sine-induced dilatation of pial arteries of cats. Blood Vessels 14: 285–293, 1977
17. Lux HD: Extracellular potassium in the CNS: relation to excitability changes. *In* Ingvar D, Lassen N (eds): Brain Work. Copenhagen, Munkgaard, pp 172–181, 1975
18. Lewis DV, Schuette WH: NADH fluorescence and K^+ changes during hippocampal electrical stimulation. J Neurophysiol 38: 405–417, 1975
19. Raberger G, Weissel M, Kraupp O: The dependence of the effects of i. cor. administered adenosine and of coronary conductance on the arterial pH, pCO_2 and buffer capacity in dogs. Arch Pharmacol 271: 301–310, 1971
20. Burnstock G: Purinergic transmission. *In* Iverson LL, Iverson SD, Snyder SH (eds): Handbook of Psychopharmacology, vol 5. New York, Plenum Publishing Corporation, pp 131–194, 1975
21. Holmsen H, Day HJ, Stormorken H: The blood platelet release action. Scand J Haemat (Suppl 8): 1–26, 1969
22. Kalendovsky Z, Austin JH: "Complicated migraine," its association with increased platelet aggregability and abnormal plasma coagulation factors. Headache 15: 18–35, 1975
23. Norwood ChW, Poole GJ, Moody D: Treatment of experimental delayed cerebral arterial spasm with a $beta_2$-adrenergic stimulator and a phosphodiesterase inhibitor. J Neurosurg 45: 491–497, 1976
24. Toda N, Hojo M, Sakae K, Usui H: Comparison of the relaxing effect of dopamine with that of adenosine, isoproterenol and acetylcholine in isolated canine coronary arteries. Blood Vessels 12: 290–301, 1975
25. Namm DH, Leader JP: Occurrence and function of cyclic nucleotides in blood vessels. Blood Vessels 13: 24–47, 1976
26. Fog-Møller F, Kemp Genefke I, Bryndum B: Changes in concentration of catecholamines in blood during spontaneous migraine attacks and reserpine-induced attacks. *In* Greene R (ed): Current Concepts in Migraine Research. New York, Raven Press, pp 115–119, 1978
27. Vane JR: Prostacyclin, an endogenous vasodilator and antiplatelet substance generated in the artery wall. *In* Second International Migraine Symposium. The Migraine Trust. London, 1978
28. Gillilan LA: Blood supply to the central nervous system. *In* Crosby EC, Humphrey T, Lauer EW (eds): Correlative Anatomy of the Nervous System. New York, MacMillan Company, pp 550–579, 1972

Local Cerebral Blood Flow in the Conscious Rat As Measured with ^{14}C-Antipyrine, ^{14}C-Iodoantipyrine and ^{3}H-Nicotine

K. OHNO, M.D., K. D. PETTIGREW, AND S. I. RAPOPORT, M.D.

SUMMARY **Local cerebral blood flow (LCBF) in the conscious rat was estimated with one of 3 radiotracers —^{14}C-antipyrine, ^{14}C-iodoantipyrine or ^{3}H-nicotine. A tracer was infused intravenously at a constant rate and blood concentration was followed until the animal was killed by decapitation. Tracer concentration was then measured in each of 14 brain regions. The Kety-Schmidt analysis was applied to the data with ^{14}C-antipyrine and ^{14}C-iodoantipyrine. The results confirmed findings of Sakurada et al. (1978) that ^{14}C-iodoantipyrine provides LCBF's that are twice those obtained with ^{14}C-antipyrine and that approximate LCBF's found with an inert gas. LCBF was calculated from the ^{3}H-nicotine data by assuming complete extraction of tracer from blood by brain. This assumption was approximated for infusion times of 50 sec or less, when LCBF's derived with ^{3}H-nicotine generally did not differ significantly from LCBF's obtained with ^{14}C-iodoantipyrine. Fifty-sec ^{3}H-nicotine-derived flows for the pineal and pituitary glands were, respectively, 2.59 ± 0.36 (SEM) cm^3 g^{-1} min^{-1} and 1.28 ± 0.08 cm^3 g^{-1} min^{-1}. LCBF's calculated for 70 sec to 240 sec of ^{3}H-nicotine infusion were lower than 50-sec values due to back-diffusion, but nevertheless were as high as ^{14}C-antipyrine LCBF's due to the marked binding of tracer by brain tissue. The results supported the conclusion of Sakurada et al. (1978) that iodoantipyrine is the non-gaseous agent of choice for measuring LCBF precisely, but also showed that short infusion schedules with nicotine provided good estimates of LCBF.**

Stroke, Vol 10, No 1, 1979

LOCAL CEREBRAL blood flow (LCBF) can be determined in experimental animals by employing an inert gas tracer, ^{131}I-trifluoriodomethane, and applying the mathematical principles of Kety to analyze blood-brain exchange.[1-4] The principles are valid because diffusional equilibrium between brain and blood is established almost instantaneously.[5, 6]

Since use of a gas tracer presents technical difficulties, attempts have been made to employ non-gaseous tracers to measure LCBF. ^{14}C-antipyrine has been used with some success, but it provides LCBF's which are less than those obtained using inert gases. Transfer of ^{14}C-antipyrine from blood to brain is limited by the comparatively low diffusion coefficient of that tracer in the brain. The cerebrovascular PS value (permeability × surface area) of ^{14}C-antipyrine is about 0.020 cm^3 sec^{-1} g^{-1}, and is not sufficiently high to satisfy the assumptions of the Kety method.[4, 5, 7-10]

Early work suggested that ^{131}I-iodoantipyrine might

From the Laboratory of Neurosciences, National Institute on Aging, Gerontology Research Center, Baltimore City Hospital, Baltimore, MD 21224 and Theoretical Statistics and Mathematics Branch, National Institute of Mental Health, Bethesda, MD 20014.

Dr. Ohno is Visiting Fellow, Department of Neurosurgery, Tokyo Medical and Dental University, Tokyo, Japan.

Reprints: Dr. Rapoport, Laboratory of Neurosciences, Gerontology Research Center, Baltimore City Hospital, Baltimore, MD 21224.

be useful for measuring cerebral blood flow,[11, 12] but it was not until recently that Sakurada et al.[4] showed that ^{14}C-iodoantipyrine, which has a higher oil/water partition coefficient than ^{14}C-antipyrine and also is more permeable at the cerebrovasculature, provides values of LCBF by the Kety method in the conscious rat that are comparable to those obtained with ^{131}I-trifluoriodomethane. Autoradiography was used to measure local brain concentrations of the ^{14}C-tracer.

In this paper, we compare LCBF's that were obtained with each of 3 tracers — ^{14}C-antipyrine, ^{14}C-iodoantipyrine and ^{3}H-nicotine — in regions of the rat brain that were dissected out according to criteria of Glowinski and Iversen[13] and Chiueh et al.[14] Knowledge of LCBF in these specific regions is required to calculate regional cerebrovascular permeability to a variety of agents.[15]

The mathematical treatment of Kety[5] was employed to estimate LCBF with both ^{14}C-antipyrine and ^{14}C-iodoantipyrine. The treatment could not be used with ^{3}H-nicotine, however, because this tracer binds to brain and is not freely exchangeable between brain and blood.[10, 16, 17] We estimated LCBF with ^{3}H-nicotine by assuming that all of the tracer was extracted from blood by brain. We chose ^{3}H-nicotine because it, like ^{14}C-iodoantipyrine, is more permeable than is ^{14}C-antipyrine at the cerebral vasculature and because its affinity for brain tissue might allow approximately complete extraction at short infusion times.[10, 17]

Methods

Male rats (Osborn-Mendel strain), weighing 250 g to 350 g, were anesthetized with Na pentobarbital (35 mg kg^{-1}, i.p.). Polyethylene catheters containing 100 IU Na heparin and 0.009 g NaCl/ml of water were implanted in the left femoral artery and vein. The skin was sutured at the catheter exit and infiltrated with 2% butacaine sulfate (Abbott Labs, N. Chicago, Ill.). The hindquarters of the animals were wrapped in a fast-setting plaster bandage (Johnson and Johnson, New Brunswick, N.J.), with hindlimbs and catheters protruding, after which the bandage was tied down on a lead block.[4] The animals were allowed to recover from anesthesia for 4 hr or more. In their harness, the conscious rats could freely move their forequarters, head and neck, and appeared comfortable.

The catheter in the femoral vein was connected to a 10-ml syringe, which was held in a constant flow pump (Model 255-2, Sage Instruments Inc., White Plains, N.Y.) that was set to deliver fluid at a rate of 0.28 ml min^{-1}. Prior to infusion, a sample of arterial blood was removed for the determination of hematocrit, and for measurement of P_{CO_2}, pH and P_{aO_2} (pH-Blood Gas Analyzer, No. 213, Instrumentation Labs, Lexington, Mass.). The femoral vein was infused for up to 4 min with isotonic saline containing 20 μC/ml of ^{3}H-nicotine-d-bitartrate (Amersham/Searle, sp. act. = 240 mC/mmol), for 60 sec with isotonic saline containing 4 μC/ml of ^{14}C-antipyrine (Amersham/Searle, sp. act. = 52 mC/mmol) and for 45 sec with isotonic saline containing 9 μC/ml of ^{14}C-iodoantipyrine (New England Nuclear, sp. act. = 50 mC/mmol). The purity of each tracer exceeded 96%, as confirmed by thin layer chromatography. Periodically during infusion, 20 μl samples of arterial blood were collected into heparinized tubes, after which 10 μl aliquots were transferred to scintillation vials.

Animals were decapitated 30 sec to 4 min after infusion began. The skull was opened and the brain was removed and placed on cold filter paper wetted with 0.9% NaCl. Large subarachnoidal and dural blood vessels were removed and discarded. Brain regions then were dissected out, according to the method of Chiueh et al.,[14] and placed in tared scintillation vials that immediately were re-weighed.

The pineal and pituitary glands were removed and the brain was hemisectioned in the midline. The caudate nucleus in the anterior horn of the lateral ventricle, and the hippocampus in the posterior-inferior horn were removed with a curved forceps. The hypothalamus and thalamus then were dissected away from the cerebral cortex and midbrain, using landmarks of the anterior commissure, massa intermedia, mamillary body and internal capsule. The cerebellum subsequently was separated from brain stem, and midbrain and brain stem were separated at the level below the inferior colliculi. In addition to sampling occipital and frontal cortical and subcortical regions, which contained gray and white matter, gray matter was separated from white matter in the temporal region and corpus callosum. Reproducibility of the dissection procedure has been published.[14]

Scintillation vials that contained blood or brain samples received 1.5 ml of Soluene 100 (Packard Instrument Co., Downers Grove, Ill.) and were shaken for 6 hr in a water bath at 60°C. Fifteen ml of Dimilume 30 (Packard Instrument Co.), a liquid scintillation fluid, were added before counting in a Packard Tricarb Liquid Scintillation Spectrometer (Model 2405). Counts per minute (cpm) was converted to disintegrations per minute (dpm), a measure of absolute radioactivity, by using external standardization and predetermined efficiency curves.

Mathematical Analysis of Data

Arterial blood concentrations (C_{blood}dpm/ml) that were measured during i.v. infusion of either ^{14}C-antipyrine or ^{14}C-iodoantipyrine were plotted against time and were fit by a non-linear, least-squares procedure with the following equation,[18]

$$C_{blood} = A + Be^{-Rt} + De^{-St} \quad (1)$$

A, B, C, R, and S are constants in Eq. 1 and A + B + C = 0, because C_{blood} = 0 at t = 0 (time when blood concentration starts to rise). Computer fitting provided numerical estimates of the constants from the arterial plasma curve (fig. 1 below).

As shown by Eckman et al.,[5] a transfer constant K can be defined as follows: where F = LCBF, λ = steady state, tissue:blood partition coefficient for a

particular tracer, and m is a constant between 0 and 1 that represents the extent to which diffusional equilibrium is established between tissue and blood,

$$K = mF/\lambda \quad (2)$$

Local diffusional equilibrium has to be established for the Kety approach to be valid, in which case m approximates 1 and $K = F/\lambda$. F then can be obtained by a least squares fit of the following equation to the data, where T = time of brain sampling and C_{brain} (T) = tracer concentration (dpm/g) in brain parenchyma (excluding intravascular concentration) at time T, and only K is unknown,

$$C_{brain}(T)/\lambda = A - (A + BK/[K - R] + DK/[K - S])e^{-KT} + (K/[K - R])Be^{-RT} + (K/[K - S])De^{-ST} \quad (3)$$

K was calculated with Eq. 3 by a non-linear iterative least squares process that employed the MLAB program on a PDP-10 computer.[17, 18] F was obtained from K by Eq. 2, letting m = 1 and $\lambda = 0.9$ for ^{14}C-antipyrine and 0.8 for ^{14}C-iodoantipyrine.[4]

Results

Figure 1 illustrates blood concentrations of ^{14}C-iodoantipyrine during 45 sec of infusion, as well as the least squares fit of Eq. 1 to these data. The constants that were derived by the fit were inserted into Eq. 3, together with m = 1, $\lambda = 0.8$[4] and C_{brain} (T). C_{brain} (T), which represents intraparenchymal brain concentration of tracer, was obtained by subtracting intravascular from net regional radioactivity when the former quantity was taken as the product of regional blood volume and blood concentration (dpm/ml). Regional volumes are as follows (% of wet wt): olfactory bulb (4.68), caudate nucleus (1.24), hippocampus (1.61), frontal lobe (2.09), occipital lobe (2.42), thalamus + hypothalamus (1.79), colliculi (2.01), cerebellum (3.46), pons (2.56), medulla (3.53), gray matter (parietal) (2.70), white matter (corpus callosum) (1.14). Regional blood volume at the pineal and pituitary glands was taken as 5% of wet wt.[15, 19]

Mean hematocrit, arterial pH, Pa_{O_2} and Pa_{CO_2} did not differ significantly between experimental groups ($P > 0.05$). For 7 animals infused either with ^{14}C-antipyrine or ^{14}C-iodoantipyrine, means ± SEM's were as follows: hematocrit = 48.0 ± 0.24%, Pa_{CO_2} = 38.4 ± 1.8 mm Hg, Pa_{O_2} = 83.8 ± 3.2 mm Hg and pH = 7.41 ± 0.013 units. For 10 animals administered ^{3}H-nicotine, means ± SEM's were: hematocrit = 47.4 ± 0.81%, Pa_{CO_2} = 39.2 ± 0.46 mm Hg, Pa_{O_2} = 84.1 ± 0.81 mm Hg and pH = 7.41 ± 0.02 units.

Table 1 lists mean LCBF's that were calculated by Eqs. 1 and 3 from data obtained with ^{14}C-antipyrine and ^{14}C-iodoantipyrine. The results confirm the original findings of Sakurada et al.[4] that ^{14}C-iodoantipyrine provides about 2-fold higher values of LCBF than does ^{14}C-antipyrine. Furthermore, the LCBF's in table 1 generally do not differ significantly from published values by Sakurada et al.,[4] although LCBF's for ^{14}C-antipyrine generally are less than ^{14}C-antipyrine LCBF's reported by Ginsberg et al.[20] As compared to the mean values in table 1, LCBF's reported by Sakurada et al.,[4] are larger at the cerebellum with ^{14}C-antipyrine, and smaller at white matter with both ^{14}C-antipyrine and ^{14}C-iodoantipyrine. Not all their data are comparable to data in table 1, however, as many regions in the table include several areas that were analyzed separately by their more discriminating autoradiographic method.

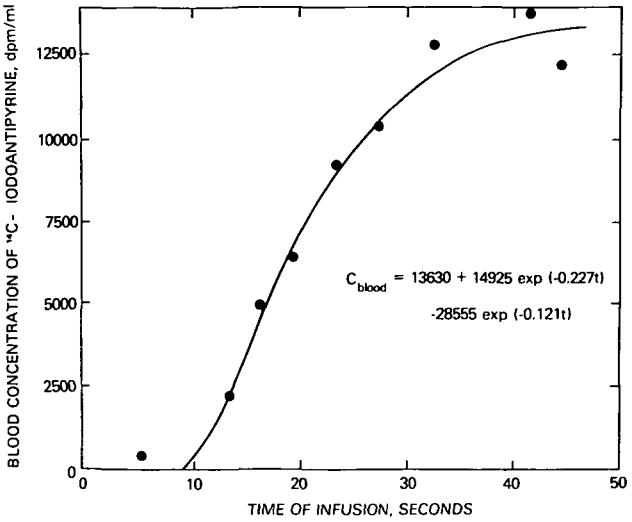

FIGURE 1. *Arterial blood ^{14}C concentration during i.v. infusion of ^{14}C-iodoantipyrine in the conscious rat. Note that t in the equation equals* Infusion Time — *8 sec.*

TABLE 1 *LCBF Calculated with ^{14}C-Antipyrine and ^{14}C-Iodoantipyrine*

Structure	Local cerebral blood flow, cm³g⁻¹min⁻¹	
	^{14}C-Antipyrine	^{14}C-Iodoantipyrine
Olfactory bulb	0.59 ± 0.03(6)[a,b]	1.02 ± 0.10(6)
Caudate nucleus	0.62 ± 0.04[b]	1.80 ± 0.19
Hippocampus	0.60 ± 0.05[b]	1.38 ± 0.16
Frontal lobe	0.73 ± 0.05[b]	1.68 ± 0.29
Occipital lobe	0.70 ± 0.06[b]	1.80 ± 0.29
Thalamus + hypothalamus	0.62 ± 0.05[b]	1.50 ± 0.17
Superior colliculus	0.67 ± 0.05[b]	1.68 ± 0.17
Inferior colliculus	0.80 ± 0.08[b]	2.04 ± 0.25
Cerebellum	0.58 ± 0.04[b]	1.02 ± 0.11
Pons	0.56 ± 0.04[b]	1.26 ± 0.12
Medulla	0.49 ± 0.05[b]	1.20 ± 0.13
Gray matter, parietal	0.86 ± 0.08[b]	2.40 ± 0.37
White matter, corpus callosum	0.43 ± 0.04[b]	1.14 ± 0.14
Pineal gland	0.61 ± 0.40	—
Pituitary gland	0.74 ± 0.16	1.04 ± 0.15

[a]Mean ± SEM (No. of experiments in column is in parenthesis).
[b]Differs significantly from ^{14}C-iodoantipyrine mean ($p < 0.05$).

The Kety analysis, which assumes diffusional equilibrium between plasma and brain, cannot be employed if the tracer is bound or incorporated within the brain as is ^{3}H-nicotine.[10, 17] We therefore estimated LCBF with ^{3}H-nicotine when assuming complete extraction by brain, and evaluated this assumption at different durations of i.v. infusion. LCBF was calculated as the ratio of net measured regional brain ^{3}H content at the time of death (T), dpm/g [parenchymal tracer C_{brain} (T) plus intravascular tracer (C_{blood} (T) × Blood Volume)], divided by the integrated blood concentration curve up to this time,

$$LCBF = \frac{\text{Net brain concentration (T)}}{\int_o^T C_{blood} dt} \quad (4)$$

Table 2 presents mean LCBF's that were calculated by Eq. 4 at infusion times of 30 to 240 sec, and figure 2 presents these means when normalized to the maximal mean for a specific region, taken as 100% and usually at 50 sec of infusion. In no instance did the 30-sec mean differ significantly from the 50-sec mean LCBF, although LCBF's derived at infusion times of 70 sec or more generally were significantly less than respective 50-sec values ($P < 0.05$). With some few exceptions (cf. table 1), 30-sec and 50-sec LCBF's derived with ^{3}H-nicotine did not differ from LCBF's derived with ^{14}C-iodoantipyrine but did exceed ^{14}C-antipyrine LCBF's.

As illustrated by figure 3, binding of ^{3}H-nicotine by brain was markedly evident at infusion times of 70 sec to 240 sec, when brain concentration of tracer exceeded blood concentration by as much as 4-fold. For infusion times of 50 sec or less, blood concentration usually exceeded brain concentration. This inequality and the large binding of intracerebral tracer reduced back-diffusion from brain to such an extent that Eq. 4 could be used to afford LCBF's that approximated those obtained with ^{14}C-iodoantipyrine.

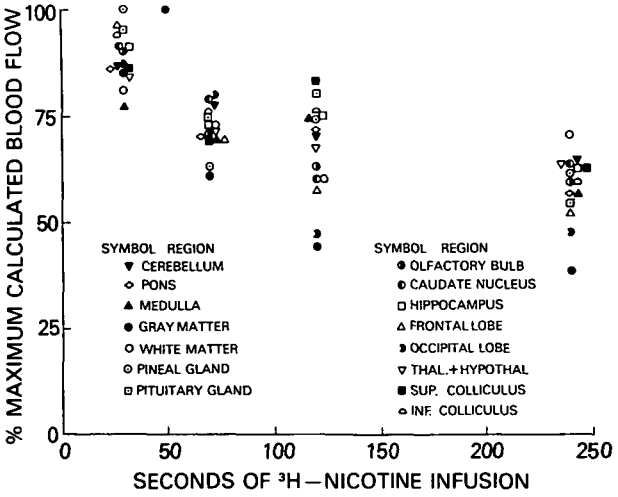

FIGURE 2. *LCBF obtained with ^{3}H-nicotine in rats infused intravenously for between 30 and 240 sec, given as percent of maximal value for each brain region.*

Discussion

These findings on dissected brain regions confirm conclusions obtained by autoradiographic procedures that ^{14}C-iodoantipyrine provides higher values of LCBF than does ^{14}C-antipyrine in the conscious rat.[4]

TABLE 2 *Calculated LCBF with ^{3}H-nicotine in Relation to i.v. Infusion Time. Mean LCBF's at Infusion Times of 30, 70, 120, 240 sec were Compared to LCBF's at 50 sec by a Multiple Comparisons Procedure, Using Bonferroni t Statistics.*[38]

Structure	Infusion time (sec) 30	50	70	120	240
	Local cerebral blood flow, cm^3 g^{-1} min^{-1}				
Olfactory bulb	1.11 ± 0.12(6)[a,c]	1.23 ± 0.08(10)[c]	0.87 ± 0.06(6)[b]	0.78 ± 0.08(3)[b]	0.73 ± 0.07(3)[b]
Caudate nucleus	1.17 ± 0.11[c,d]	1.28 ± 0.08[c,d]	1.01 ± 0.10[c]	0.77 ± 0.19[b]	0.81 ± 0.08[b]
Hippocampus	1.04 ± 0.10[c]	1.14 ± 0.08[c]	0.84 ± 0.07	0.85 ± 0.11	0.71 ± 0.06[b]
Frontal lobe	1.74 ± 0.16[c]	1.82 ± 0.17[c]	1.23 ± 0.15	1.04 ± 0.28	0.95 ± 0.07[b]
Occipital lobe	1.57 ± 0.15[c]	1.80 ± 0.12[c]	1.44 ± 0.13[c]	0.85 ± 0.17[b]	0.84 ± 0.07[b]
Thal. + hypothal.	1.03 ± 0.08[c,d]	1.23 ± 0.07[c]	0.87 ± 0.06[b]	0.83 ± 0.15[b]	0.77 ± 0.04[b]
Sup. colliculus	1.08 ± 0.12[c,d]	1.26 ± 0.08[c,d]	0.86 ± 0.05[b]	1.04 ± 0.16	0.78 ± 0.04[b]
Inf. colliculus	1.28 ± 0.16[c]	1.36 ± 0.07[c]	1.03 ± 0.06	1.03 ± 0.11	0.80 ± 0.04[b]
Cerebellum	0.79 ± 0.09	0.91 ± 0.04[c]	0.71 ± 0.04[b]	0.64 ± 0.08[b]	0.58 ± 0.05[b]
Pons	0.95 ± 0.07[c]	1.11 ± 0.07[c]	0.78 ± 0.05[b]	0.80 ± 0.07[b]	0.62 ± 0.03[b]
Medulla	0.85 ± 0.09[c]	1.10 ± 0.06[c]	0.75 ± 0.05[b]	0.81 ± 0.05[c]	0.62 ± 0.04[b]
Gray matter	2.38 ± 0.19[c]	2.81 ± 0.21[c]	1.72 ± 0.16[b]	1.23 ± 0.37[b]	1.08 ± 0.03[b]
White matter	0.59 ± 0.05[d]	0.73 ± 0.06[c,d]	0.53 ± 0.05[b]	0.44 ± 0.12[b]	0.51 ± 0.03
Pineal gland	4.16 ± 1.54	2.59 ± 0.36	1.63 ± 0.53	1.91 ± 1.04	1.57 ± 0.21
Pituitary gland	1.21 ± 0.24	1.28 ± 0.08	0.96 ± 0.08	1.03 ± 0.19	0.69 ± 0.15

[a]Mean ± SEM (No. of experiments in column is in parenthesis); [b]Differs significantly from 50-sec ^{3}H-nicotine mean ($p < 0.05$); [c]Differs significantly from ^{14}C-antipyrine mean in table 1 ($p < 0.05$); [d]Differs significantly from ^{14}C-iodoantipyrine mean in table 1 ($p < 0.05$), where only 30-sec and 50-sec ^{3}H-nicotine means are considered.

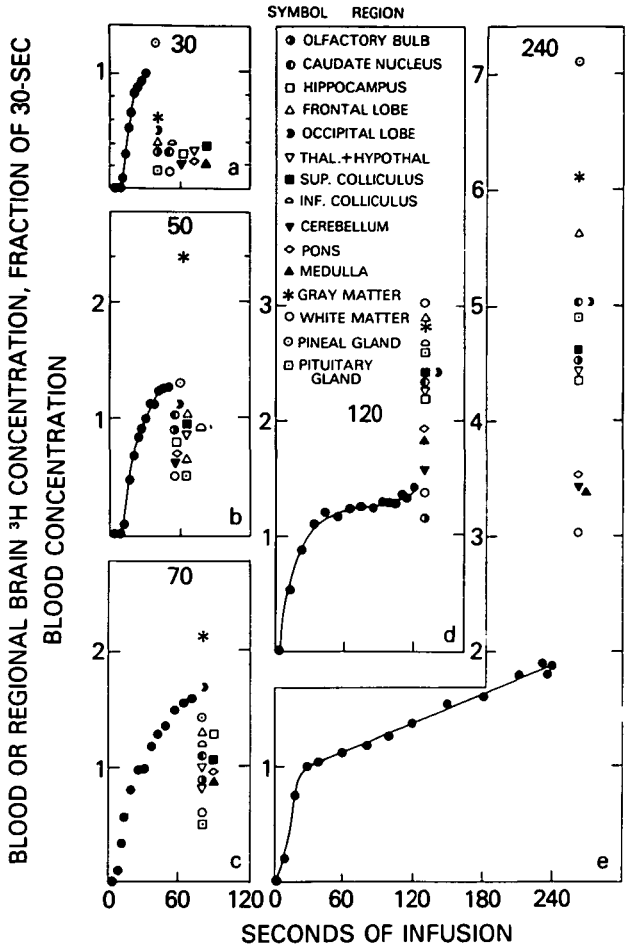

FIGURE 3. *³H concentration (dpm/ml) in whole blood of rats during i.v. infusion of ³H-nicotine for 30 sec to 240 sec, as well as regional brain parenchymal concentrations, C_{brain} dpm/g, at time of death in individual experiments. Concentrations were normalized to the 30-sec blood concentration (taken as 1).*

¹⁴C-iodoantipyrine also furnishes higher LCBF's than does the hydrogen clearance method. For example, at the frontal cortex of the conscious rat, LCBF equals 0.79 ± 0.22 (SEM) $cm^3g^{-1}min^{-1}$ with the hydrogen clearance method,[21] but equals 1.68 ± 0.29 $cm^3g^{-1}min^{-1}$ with ¹⁴C-iodoantipyrine (table 1).

The Kety method[1, 2] was designed for use with an inert gas, such as ¹³¹I-trifluoriodomethane, that can reach almost instantaneous equilibrium between brain and blood. This condition is not satisfied by ¹⁴C-antipyrine, which gives low LCBF's, because its entry into brain is somewhat limited by diffusion at the capillary and does not depend only on blood flow.[4-6, 8, 17] Diffusional equilibrium presumably is reached, on the other hand, by the more lipid-soluble ¹⁴C-iodoantipyrine, which provides LCBF's that compare with those obtained with ¹³¹I-trifluoriodomethane.[4] ¹⁴C-iodoantipyrine would appear to be the non-gaseous agent of choice for exact measurement of LCBF, not only because it gives values comparable to those with the inert gas, but also because it is long-lived within blood, not significantly metabolized within 1 min, commercially available and exhibits a tissue-blood partition coefficient that is uniform throughout the brain and is independent of hematocrit.[4]

Due to its possible back-diffusion from brain to blood, ³H-nicotine cannot be used with certainty to obtain LCBF. However, the equivalent values of LCBF as derived with 30 or 50 sec of infusion of ³H-nicotine, as compared to those provided by ¹⁴C-iodoantipyrine and the Kety analysis, demonstrate that back-diffusion was minimal for infusion times of up to 50 sec, and that Eq. 4 then can be used to derive LCBF precisely. The lower LCBF's obtained with ³H-nicotine than with ¹⁴C-iodoantipyrine in occasional regions (thalamus + hypothalamus at 30 sec of infusion; superior colliculus, caudate nucleus and white matter at 30 and 50 sec), may have been due to incomplete extraction of ³H-nicotine by those regions or to some back-diffusion into blood.

For 120 sec or 240 sec of ³H-nicotine infusion, delivery rate may be less important than tissue uptake in calculating LCBF. The higher calculated LCBF of gray as compared to white matter reflects in part specific, reversible uptake of ³H-nicotine by cell bodies and nerve endings, in part at cholinergic receptors. Uptake is lowered *in vivo* by pentobarbital anesthesia, and is reduced in brain slices by lowering temperature.[16, 22-27] Most of the ³H in the brain at 240 sec is associated with ³H-nicotine; less than 3% is due to ³H-cotinine, a metabolic derivative of ³H-nicotine that is produced in liver and kidney and appears in blood about 180 sec after an intravenous injection, but is poorly permeable at the blood-brain barrier.[26, 28]

The pineal and pituitary glands are outside of the blood-brain barrier system.[29] Local blood flows in these glands, as measured with ¹⁴C-iodoantipyrine and ³H-nicotine, are comparable to flows derived by analyzing the distribution of cardiac output to these glands — 2 to 3.43 $cm^3g^{-1}min^{-1}$ at the pineal gland, 1.01 $cm^3g^{-1}min^{-1}$ at the anterior pituitary and 3.74 $cm^3g^{-1}min^{-1}$ at the posterior pituitary.[19, 30, 31] ¹²⁵I-antipyrine gives flows of 1.2 $cm^3g^{-1}min^{-1}$at the anterior pituitary and 2.36 $cm^3g^{-1}min^{-1}$ at the posterior pituitary of the conscious rat.[32]

LCBF closely correlates with regional cerebral metabolism under many physiological conditions in the same animal, and when comparing brains of different species.[33] Both LCBF and cerebral energy metabolism are higher, for example, in the rat than in many other species, possibly because cellular packing is greater in the rat brain.[4, 34-36] If ³H-nicotine binding by brain is a measure of density of cells and nerve endings, then short-term experiments (50 sec or less) with nicotine might be used to estimate LCBF and long-term experiments (5 min or more) to estimate density of cells and nerve endings.[16, 22-27] Nicotine, when incorporating the positron emitter ¹³N, might also be used in man to measure regional blood flow by means of emission computed tomography.[37]

Acknowledgment

We thank Drs. O. Sakurada and L. Sokoloff for providing us with a preprint of their publication on LCBF, and for their helpful suggestions and criticism.

References

1. Kety SS: The theory and applications of the exchange of inert gas at the lungs and tissues. Pharmacol Rev **3**: 1–41, 1951
2. Kety SS: Measurement of local blood flow by the exchange of an inert, diffusable substance. Methods Med Res **8**: 228–236, 1960
3. Freygang WH Jr, Sokoloff L: Quantitative measurement of regional circulation in the central nervous system by the use of radioactive inert gas. Adv Biol Med Phys **6**: 263–279, 1958
4. Sakurada O, Kennedy C, Jehle J, Brown JD, Carbin GL, Sokoloff L: Measurement of local cerebral blood flow with iodo [^{14}C] antipyrine. Am J Physiol **234**(1): H59–H66, 1978
5. Eckman WW, Phair RD, Fenstermacher JD, Patlak CS, Kennedy C, Sokoloff L: Permeability limitation in estimation of local brain blood flow with [^{14}C] antipyrine. Am J Physiol **229**: 215–221, 1975
6. McHenry LC Jr, Jaffe ME, Goldberg HI: Regional cerebral blood flow measurement with small probes. I. Evaluation of the method. Neurology (Minneap) **19**: 1198–1206, 1969
7. Crone C: The permeability of brain capillaries to nonelectrolytes. Acta Physiol Scand **64**: 407–417, 1965
8. Eklof B, Lassen NA, Nilsson L, Norberg K, Siesjö BK, Torlöf P: Regional cerebral blood flow in the rat measured by the tissue sampling technique; a critical evaluation using four indicators C^{14}-antipyrine, C^{14}-ethanol, H^{3}-water and Xenon^{133}. Acta Physiol Scand **91**: 1–10, 1974
9. Oldendorf WH: Lipid solubility and drug penetration of the blood-brain barrier. Proc Soc Exp Biol Med **147**: 813–816, 1974
10. Oldendorf WH: Brain concentrations of ^{14}C-nicotine and ^{14}C-antipyrine after intravenous injection. pp 103–109. *In* Langfitt TN, McHenry LC, Reivich M, Wallmann H (eds) Cerebral Circulation and Metabolism. New York, Springer-Verlag 1975
11. Sapirstein LA: Regional blood flow by fractional distribution of indicators. Am J Physiol **193**: 161–168, 1958
12. Reinmuth OM, Scheinberg P, Bourne B: Total cerebral blood flow and metabolism. Arch Neurol **12**: 49–66, 1965
13. Glowinski J, Iversen LL: Regional studies of catecholamines in the rat brain. I. The disposition of [^{3}H] norepinephrine, [^{3}H] dopamine and [^{3}H] DOPA in various regions of the brain. J Neurochem **13**: 655–669, 1966
14. Chiueh CC, Sun CL, Kopin IJ, Fredericks WR, Rapoport SI: Entry of ^{3}H-norepinephrine, ^{125}I-albumin and Evans blue from blood into brain following unilateral osmotic opening of the blood-brain barrier. Brain Res **145**: 291–301, 1978
15. Ohno K, Pettigrew KD, Rapoport SI: Lower limits of cerebrovascular permeability to nonelectrolytes in the conscious rat. Am J Physiol **253** (3), H299–H307, 1978
16. Schmiterlöw RC, Hansson E, Andersson G, Applegren L-E, Hoffmann PC: Distribution of nicotine in the central nervous system. Ann NY Acad Sci **142**: 2–14, 1967
17. Bradbury MWB, Patlak CS, Oldendorf WH: Analysis of brain uptake and loss of radiotracers after intracarotid injection. Am J Physiol **229**: 1110–1115, 1975
18. Knott GD, Shrager RI: On-line modeling by curve fitting. pp 138–151. *In* Computer Graphics: Proceedings of the SIGGRAPH Computers in Medicine Symposium 6 (no 4). ACM, SIGGRAPH Notices, 1972
19. Goldman H, Wurtman RJ: Flow of blood to the pineal body of the rat. Nature **203**: 87–88, 1964
20. Ginsberg MD, Medoff R, Reivich M: Heterogeneities of regional cerebral blood flow during hypoxia-ischemia in the rat. Stroke **7**: 132–134, 1976
21. Haining JL, Turner MD, Pantall RM: Measurement of local cerebral blood flow in the unanesthetized rat using a hydrogen clearance method. Circ Res **23**: 313–324, 1968
22. Alderdice MT, Weiss GB: Effects of pharmacological agents on [^{14}C]-nicotine distribution and movements in slices from different rat brain areas. Neuropharmacology **14**: 811–817, 1975
23. Weiss GB, Alderdice MT: Characterization of [^{14}C]-nicotine accumulation and movements in slices from different brain areas. Neuropharmacology **14**: 265–273, 1975
24. Tsujimoto A, Nakashima T, Tanino S, Dohi T, Kurogochi Y: Tissue distribution of [^{3}H] nicotine in dogs and rhesus monkeys. Toxicol Appl Pharmacol **32**: 21–31, 1975
25. Eterović VA, Bennett EL: Nicotinic cholinergic receptor in brain detected by binding of α-[^{3}H] bungarotoxin. Biochim Biophys Acta **362**: 346–355, 1974
26. Appelgren L-E, Hansson E, Schmiterlöw CG: The accumulation and metabolism of C^{14}-labelled nicotine in the brain of mice and cats. Acta Physiol Scand **56**: 249–257, 1962
27. Brown DA, Halliwell JV: Intracellular pH in rat isolated superior cervical ganglia in relation to nicotine-depolarization and nicotine-uptake. Br J Pharmacol **45**: 349–359, 1972
28. Turner DM: Metabolism of small multiple doses of [^{14}C]-nicotine in the cat. Br J Pharm **41**: 521–529, 1971
29. Rapoport SI: Blood-Brain Barrier in Physiology and Medicine. New York, Raven Press, 1976
30. Goldman H: The nervous control of blood flow to the pineal body. Life Sci **6**: 2071–2077, 1967
31. Goldman H: Failure of cervical sympathectomy to alter pituitary blood flow. Endocrinology **83**: 603–606, 1968
32. Lichardus B, Albrecht I, Ponec J, Linhart L: Water deprivation for 24 hours increases selectively blood flow in posterior pituitary of conscious rats. Endocrinol Exp (British) **11**: 99–104, 1977
33. Reivich M, Sokoloff L: Application of the 2-deoxy-D-glucose method to the coupling of cerebral metabolism and blood flow. Neurosci Res Program Bull **14**: 474–475, 1976
34. Landau WM, Freygang WH, Roland LP, Sokoloff, Kety SS: The local circulation of the living brain; values in the unanesthetized and anesthetized cat. Trans Am Neurol Assoc **80**: 125–129, 1955
35. Nilsson B, Siesjö BK: A method for determining blood flow and oxygen consumption in the rat brain. Acta Physiol Scand **96**: 72–82, 1976
36. Sokoloff L, Reivich M, Kennedy C, Des Rosiers MH, Patlak CS, Pettigrew KD, Sakurada O, Shinohara M: The [^{14}C] deoxyglucose method for the measurement of local cerebral glucose utilization: theory, procedure, and normal values in the conscious and anesthetized albino rat. J Neurochem **28**: 897–916, 1977
37. Phelps ME: Emission computed tomography. Seminars in Nuclear Medicine **7**: 337–365, 1977
38. Miller RG: Simultaneous Statistical Inference. pp 67–70. New York, McGraw-Hill

Local cerebral blood flow in the conscious rat as measured with 14C-antipyrine, 14C-iodoantipyrine and 3H-nicotine.

K Ohno, K D Pettigrew and S I Rapoport

Stroke. 1979;10:62-67
doi: 10.1161/01.STR.10.1.62
Stroke is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231

Print ISSN: 0039-2499. Online ISSN: 1524-4628