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Effect of Hypocapnia on Local Cerebral Glucose Utilization in Rats

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The effect of hypocapnia on regional cerebral glucose utilization (L-CMRg) was studied in 14 Sprague Dawley rats. After cannulation of femoral vessels, halothane was discontinued and anesthesia was maintained with 70% N_2O in oxygen. The animals' lungs were mechanically ventilated to achieve normocapnia (Pa_{CO_2} = 40 ± 2 mmHg) in group A or hypocapnia (Pa_{CO_2} = 25 ± 2 mmHg) in group B. L-CMRg was measured by the ^{14}C-2-deoxyglucose autoradiographic method. Twenty-six anatomically discrete structures representing cortical, subcortical, limbic, and brainstem areas were studied. In hypocapnic animals, mean values for L-CMRg were higher in 25 out of 26 structures studied. The increase in L-CMRg was heterogenous. The structures that had higher L-CMRg during normocapnia showed the greatest increase in L-CMRg. When the two groups were compared using a profile analysis, in six regions (lateral and ventral thalamus, inferior colliculus, lateral habenulla, medial geniculate body, and auditory cortex), a value of $P < 0.05$ was obtained. (Key words: Brain: cerebral glucose utilization; cerebral metabolism. Ventilation: hypocapnia.)

IT HAS BEEN WELL RECOGNIZED that changes in blood carbon dioxide tension (Pa_{CO_2}) profoundly affect neuronal function. This effect of hypercapnia and hypocapnia is partly due to accompanying changes in cerebral blood flow and partly due to alterations in the carbohydrate, amino acid, and energy metabolism at various levels of CNS. Wide variations in Pa_{CO_2} occur in many clinical disorders and laboratory models of stroke and head injury. Moreover, hyperventilation is commonly used during intracranial surgical procedures. Therefore, knowledge of the effects of hyper- and hypocapnia on measured parameters in any clinical or laboratory investigation is of utmost importance for proper interpretation of results. The [^{14}C-]2-deoxyglucose (^{14}-C-2DG) autoradiographic method recently popularized by Sokoloff *et al.*[1] has provided a tool for study of functional activity of CNS. This technique is currently being used for a variety of laboratory investigations in both conscious and anesthetized animals. Effect of hypercapnia on regional cerebral glucose utilization using this technique has already been reported.[1,2]

The purpose of this investigation was to study the effect of hypocapnia on regional glucose utilization.

Materials and Methods

Institutional approval of the experimental protocol was obtained and guidelines of the National Institute of Health for the care and use of the laboratory animals were followed. Fourteen Sprague-Dawley male rats between 270–330 g were randomly assigned to two groups of seven each. All animals were anesthetized with halothane in a mixture of nitrous oxide and oxygen (70:30). After cannulation of a femoral artery and vein, the trachea was intubated and mechanical ventilation with a rodent ventilator (Harvard Model 683) started. Halothane was discontinued and anesthesia was maintained with 70% nitrous oxide and oxygen. Paralysis was maintained with pancuronium bromide. Rate of mechanical ventilation was adjusted to achieve a Pa_{CO_2} of 40 ± 2 mm in group A (normocapnic) and 25 ± 2 mm in group B (hypocapnic) animals. Arterial blood pressure and rectal temperature were continuously monitored. Rectal temperature was maintained at 37° C with the aid of a heat lamp and a heating pad when necessary. Arterial blood gas values were determined at 30-min intervals. A steady Pa_{CO_2} was maintained for at least 30 min prior to measurement of L-CMRg and was reconfirmed 35 min after the injection of ^{14}C-2DG. Halothane was discontinued for at least 2 h before L-CMRg measurement. Inspired concentration of oxygen was continuously monitored and maintained at 30% throughout the experiment.

The measurement of L-CMRg was initiated by an intravenous injection of 125 uci/kg body weight of ^{14}C-2-DG (specific activity 55-mCi/mM: American Radiolabeled Chemicals, Inc., St. Louis, MO). Timed samples of arterial blood were collected during the next 45 min. Blood samples were immediately centrifuged and the plasma assayed for ^{14}C-2-DG activity (liquid scintillation spectrometer Model 1209 manufactured by LKB-Gathersberg, MD) and glucose concentration (glucose analyzer II Beckman Instruments, Brea, CA). Forty-five minutes later, the an-

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TABLE 1. Experimental and Physiological Parameters in Two Groups of Animals

	Group A (Normocapnic)	Group B (Hypocapnic)
Duration of halothane administration (minutes)	59 ± 16.2	66 ± 16.0
Interval between discontinuation of halothane and measurement of L-CMRg (minutes)	177 ± 38.1	177 ± 41.5
Mean arterial blood pressure (mmHg)	142 ± 23.2	132 ± 14.3
Hematocrit (%)	49 ± 3.9	49 ± 4.6
Arterial blood gasses		
*p*H	7.38 ± 0.05	7.47 ± 0.05*
Pa_{CO_2} (mmHg)	40 ± 1.9	25 ± 1.3*
P_{O_2} (mmHg)	99 ± 12.1	128 ± 12.4*
Plasma glucose (mg/dl)	248 ± 32.4	260 ± 21.9

Values are mean ± SD.
* Indicate $P < 0.05$.

imals were killed by a rapid intravenous injection of sodium pentobarbital. The brains were immediately removed, frozen in isopentane at −45° C, coated with chilled embedding matrix (M-1 embedding matrix manufactured by Lipshaw Manufacturing Co., Detroit, MI), and stored at −80° C until sectioning. At a later date, the frozen brains were cut into 20-micron-thick coronal sections in a cryostat at −22° C. The cut sections were thaw-mounted on glass coverslips and dried on a hot plate at 60° C. X-ray films (OM-I manufactured by Kodak®) were exposed to mounted brain sections along with previously calibrated methyl methacrylate standards (supplied by Amersham, Arlington Heights, IL) for 7 days. Optical densities of the autoradiograms were determined with an auto-scanning microdensitometer with an aperture of 345 mm (Photoscan P-1000 scanning densitometer manufactured by Optronics International, Inc., Chelmsford, MA). Local rates of glucose utilization were computed from the optical densities, using the operational equation of the method described by Sokoloff *et al.* Six separate determinations of L-CMRg were made for each structure on both left and right side of the brain in each animal.

STATISTICAL ANALYSIS

L-CMRg measurement in our study constituted a multivariate response because measurements were taken for 26 structures in each animal. Thus structures were not independent and a structure to structure correlation must be considered in any analysis to compare the two treatment groups. To accomplish this, numerical data for L-CMRg for all structures were analyzed in a fashion analogous to a two-factor repeated measures design (called profile analysis in the multivariate context) with two factors defined as: 1) treatment group at two levels (normocapnia and hypocapnia), and 2) structures at 26 levels (for 26 structures identified). This permits a univariate analysis of a multivariate response.[3,4] If profile analysis revealed a significant interaction between the two defined factors, then comparisons for individual structures were performed by first combining the appropriate error terms from the analysis of variance table to obtain the proper denominator for the *t* statistic.

Results

Experimental and physiological parameters in the two groups are shown in table 1. There were no significant differences in the duration of halothane administration, interval between discontinuation of halothane and measurement of L-CMRg, mean arterial pressure, hematocrit, and plasma glucose concentration in the two groups of animals. A significant difference in Pa_{CO_2} (variable to be

TABLE 2. Local Rates of Glucose Utilization μmoles/100 gm

Structure	Rates of Glucose Utilization: Normocapnia	Rates of Glucose Utilization: Hypocapnia	Difference Between Means	*P**
Corpus callosum	50.1 ± 7	49.7 ± 8	−0.4	.970
Internal capsule	37.8 ± 5	39.0 ± 5	1.2	.867
Amygdala	48.1 ± 6	50.1 ± 6	2.0	.780
Dentate gyrus	59.5 ± 9	61.7 ± 8	2.2	.764
Med. nabernulle	76.2 ± 10	78.4 ± 7	2.2	.762
Hypothalamus	53.7 ± 8	56.6 ± 11	2.9	.700
Substantia nigra	72.4 ± 11	75.3 ± 10	2.9	.698
Superior colliculus	87.1 ± 15	90.2 ± 14	3.1	.671
Cerebellar cortex	47.3 ± 10	51.9 ± 11	4.6	.540
Visual cortex	92.7 ± 12	97.4 ± 17	4.7	.528
Globus pallidus	56.6 ± 9	61.9 ± 9	5.3	.477
Hippocampus	88.9 ± 17	94.8 ± 15	5.9	.426
Pontine gray	64.6 ± 10	71.8 ± 11	7.2	.336
Lat. geniculate body	90.8 ± 14	100.0 ± 10	9.2	.217
Lat. lemniscus	80.8 ± 12	90.5 ± 12	9.7	.200
Septal nucleus	55.2 ± 12	65.1 ± 15	9.9	.192
Sup. olivary nucleus	84.2 ± 14	94.1 ± 15	9.9	.192
Pyriform cortex	89.5 ± 13	102.6 ± 11	13.1	.087
Frontal cortex	104.4 ± 14	118.2 ± 29	13.8	.072
Caudate nucleus	104.7 ± 16	119.1 ± 20	14.4	.061
Ventral thalamus	112.1 ± 17	128.0 ± 18	15.9	.040
Lat. thalamus	97.0 ± 13	113.1 ± 15	16.1	.038
Inferior colliculus	102.0 ± 13	119.2 ± 14	17.2	.027
Med. geniculate body	111.4 ± 20	129.5 ± 18	18.1	.021
Auditory cortex	118.0 ± 22	137.2 ± 24	19.2	.015
Lat. habenulla	106.7 ± 18	126.5 ± 12	19.8	.012

Values are mean ± SD. Structures are rank ordered from low to high based on magnitude of treatment group differences.
* Two-tailed level of significance by the *t* test for simple effects in a profile analysis.

studied) was accompanied by significant differences in *p*H and P_{O_2} between the two groups. Group B had a higher *p*H (mean = 7.47 *versus* 7.38) and P_{O_2} (mean = 128 mmHg *versus* 99 mmHg) than group A. L-CMRg was determined in 26 anatomically discrete structures of the brain. Numerical values for L-CMRg are shown in table 2. Mean values of L-CMRg in 25 out of 26 structures studied were higher in rats in group B than in those in group A. The increase in L-CMRg with hypocapnia was heterogenous. Those structures which had higher L-CMRg values during normocapnia showed a greater increase with hypocapnia. The observed *P* values in table 2 are for two-tailed tests since directional differences were not specified. In six regions, namely, lateral and ventral thalamus, inferior colliculus, medial geniculate body, auditory cortex, and lateral habenulla, an observed significance level of $P < 0.05$ was seen.

Discussion

The aim of this investigation was to study the effect of hypocapnia on L-CMRg. Hypocapnia was produced by mechanical hyperventilation in lightly anesthetized rats. Nitrous oxide was chosen as the primary anesthetic because it has been previously shown that, in contrast to barbiturates and potent inhalation anesthetics, which are known to cause significant dose-related decrease in L-CMRg, nitrous oxide causes only minimal change in L-CMRg.[5–10] Experimental conditions in two groups of animals in our study were similar (table 1). Significant differences were seen in P_{O_2}, Pa_{CO_2}, and *p*H. P_{O_2} in hypocapnic animals was 29 mmHg higher than that in normocapnic group. Torbati *et al.*[11] have shown that increasing inspired concentration of oxygen from 21% (room air) to 100% increased L-CMRg in only one structure, *i.e.*, superior olivary nucleus. Therefore, the small difference in P_{O_2} (128 *versus* 99 mmHg) between the two groups of animals in our study, is unlikely to have a significant effect on L-CMRg.

Although mean L-CMRg values in hypocapnic group in our study were higher than those in normocapnic animals in 25 out of 26 structures, statistical significance was achieved in only six of the 26 structures. When structures were rank ordered based on the difference between mean L-CMRg values in the two groups, a trend became clear that those structures that have higher L-CMRg in normocapnic animals show a greater increase in L-CMRg with hypocapnia. Statistical analysis of such a dataset becomes somewhat complicated. We analyzed our data with a two factor repeated measures design (profile analysis). The initial hypothesis to be tested was that the treatment group by structure interaction is zero. If this hypothesis is true, then a plot of mean values of L-CMRg for the two treatment groups should be parallel. In our study, response patterns for two graphs were not parallel as indicated by the statistical significance of the group by structure interaction ($F_{25,300} = 2.17$, $P = 0.0013$). Thus the test for individual structures was performed. As recommended in recent statistical literature,[12] we did not assign an *a priori* value of *P* for the level of statistical significance. It can be seen (table 2) that the brain structures included in our study could be divided into three distinct groups. In nine of these, a value of $P < 0.10$ was observed, while 13 regions yielded a value of $P > 0.33$ with the remaining four structures showing a *P* value close to 0.2.

Effect of carbon dioxide on brain metabolism has previously been studied using biochemical methods.[13] These studies have reported only global effects of hypocapnia and shown that profound hypocapnia ($Pa_{CO_2} < 18$ mmHg) leads to an increase in (global) cerebral glucose consumption in both man[14] and experimental animals.[15]

To the best of our knowledge, no study of effect of hypocapnia on L-CMRg has been previously published. Hypercapnia has been shown to decrease L-CMRg in many of the brain structures (included in our study) when studied with ^{14}C-2DG autoradiographic technique. In our study, although mean values for L-CMRg in hypocapnic rats were higher than those in normocapnic rats, a value of $P < 0.05$ was achieved in only six structures. Compared to previous biochemical studies that show an increase in (global) glucose utilization with profound hypocapnia, our data suggests that a small number of structures with high metabolic rates of glucose show increased rates of glucose utilization even with modest hypocapnia ($Pa_{CO_2} = 25$). However, the difference in rates of glucose utilization is not significantly changed in the majority of structures as Pa_{CO_2} is decreased from 40 mmHg to 25 mmHg.

Based on the data presented, we conclude that modest hypocapnia results in a slight increase in L-CMRg. This increase in L-CMRg is heterogenous—those structures which have higher rates of glucose utilization during normocapnia show maximum increase in L-CMRg.

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