Experimental Increase in Brain HIPDM Uptake by Hypercapnia

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The 30-min brain uptake of [^{125}I]HIPDM was measured in conscious rats—normocapnic (n = 8), hypercapnic (n = 12), and hyperoxic (n = 6). A mean 41.2% higher uptake was found in the brains of hypercapnic animals (p < 0.01). In the three groups of rats, brain HIPDM uptake had a negative correlation with body weight (p < 0.001) and a positive correlation with arterial pCO_2 (p < 0.01), when adjusted for body weight. These results indicate that HIPDM uptake with hypercapnia may be used as a provocative test to measure cerebral blood flow reserves.

Single photon emission computed tomography (SPECT) has been successfully applied in imaging and semiquantitation of regional cerebral blood flow (rCBF) (*1-8*). The value of rCBF studies in borderline vascular lesions is limited (*9-12*). It is known, however, that normal arteries allow up to several times the baseline blood flow following a vasodilatory stimulus, whereas a stenotic vessel does not respond sufficiently, although at rest it may allow normal flow (*4,12-19*). A method to increase rCBF above baseline levels by pharmacological or physiological means would be useful in clinical practice. Such a test will show the "cerebral perfusion reserve" which is reduced in borderline lesions.

Changes in arterial blood gases in animals and humans induce alterations in the CBF and cerebral vascular resistance (*13-18*). Carbon dioxide is the most powerful cerebral vasodilator known (*14,15,17,19*). When arterial CO_2 pressure (pCO_2) is increased, molecular CO_2 diffuses across the blood–brain barrier, increases the local CO_2 pressure of vascular muscle, and reduces extracellular fluid pH in this location thus producing vasodilatation by relaxation of vascular muscle. The reverse occurs when pCO_2 is reduced (*17-23*).

The relationship between total CBF and pCO_2 is curvilinear (*14*). Maximum increases in CBF have been reported by different investigators to occur when pCO_2

is greater than 60 mmHg (*24*), 80 mmHg (*25*), and 120–150 mmHg (*14*). The vasodilatory effect of CO_2 may be threshold phenomenon (*26*). Contrary to humans (*27*), rats and mice exposed to high (30–40%) CO_2 concentrations could have seizures (*28*).

The monoamine, N-isopropyl-p-iodoamphetamine (IMP) and the diamine N,N,N'-trimethyl-N-[2-hydroxy-3-methyl-5-iodobenzyl]-1,3-propanediamine (HIPDM) have distributions in brain reflecting rCBF in autoradiographic and SPECT studies (*1-6,29-31*). The mechanism of uptake of these amines in normal brain is not completely understood. Lucignani et al. have studied the metabolic degradation and the cerebral kinetics of iodine-125 (^{125}I) HIPDM in conscious adult male rats (*31*). IMP and HIPDM have been studied in the normal state and compared with microspheres (*31, 32*) and iodoantipyrine (*31,33*).

Burt et al. (*34*) recently published preliminary work in patients indicating that acetazolamide (diamox) enhanced the sensitivity of HIPDM in detecting rCBF changes. Acetazolamide (A) is a potent inhibitor of the carbonic anhydrase, which catalyzes the reaction $CO_2 + H_2O = [H]^+ + [HCO_3]^-$. It has been found that A increases CBF (*35*) by a selective vasodilatory action on the cerebral resistance vessels, without effect on the systemic blood pressure, the heart rate, the cardiac output, or the vascular resistance of other major components of the arterial circulation (*36*).

The exact mechanism of A action on the CBF is not well known. It may be related exclusively to the chemical regulation of CBF or it may involve the autoregu-

lation as well. Low dose A (7.5–8.5 mg/kg i.v.) increases CBF without detectable alterations in pCO_2, while allowing the independent action of pCO_2 on CBF (37). High-dose A (30 mg/kg i.v.) markedly increases CBF and (disproportionately less) pCO_2, and blocks the independent effect on CBF of further increases of pCO_2, but it does not abolish the autoregulatory CBF mechanism (36,38). High dose A also induces an apparent decrease in the cerebral metabolic rate of oxygen, which is in reality a decrease in oxygen delivery to the brain, resulting from an effect of A on the erythrocyte carbonic anhydrase (interference with the Bohr shift, the mechanism that augments oxygen release from hemoglobin via acidic pH shift) (36). These observations suggest that A may increase CBF: (a) partly because of a carbonic anhydrase inhibition (36–38), and (b) partly through other mechanisms, such as cerebral hypoxia (36) or a direct action on the smooth muscle cells of the cerebral arterioles (37).

Brain HIPDM uptake in hypercapnic and hyperoxic animals as compared to normocapnic animals and the influence of body weight were evaluated in the work reported in this paper.

MATERIALS AND METHODS

A total of 26 Sprague-Dawley male rats were studied. The animals were chosen to have increasing body weight within each group and were appropriately randomized between groups. Their weight ranged between 189 g and 432 g. Following anesthesia (5 mg ketamine per 100 g body weight), the femoral artery and vein were cannulated with polyethylene PE-50 catheters (Clay Adams) for anaerobic withdrawal of arterial blood samples (400–500 μl) and for i.v. injections. The catheters were filled with heparinized saline (10 U.S.P. units/ml). The animals were immobilized in the supine position in a Plexiglas chamber (72 cm × 30 cm × 20 cm) at normal atmospheric pressure. The ambient temperature of the chamber was kept above the thermoneutral zone of the rat (28–230°C) in order to maintain the temperature of the animal stable (36° ± 1°C) during the experiment.

Thirty minutes following complete recovery from anesthesia, rats were exposed for a total of 1 hr before death to one of the following three chamber environments: (a) atmospheric air (normocapnic, n = 8); (b) 10–30% CO_2 mixture with atmospheric air (hypercapnic, n = 12); and (c) 40–80% O_2 mixture with atmospheric air (hyperoxic, n = 6).

During the initial 30 min, all animals remained in the controlled environment awake and freely breathing, but immobilized. At 30 min, blood samples were drawn and the radiopharmaceutical was injected as follows: Arterial blood sampling was performed for measurements of hematocrit (microhematocrit method), arterial blood gasses (pCO_2, pO_2), and pH (pH/Blood gas analyzer 113 and 127 temp. bath, Instrumentation Laboratory, Inc.) The volume of heparinized saline in arterial blood sample was smaller than 2% of the volume of the sample (39). Immediately following blood sampling, 5 μCi (25 μg) of [^{125}I]HIPDM in 0.20–0.30 ml

normal saline were injected into the femoral vein, followed by 0.50 ml normal saline to wash the i.v. lines.

The animals remained in the experimental environment for another 30-min period and were killed following a second determination of blood gases and pH. A volume of normal saline equal to the blood volume (40) of the animal was infused i.v. to wash the blood out of the organs.

The brain and other organs of interest were quickly excised. After careful removal of the leptomeninges of the brain, the hemispheres, cerebellum, and brain stem were prepared, weighed, and counted appropriately against a standard in a scintillation counter.

The labeling efficiency of [^{125}I]HIPDM was 97% ± 1%. The same batch of [^{125}I]HIPDM and the same standard were used for the entire group of 26 rats.

The percent of the dose taken up (uptake, U) by the organs was calculated by comparing brain counts (total, hemispheric, cerebellar, stem), or other organ counts to counts of appropriately diluted aliquots of the injected material. The brain and other organ activity was also expressed as uptake per gram of tissue (Ug).

One way statistical analysis of variance between the three groups was performed. Differences among means were tested using the Bonferroni procedure. Regression analysis was used to examine the relationship between HIPDM U, or Ug and the other variables, body and brain weight, pCO_2, pO_2, pH. Brain HIPDM U and Ug were adjusted for body weight by covariance analysis.

RESULTS

Mean body (BoW), brain (BrW), hemispheric (HW), cerebellar (CW), and brain stem weight (SW) and pCO_2, pO_2, pH at 30 and 60 min in the controlled air environment, in the three groups, normocapnic (N), hypercapnic (C), and hyperoxic (O), are listed in Table 1. Mean values of [^{125}I]HIPDM uptake by the brain and its parts are in Table 2 and Figure 1. The mean uptake by the other organs are in Table 3. One hypercapnic rat had to be removed from the analysis because the uptake values were 4.3 s.d. above mean uptake for the remaining animals in that group; the uptake distribution in the three parts of the brain was similar to the group. Removing this animal was a justified conservative approach.

One-way analysis of variance between the groups showed statistically significant higher brain HIPDM uptake in the hypercapnic rats as compared to the other two groups of animals. This increase was true for the total uptake by the brain (41.2%), the per gram of tissue uptake (34%) and the per gram of tissue uptake normalized against BoW and BrW (46%) (Table 2). The increase in uptake was significant for the entire brain (p = 0.0023), the hemispheres (p = 0.0049), the cerebellum (p = 0.001) and the brain stem (0.0014). There was no statistically significant difference in brain HIPDM uptake between the normocapic and hyperoxic

TABLE 1
Mean (± s.d.) Body and Brain Weight, Arterial pH and Arterial Blood Gases in the Three Groups of Rats

	BoW*	BrW[†]	HW[‡]	CW[§]	SW[¶]	pH 30 min	pH 60 min	pCO$_2$ (mmHg) 30 min	pCO$_2$ (mmHg) 60 min	pO$_2$ (mmHg) 30 min	pO$_2$ (mmHg) 60 min
N**	307.88	1.840	1.304	0.274	0.243	7.36	7.37	34.4	38.2	102.1	98.0
	(83.36)	(0.178)	(0.191)	(0.043)	(0.093)	(0.04)	(0.07)	(6.6)	(5.3)	(12.0)	(21.0)
C[††]	326.20	1.854	1.335	0.277	0.242	7.10	6.97	79.8	112.0	131.3	110.5
	(55.10)	(0.150)	(0.102)	(0.038)	(0.055)	(0.18)	(0.16)	(25.1)	(41.1)	(48.1)	(48.1)
O[‡‡]	318.50	1.899	1.381	0.273	0.225	7.33	7.35	37.3	33.7	222.0	285.2
	(84.71)	(0.146)	(0.168)	(0.065)	(0.061)	(0.06)	(0.07)	(2.7)	(2.4)	(56.2)	(77.9)

* Body weight.
[†] Brain weight.
[‡] Hemispheric weight.
[§] Cerebellar weight.
[¶] Brain stem weight in grams.
** Normocapnic.
[††] Hypercapnic.
[‡‡] Hyperoxic animals.

rats, although the mean U and Ug were lower in the latter.

The arterial pH decreased (p = 0.003) and the pCO$_2$ increased significantly (p < 0.0001) in the hypercapnic group but it was not different among the other two groups. The pO$_2$ increased significantly (p < 0.005) in the hyperoxic group but it did not differ in the other two.

The animals were chosen to have increasing weight within each group but the span of weight among the groups was similar (no statistical difference between the groups). In order to see if there was an effect of body weight on brain weight, pH, pCO$_2$, pO$_2$, and brain uptake, regression analysis was performed.

There was a positive correlation between brain weight (range 1.662–2.220 g) and body weight (range 189–432 g) in the three groups (slope 0.006 g BrW/BoW p < 0.001). After adjusting for body weight, there was no significant association between total brain uptake and brain weight within the groups separately or combined.

In the hypercapnic group the body weight had a positive correlation with pCO$_2$ at 30 min (slope 0.408, p < 0.001) and a negative but not significant correlation with pH (slope −0.002, p < 0.15), that is the heavier animals had higher arterial pCO$_2$ levels and lower pH when placed in the same environment with smaller animals.

In the three groups the body weight had a negative correlation with brain HIPDM uptake (slope −0.005 and p < 0.001 for total brain U and slope −0.004 and p < 0.001 for the Ug in pooled estimates) (Fig. 2). The same was true for all parts of the brain HIPDM U or Ug.

When brain HIPDM uptake was adjusted for body

TABLE 2
Brain (and Parts) Mean (±s.d.) HIPDM Uptake in the Three Groups of Rats

	Total brain (U)*	Total brain (Ug)[†]	Total brain (Y)[‡]	Hemispheres (U)	Hemispheres (Ug)	Cerebellum (U)	Cerebellum (Ug)	Brain stem (U)	Brain stem (Ug)
N[§]	2.062	1.150	179.65	1.567	1.255	0.232	0.825	0.187	0.932
	(0.519)	(0.388)	(29.50)	(0.377)	(0.451)	(0.059)	(0.288)	(0.049)	(0.298)
C[¶]	2.912	1.545	262.59	2.197	1.647	0.358	1.308	0.282	1.285
	(0.442)	(0.269)	(23.37)	(0.354)	(0.304)	(0.047)	(0.189)	(0.040)	(0.232)
CD**	41.2%	34%	46%	40.2%	31.2%	54%	58.5%	50.8%	37.9%
O[††]	1.050	0.991	170.52	1.604	1.064	0.234	0.919	0.196	0.921
	(0.670)	(0.279)	(37.70)	(0.545)	(0.324)	(0.067)	(0.483)	(0.076)	(0.430)

* % of the dose uptake.
[†] Per gram uptake.
[‡] Corrected for body weight uptake of HIPDM.
[§] Normocapnic.
[¶] Hypercapnic.
** Difference from normocapnic.
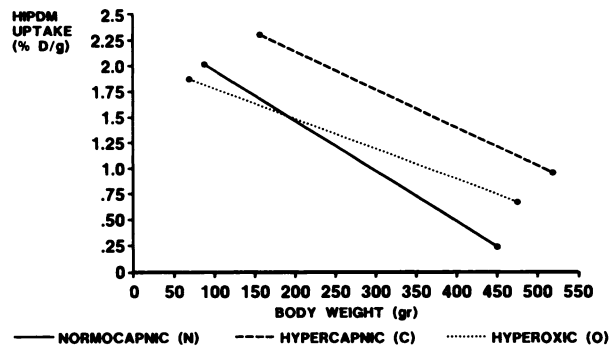[††] Hyperoxic animal values.
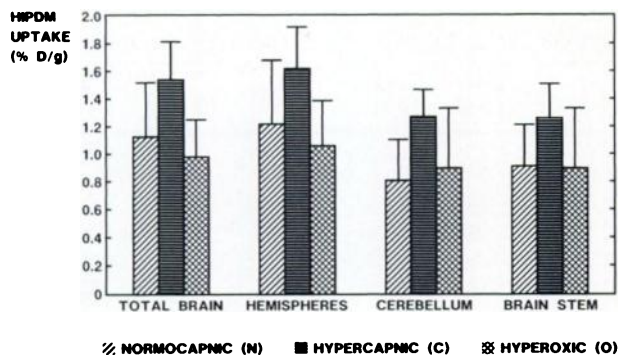
FIGURE 1
Brain HIPDM uptake (Ug) in the three groups of animals (mean and s.d.). Differences between N and C and between O and C were significant (see text). ▨ Normocapnic (N): ■ Hypercapnic (C): ■ Hyperoxic (O).

FIGURE 2
Correlation between brain HIPDM uptake (Ug) and body weight in the three groups of animals (fitted lines from regression analysis). Body weight had a negative correlation with Ug in all groups but the regression line for the hypercapnic (C) animals showed statistically significant higher uptake in this group (see text). (—) Normocapnic (N); (---) Hypercapnic (C); (···) Hyperoxic (O).

weight by covariance analysis the following results were obtained.

1. In the hypercapnic animals the increase in brain HIPDM uptake (per organ and per g, for the entire brain and for its parts) was still evident and significant at least at the same probability levels as before the adjustment.

2. In all animals (pooled estimates) after adjustment for body weight, there was a positive correlation between pCO_2 (30 and 60 min) and total brain HIPDM uptake (r = 0.68, p < 0.01) (Fig. 3).

Analysis of the HIPDM uptake by other than the brain organs indicated that hypercapnia induced a significant decrease in the per gram tissue uptake of HIPDM in the lungs, while in hyperoxic animals there was a significant increase in HIPDM lung uptake (Table

3). There was no statistically significant difference in the findings from the other organs.

DISCUSSION

Brain Uptake

Hypercapnia increases regional cerebral blood flow (16,41–45). In rats this has been investigated using xenon-133 (^{133}Xe) (16,41,44–47), iodoantipyrine (42), microspheres (43), and carbon-14 (^{14}C) ethanol (46). Radio-iodinated amines (HIPDM, IMP) are currently used to obtain information about rCBF in patients. The work reported in this paper showed that under the experimental conditions chosen, hypercapnia increases

TABLE 3
Mean (±s.d.) Organ or Tissue Weight and Per Gram HIPDM Uptake in the Three Groups of Rats

Organ or tissue	Weight of organ or tissue			HIPDM uptake per gram (Ug)		
	Normocapnic	Hypercapnic	Hyperoxic	Normocapnic	Hypercapnic	Hyperoxic
Eye (1)	0.15 (±0.05)	0.14 (±0.02)	0.13 (±0.02)	0.21 (±0.05)	0.23 (±0.05)	0.24 (±0.13)
Lungs	1.56 (±0.60)	2.07 (±0.55)	1.51 (±0.34)	9.03 (±4.51)	5.97 (±2.37)*	10.13 (±2.83)†
Heart	1.02 (±0.33)	1.08 (±0.21)	1.01 (±0.36)	0.47 (±0.16)	0.43 (±0.11)	0.71 (±0.62)
Blood	19.74 (±4.91)	20.50 (±3.20)	19.19 (±4.98)	0.09 (±0.04)	0.10 (±0.05)	0.09 (±0.04)
Liver	11.87 (±2.80)	12.30 (±2.14)	11.79 (±3.55)	1.03 (±0.20)	1.39 (±0.60)	1.55 (±0.80)
Spleen	0.55 (±0.13)	0.64 (±0.12)	0.59 (±0.18)	2.24 (±0.74)	2.82 (±0.71)	3.07 (±2.33)
Intestine (small)	0.69 (±0.36)	0.56 (±0.19)	0.80 (±0.27)	1.70 (±1.09)	1.86 (±0.56)	2.16 (±0.94)
Pancreas	0.44 (±0.17)	0.46 (±0.17)	0.43 (±0.25)	1.45 (±0.94)	0.93 (±0.49)	1.75 (±1.46)
Kidney	2.15 (±0.62)	2.77 (±0.47)	2.35 (±0.84)	2.05 (±0.64)	1.69 (±0.76)	3.14 (±1.98)
Testes	2.25 (±0.78)	2.70 (±0.32)	2.66 (±0.42)	0.21 (±0.09)	0.19 (±0.10)	0.17 (±0.09)
Skin	1.00 (±0.40)	0.86 (±0.22)	0.81 (±0.20)	0.15 (±0.05)	0.12 (±0.03)	0.14 (±0.05)
Fat	0.76 (±0.37)	0.80 (±0.20)	0.94 (±0.22)	0.14 (±0.08)	0.12 (±0.08)	0.15 (±0.05)
Muscle	1.20 (±0.56)	1.09 (±0.41)	1.44 (±0.75)	0.15 (±0.05)	0.13 (±0.04)	0.21 (±0.10)
Bone and marrow	0.41 (±0.23)	0.33 (±0.14)	0.40 (±0.16)	0.28 (±0.11)	0.30 (±0.13)	0.30 (±0.07)

* Difference from normocapnic p = 0.05.
† Difference from hypercapnic p < 0.01 (Mann-Whitney U-test).
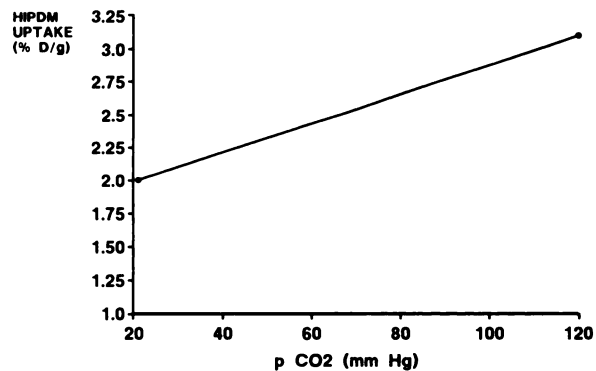
FIGURE 3
Regression line of brain HIPDM uptake (Ug) versus pCO$_2$ after correction for body weight.

HIPDM brain uptake in the rats (Table 2, Fig. 1). Thus, HIPDM appears to be a tracer suitable for cerebral perfusion reserve testing in the rat. In this animal model, total brain blood flow, hence HIPDM uptake, represent gray matter values because the brain of the rat contains small amounts of white matter. A recent publication (34) indicated that premedication with acetazolamide amplified by a not well known mechanism (35-38) the sensitivity of HIPDM in diagnosing brain ischemia in man. Both hypercapnia and acetazolamide most probably involve to some extent the same vasodilatory mechanism.

Studies with ^{133}Xe, iodoantipyrine, [^{14}C]ethanol, microspheres had shown a 40-700% increase in rCBF depending on the pCO$_2$. These studies based their measurements on the washout of the first pass uptake of Xe, or on 1 min postinjection data of the other tracers. In clinical practice, however, brain imaging with HIPDM or IMP is performed 15-60 min after i.v. injection because there is a stable concentration of these amines in the brain at this time (3,48). To investigate the problem under clinical conditions the 30 min postinjection sampling was considered appropriate. Further work may be needed to investigate potential temporal changes in brain amine uptake in hypercapnia during the scanning period (15-60 min).

The magnitude of increase in brain HIPDM uptake observed under the conditions of this experiment (40% for the entire brain, Table 2, Fig. 1) is less than expected based on results from previous reports (16,41,44,45-47). This may be due to the biochemical properties of HIPDM: Under the experimental conditions, hypercapnia was associated with a decrease in arterial pH (Table 1). Although pH is better buffered in the cerebral intracellular space (49), it is possible that in hypercapnia the blood-brain pH difference decreases as compared to the normocapnic state. This decrease may account for the lower than expected brain HIPDM uptake in the hypercapnic state (pH shift hypothesis) (30,50). Other factors, such as receptor saturation and diffusion

changes may also be responsible for the lower than expected increase (51). It should be stressed that diffusion limitation causes a nonlinear relationship of tracer concentration as compared to CBF; as flow increases the amines accumulate at a lower rate and the difference is more pronounced at higher CBF rates (51). Thus diffusion limitation should be considered as a possibility of the blunted response of the HIPDM concentration in hypercapnia found in this experiment. Immobilization induced decrease in CBF might also play some role (52).

As expected, a positive correlation between brain HIPDM uptake and pCO$_2$ was found (Fig. 3). Using 2-7% CO$_2$ in air, pCO$_2$ levels between 38-60 mmHg could be achieved in man (13,53-55). These levels were within the range of our experiments; indeed at a pCO$_2$ of 44-65 mmHg an increase in brain HIPDM was noticed in individual hypercapnic animals.

The HIPDM uptake in the brain of the first group of animals (normocapnic rats) followed the regional pattern of iodoantipyrine (31). All three parts of the brain studied showed a hypercapnia-related increase in HIPDM uptake. The cerebellum had the highest relative increase (0.825 to 1.308 or 58.5%) followed by the brain stem (0.93 to 1.285 or 38%) and the hemispheres (1.285 to 1.647 or 31%). Similar hierarchy of responsibility was found in the rabbit and was attributed to the relatively higher gray matter content of the cerebellum (56).

Hyperoxic animals had a mean of pO$_2$ higher than in the other groups (p < 0.001). There was no significant difference, however, from the normocapnic group in the brain HIPDM uptake, although the mean decreased (Table 2). At the levels of pO$_2$ achieved in these experiments (285 ± 78 mmHg) a pronounced change in rCBF was not expected since previous work had shown that a decrease in CBF occurs at very high pO$_2$ levels (13,16,57).

The total organ uptake (% of the injected dose, U) and the uptake per gram of tissue (Ug) employed in this work are acceptable units to express organ (U) or tissue (Ug) accumulation of HIPDM in experimental animals (25,30,58,59). This approach does not measure CBF or rCBF in ml/min/100 g, which is the standard unit. It reflects, however, the fraction of cardiac output (COP) perfusing the brain. It should be stressed that using percentage uptake of tracer to evaluate blood flow distribution assumes the microsphere principle which is only partially operating in the case of HIPDM (31). Indeed more accurate quantitation will require arterial blood profiles of the amine and its metabolites (31). Despite these limitations the percentage uptake has been used in experimental work and in semiquantitative studies in patients (1,5).

In the age range of the animals used, the brain grows at a disproportionally lower rate than the body of the

animal, and this was noticed among the animals in this experiment (slope 0.006 gr BrW/BoW). It appears that as the animals grow, a smaller fraction of COP reaches the brain, which explains the negative correlation between BoW and brain U or Ug of HIPDM; overwhelmed by the greater increase in BoW, BrW had no effect on HIPDM accumulation. Furthermore, when corrected for BrW and BoW brain uptake values (Y) did not differ among the animals of the same group (Table 2, corrected total brain values, Y). Thus, it is essential in similar experiments to either use animals of the same weight (age) (59), or correct for body weight. Following such correction, total brain (or H, C, S) U or Ug of HIPDM was still higher in hypercapnic than in normocapnic animals ($p < 0.005$) and was related to pCO_2 ($p < 0.01$). A correlation between BoW and pCO_2 found in the hypercapnic group suggested that smaller animals tolerated high levels of CO_2 in the breathing air better than larger (older) rats. This interesting finding cannot be explained with the data available.

Cardiac output or blood pressure were not monitored in this experiment to avoid further stressing of the animals. It is known however that in the rat CBF remains practically unchanged for a mean arterial blood pressure (mABP) ranging between 80-160 mmHg, apparently because of autoregulatory mechanisms in the brain (41). It has also been reported that mABP (and cerebral perfusion pressure) did not show significant change in hypercapnic rats, whereas CBF increased significantly (14,45). Anesthesia is known to have an effect on CBF and metabolism (60). The three groups of animals, however, were treated identically; the dose of 50 mg/kg intramuscularly induces only light anesthesia and complete recovery occurs 1-1.5 hr after injection of Ketamine, whereas the study started at 2 hr, when the rats were completely awake.

Other Organs

Previous work has shown that in the normocapnic rats the HIPDM lung uptake was approximately 35% of the injected dose at 2 min, falling rather slowly to ~20% of the injected dose at 1 and 2 hr (3). Our findings in the normocapnic animals are in agreement. Hypercapnia was associated with a significantly lower lung HIPDM content at 30 min postinjection as compared with normocapnia or hyperoxia (Table 3). It is known that amines are removed from the circulation by the lungs and the lung uptake depends on the pH of the perfusion medium. Higher values of pH of the perfusion medium increase the uptake of amines by the lung and vice-versa (61). Hypercapnia induces pulmonary vasoconstriction (62,63) and reduces the pH of the arterial blood, which may explain the decrease in lung HIPDM (pH-shift hypothesis). A small increase in lung HIPDM uptake was found in hyperoxic animals (Table 3) and can also be attributed to pH changes (61).

In conclusion, HIPDM brain uptake increases significantly in CO_2 induced hypercapnia in healthy rats of different body weight (age). This pharmaceutical may have a place in studying brain blood flow reserves in cerebrovascular disease.

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