf), calculated as mean protein content of all slices per milligram of protein of the single slice, was introduced to normalize taken-up radioactivity to slice size:

$$^{99m}\text{Tc-HMPAO uptake (\%)} = \frac{\text{cpm slice}}{\text{cpm slice + cpm medium at end of uptake period}} \times \text{cf.}$$

In release studies, 12 slices were preloaded at 37°C in a beaker containing 12 mL control medium bubbled with 95% O_2 and 5% CO_2 and 6.2 MBq ^{99m}Tc-HMPAO. After 20 min, slices were carefully washed and were placed in different test tubes. After another 20 min, slices were carefully washed and were transferred into counting tubes containing 1 mL 2 N NaOH solution to dissolve the tissue. A 0.6-mL aliquot of the incubation medium was picked up from each test tube and was added to 0.4 mL 2 N NaOH solution in counting tubes. The radioactivity released by the slices was calculated as the ratio between the cpm in the medium to the cpm in the slice at the beginning of the experiment (cpm medium plus cpm left in the slice at the end of the release period):

$$^{99m}\text{Tc-HMPAO release (\%)} = \frac{\text{cpm medium}}{\text{cpm left in slice + cpm medium}}.$$

Determination of Net Content of ^{99m}Tc-HMPAO in Brain Slices

When the same experimental condition affected both uptake and release leading to opposite final effects on radiotracer net retention, ^{99m}Tc-HMPAO uptake and release experiments were performed in sequence. Hence, slices first underwent a period of uptake and then were washed and transferred in test tubes for a subsequent period of release under the same experimental condition. The cf, previously described for uptake experiments, was introduced to normalize radioactivity content to slice size. Slice radioactivity content was expressed as percentage of counts added to the medium at the beginning of the experiments:

$$^{99m}\text{Tc-HMPAO slice net content (\%)} = \frac{\text{cpm slice}}{\text{cpm added to medium at beginning of experiment}} \times \text{cf.}$$

Determination of Endogenous Glutamate Efflux from Brain Slices

At the end of every experiment, 50 µl medium was picked up from each test tube and was used to assess the glutamate released from the slices by high-performance liquid chromatography (HPLC) and fluorimetric detection after precolumn derivatization with o-phthalaldehyde (*26*). The HPLC system has been described previously (*27*). The column was perfused with a mobile phase containing 0.1 mol/L sodium acetate, 20% methanol and 2.5% tetrahydrofuran (pH 6.5). Under these experimental conditions, the retention time for glutamate was about 1.5 min. Glutamate efflux in the test tubes was expressed as pmol released/mg of slice protein/min.

Determination of Lactate Dehydrogenase Levels in the Medium

Lactate dehydrogenase (LDH) levels (a marker of irreversible cellular damage [*28*]) were measured in the medium by a direct colorimetric method (Technicon DAX system; Technicon, Tarrytown, NY), as described by Wroblewski and LaDue (*29*), with minor modifications as suggested by the Committee on Enzymes of the Scandinavian Society for Clinical Chemistry and Clinical Physiology (*30*). LDH activity released by slices was normalized to slice size and was expressed as U/L/mg of slice protein/min.

Data Analysis

The results were presented as mean ± SD of n experiments. Data were statistically analyzed using one-way analysis of variance (ANOVA) followed by the Dunnett test for multiple comparisons (*31*) or the *t* test for unpaired data, as appropriate; *P* values lower than 0.05 were considered to be significant.

Drugs

All reagents and drugs were purchased from Merck (Darmstadt, Germany) except for glutathione, which was obtained from Sigma (Sigma Chemical Co., St. Louis, MO).

RESULTS

Effect of Metabolic Insults on ^{99m}Tc-HMPAO Uptake and Release

Table 3 summarizes the changes in ^{99m}Tc-HMPAO uptake and release under the four tested experimental conditions.

Under control conditions, slices took up 7.0% ± 1.4% of ^{99m}Tc-HMPAO contained in the medium and released 10.8% ± 2.6% of preloaded tracer content. Adding 5 mmol/L azide to the incubation medium significantly inhibited the uptake of ^{99m}Tc-HMPAO and increased the release of radioactivity from preloaded slices. In vitro ischemia did not significantly modify tracer uptake, whereas it significantly increased the release of ^{99m}Tc-HMPAO from preloaded slices. Under cell lysis condition, a 2-fold rise in the amount of radioactivity taken up by the slices could be demonstrated. Cell lysis also caused a 5-fold increase in ^{99m}Tc-HMPAO release from preloaded slices.

Effect of Metabolic Insults on Glutamate and Lactate Dehydrogenase Efflux

Under control conditions, the amount of glutamate and LDH released from rat cerebral cortex slices were 49.1 ± 21.6 pmol/mg protein/min and 4.8 ± 0.9 U/L/mg protein/min, respectively. Adding 5 mmol/L azide to the incubation medium doubled glutamate efflux from the slices, without any significant modification in LDH efflux (Table 3). In vitro ischemia evoked a far higher glutamate efflux, reaching ~18-fold of the control values, but no statistically significant increase in LDH efflux was found (Table 3). As expected, brain slices incubated in hypotonic solution gave rise to a massive outflow of glutamate and to a significant increase in LDH efflux, reaching 4.5-fold more than the control values (Table 3).

^{99m}Tc-HMPAO Net Content Under Hypotonic Medium Condition

Because cell lysis affected both ^{99m}Tc-HMPAO uptake and release, leading to opposite final effects on radiotracer net retention, the net content protocol was applied. Under the control condition, ^{99m}Tc-HMPAO net retention in the slices at the end of the experiment was 6.5% ±1.3% of that initially added to the medium. Under the cell lysis condition, ^{99m}Tc-HMPAO net retention in the slices at the end of the experiment was 12.3 ± 2.3 ($P < 0.01$ versus control) (Fig. 1, left bars). Also, $Na^{99m}TcO_4$ net retention was studied under control and cell lysis conditions and was found to be very low in both cases (1.0% ± 0.3% and 0.9 ± 0.4, respectively; n = 3). Moreover, we evaluated the importance of ^{99m}Tc-HMPAO lipophilia by studying the net retention of the hydrophilic derivatives of the tracer. Because paper chromatography demonstrated that after 5 min of incubation with 1 mmol/L glutathione almost all of the radiotracer (>95%) was converted into its hydrophilic form(s), ^{99m}Tc-HMPAO was incubated with 1 mmol/L glutathione before the beginning of the experiments. The net retention of the hydrophilic ^{99m}Tc-HMPAO derivatives was nearly 4-fold lower than that of native lipophilic ^{99m}Tc-HMPAO and was not significantly different under control and cell lysis conditions (Fig. 1, right bars).

DISCUSSION

Under the control condition, glutamate levels were consistent with good cell viability (*20*). LDH levels, however, were detectable. This can be explained by considering cell membrane disruption occurring during brain slice preparation in neurons immediately below the cut surface. ^{99m}Tc-HMPAO uptake by control slices appeared to be efficient, especially considering the extremely high volume-to-volume ratio between the medium and the slice. On the other hand, only 10.8% of slice tracer content was released in the medium, indicating an effective trapping mechanism.

Metabolic poisoning with azide, as already reported in the literature (*15*), decreased ^{99m}Tc-HMPAO uptake and en-

TABLE 3
Effect of Metabolic Insults on ^{99m}Tc-HMPAO Uptake and Release

Incubation condition	Analyzed variables			
	^{99m}Tc-HMPAO uptake (%)	^{99m}Tc-HMPAO release (%)	Glutamate (pmol/mg protein/min)	LDH (U/L/mg protein/min)
Control	7.0 ± 1.4	10.8 ± 2.6	49.1 ± 21.6	4.8 ± 0.9
Azide	5.8 ± 0.9*	15.8 ± 3.3†	103.1 ± 37.1*	6.9 ± 2.0
Ischemia	6.9 ± 2.1	18.3 ± 6.2†	886.9 ± 298.7†	4.3 ± 1.1
Cell lysis	14.7 ± 3.9†	56.0 ± 10.2†	3049.1 ± 977.1†	21.4 ± 4.6†

*$P < 0.05$ vs. control condition.
†$P < 0.01$ vs. control condition.
HMPAO = hexamethyl propyleneamine oxime; LDH = lactate dehydrogenase.
Values are mean ± 1 SD of five experiments.

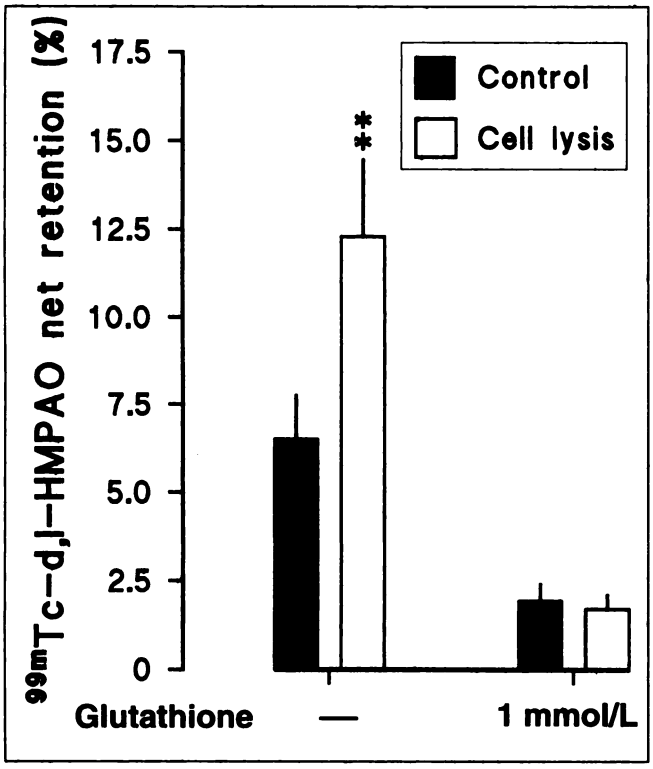

FIGURE 1. ^{99m}Tc-HMPAO net retention (%) under control and cell lysis conditions. Under cell lysis condition, native lipophilic ^{99m}Tc-HMPAO massively accumulates into cell. Presence of 1 mmol/L glutathione significantly reduces retention of tracer under control condition and completely prevents cell accumulation of ^{99m}Tc-HMPAO under cell lysis condition. Values are mean ± SD of four experiments. $^{**}P < 0.01$ versus control condition.

hanced its release. The net retention of the tracer by the slices was clearly reduced. In vitro ischemia displayed a similar net result, but it significantly affected only ^{99m}Tc-HMPAO release, suggesting that the factors involved in ^{99m}Tc-HMPAO uptake mechanism (which were sensitive to azide but not to in vitro ischemia) differ from those involved in ^{99m}Tc-HMPAO release (which were sensitive to both treatments). Furthermore, the difference between azide poisoning and in vitro ischemia was even greater where glutamate outflow was concerned. It was slightly (2-fold) and massively (18-fold) increased by the former and latter treatment, respectively. Hence, azide poisoning did not faithfully reproduce the metabolic effects of a cerebrovascular accident, because, in the ischemic cerebral tissue, glutamate extracellular levels are dramatically raised (*17*), causing delayed neuronal death (*22*). On the other hand, both azide poisoning and in vitro ischemia did not increase LDH levels, indicating the occurrence of a reversible tissue injury (at least within the 20-min period of treatment).

Under the cell lysis condition, the massive outflow of ^{99m}Tc-HMPAO, glutamate and LDH can be easily explained by the leakage of cytoplasmic contents from cells undergoing osmotic lysis. However, under the same experimental condition, a 2-fold increase of ^{99m}Tc-HMPAO uptake was also found. The extrapolation of a net retention forecast from isolated uptake and release experiments is in this case untrustworthy. The ^{99m}Tc-HMPAO released by the lysed tissue had been previously taken up and trapped by slices under control conditions. Because intracellular trapping of native lipophilic ^{99m}Tc-HMPAO involves its reduction (mainly by glutathione) to hydrophilic derivatives (*2,4,5*), the important differences in term of chemical properties between the native and reduced tracer should be kept in mind. In fact, the massive release of ^{99m}Tc-HMPAO observed under the cell lysis condition dealt with the reduced hydrophilic derivatives of the tracer, which readily diffused in the aqueous medium (Fig. 2A). Conversely, during net retention experiments, native lipophilic ^{99m}Tc-HMPAO was allowed to meet an already osmotically lysed tissue. These experiments demonstrate an important increase (nearly 2-fold) in ^{99m}Tc-HMPAO net retention by lysed slices. Moreover, the increased retention was specifically due to the HMPAO part of the compound, because $Na^{99m}TcO_4$ net retention was nearly identical under control and cell lysis conditions. It can be hypothesized that native lipophilic ^{99m}Tc-HMPAO dissolves more easily throughout a lysed cell, soaking into the large amount of intracytoplasmic membranes disclosed after cell membrane damage (Fig. 2B). ^{99m}Tc-HMPAO is the brain perfusion tracer displaying the higher octanol/water partition coefficient (pc = 80 [*32*]). Accordingly, it was demonstrated that ^{99m}Tc-HMPAO is mainly found attached to cell organelles in homogenates of rat brain (*3,6*). The "lipophily hypothesis" is further corroborated by the decrease in ^{99m}Tc-HMPAO net retention observed after tracer reduction to its hydrophilic derivatives by glutathione (Fig. 1). In fact, hydrophilic ^{99m}Tc-HMPAO was no longer able to cross cell membranes easily (see the dramatic decrease in tracer net retention under the control condition) and to soak into the membranes of the damaged tissue (see the complete prevention of the nearly two-fold increase in tracer net retention under the cell lysis condition).

In trying to broaden the meaning of our data to in vivo SPECT imaging, one has to keep in mind the high degree of oversimplification inherent in an in vitro slice model. Nevertheless, our findings may help to explain some misleading flow-independent changes in ^{99m}Tc-HMPAO distribution during the acute and subacute phase of stroke.

In the acute phase of stroke, both ^{99m}Tc-HMPAO hypocaptation (counting rates below the actual rCBF demonstrated by PET [*10*]) and filling out (leakage of the tracer in delayed images of mild cerebral ischemia [*9*]) have been reported. These findings can be explained by the present data, because ^{99m}Tc-HMPAO release from brain slices is enhanced during in vitro ischemia. This may be due to a decrease in the resynthesis of reduced glutathione (the main cause for ^{99m}Tc-HMPAO intracellular trapping) secondary to ATP depletion (*15*). However, in this phase of stroke, particularly high counting rates of ^{99m}Tc-HMPAO have sometimes been reported (*33*). As demonstrated using PET and ^{133}Xe blood-

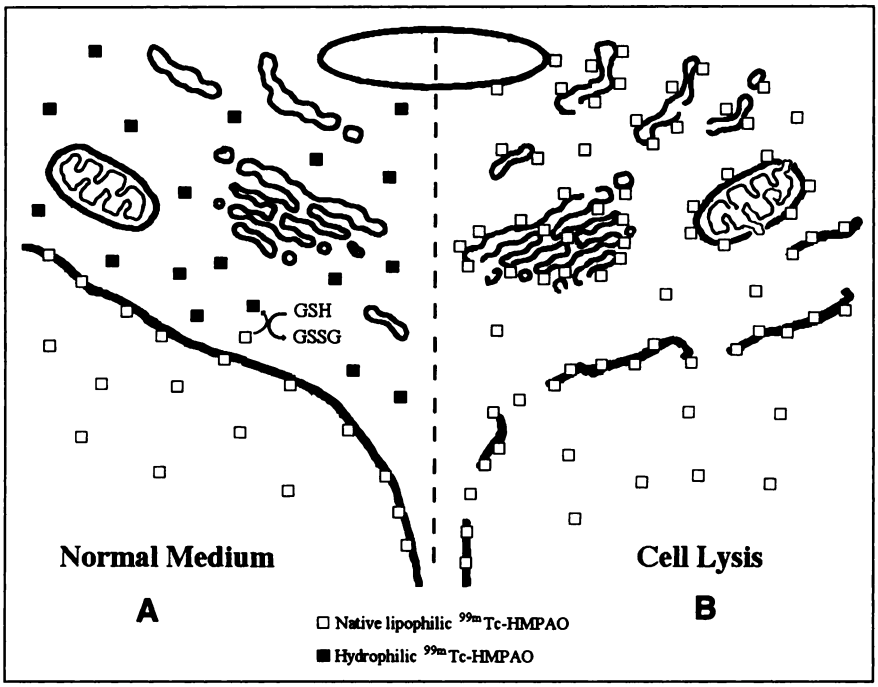

FIGURE 2. (A) Under control conditions, ^{99m}Tc-HMPAO retention depends mainly on reduced glutathione (GSH) activity, which traps lipophilic tracer in cells converting it into its hydrophilic derivatives. (B) Under cell lysis conditions, ^{99m}Tc-HMPAO remains in lipophilic native form and can soak into cytoplasmic membrane and into many intracellular membranous sites, easily reachable after cell membrane disruption.

flow techniques (*34–36*), these high counting rates are probably associated with true reperfusion hyperemia and are often linked to tissue viability preservation in the chronic phase of stroke (*12,14*). However, Shimosegawa et al. (*37*) reported two cases of high counting rates of ^{99m}Tc-HMPAO within 6 h after the onset of cerebral infarction corresponding to low-density necrotic areas on follow-up CT scans. Therefore, it cannot be excluded that in some cases high counting rates could be due to enhanced soaking of ^{99m}Tc-HMPAO into an already necrotic tissue (see present data) in the absence of significant hyperemic changes.

In the subacute phase of stroke, high counting rates of ^{99m}Tc-HMPAO (higher than the contralateral healthy hemisphere) represent a far more common finding. This phenomenon is encountered in 20%–48% of cases according to the different studies (*14,38*) and is typically located inside the infarcted region (*12*). Interestingly, Sperling and Lassen (*12*) demonstrated an evident inconsistency between high counting rates of ^{99m}Tc-HMPAO and actual rCBF measures obtained by ^{133}Xe: ^{99m}Tc-HMPAO hyperfixation intervened despite the absence of any hyperemic phenomenon. Moreover, in a subsequent study (*11*), they reported that 13 of 14 cases of high counting rates of ^{99m}Tc-HMPAO in the subacute stroke were due to tracer hyperfixation by the tissue. Unfortunately, because the number of studies comparing rCBF measures by ^{133}Xe or $C^{15}O_2$ with ^{99m}Tc-HMPAO distribution is rather limited in the literature, ^{99m}Tc-HMPAO hyperfixation cannot be definitively proved in many cases of high counting rate detection. Nevertheless, our data clearly indicate that when SPECT imaging of reperfused strokes displays high counting rates per hyperfixation of ^{99m}Tc-HMPAO (for example the classical "hot spot"), tissue necrosis must be suspected. In fact, as demonstrated for slices under the cell lysis condition, the necrotic evolution of the infarcted tissue and the fall to the floor of reduced glutathione levels predict an enhanced soaking into the tissue of the native lipophilic ^{99m}Tc-HMPAO. This consideration leads to an important inference concerning the clinical meaning of ^{99m}Tc-HMPAO hyperfixation. It is likely due to a deeply damaged tissue with poor (if any) potential recovery.

In regard to the prognostic value of high counting rates of ^{99m}Tc-HMPAO in SPECT imaging, conflicting results are reported in the literature, probably because of the difficulties in discriminating between reperfusion hyperemia and hyperfixation in the different phases (acute, subacute and chronic) of stroke. However, several cases of ^{99m}Tc-HMPAO hyperfixation, later reexamined in the chronic phase of stroke, showed hypoperfusion, suggesting loss of viable tissue (*11*). Similarly, high counting rates of ^{99m}Tc-HMPAO are often followed by hypoperfusion in the chronic phase of stroke (*38,39*), suggesting a previous hyperfixation by necrotic tissue. Moreover, occasional normal CT finding (preservation of gross tissue structure) in the chronic phase of subacutely hyperfixating small infarcts does not exclude loss of small groups of neurons, as suggested by hypoperfusion demonstrated by SPECT in the chronic phase (*11*). It is noteworthy that Papazyan et al. (*40*) reported high counting rates of ^{99m}Tc-HMPAO in grade III astrocytoma, whereas ^{201}Tl-SPECT and PET showed poor metabolic activity in the same area, suggesting a probable ^{99m}Tc-HMPAO hyperfixation by the necrotic center of the tumor. The availability of different imaging techniques (CT, SPECT, PET and MRI) will probably help to overcome limitations related to a single modality of investigation.

CONCLUSION

In conclusion, our data confirmed and extended previous observations that ^{99m}Tc-HMPAO net retention in neurons depended not only on rCBF but also on the metabolic status of the tissue. This study also provided evidence that poisoning of the tissue and in vitro ischemia induced a reduction of ^{99m}Tc-HMPAO retention; however, the effects of these treatments were not superimposable. Finally, we demonstrated that injuries inducing cell membrane disruption increase ^{99m}Tc-HMPAO net retention. This suggests that SPECT images displaying high counting rates per hyperfixation of this tracer may be the hallmark of a deeply damaged tissue with poor (if any) potential of recovery.

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