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Local Cerebral Glucose Utilization during Nitrous Oxide and Pentobarbital Anesthesia in Rats

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Local cerebral glucose utilization was measured in rats during nitrous oxide and pentobarbital anesthesia, using the 2-[^{14}C]-deoxyglucose method. During nitrous oxide anesthesia, 67%, marked heterogeneity of glucose utilization was observed. During pentobarbital anesthesia (30 mg/kg), glucose utilization decreased, the decrease being pronounced in the structures where glucose utilization was high during nitrous oxide anesthesia. During combined use of nitrous oxide and pentobarbital (30 mg/kg), with an electroencephalogram (EEG) consisting of 4–6 Hz wave superimposed by 10–15 Hz wave, glucose utilization was higher in many brain structures, including the midbrain reticular formation, than that observed during pentobarbital (30 mg/kg) anesthesia alone. With pentobarbital, 125 mg/kg, the EEG became nearly flat and a dose-related decrease in glucose utilization was observed in the cerebral cortices and inferior colliculus but not observed in any other structures. During the combined use of nitrous oxide and pentobarbital (125 mg/kg), the EEG was nearly flat, and no statistically significant differences in glucose utilization were observed as compared with those during pentobarbital (125 mg/kg) anesthesia in any of the structures examined. The results suggest that nitrous oxide and pentobarbital affect local cerebral glucose metabolism differently and that nitrous oxide acts as cerebral metabolic stimulant in the presence of cortical function during pentobarbital anesthesia. (Key words: Anesthetics, gases: nitrous oxide. Anesthetics, intravenous: pentobarbital. Brain: glucose metabolism.)

THEYE AND MICHENFELDER[1] first reported that nitrous oxide increases cerebral metabolic rate for oxygen (CMR_{O_2}) and cerebral blood flow in dogs. This finding has been confirmed by subsequent studies in animals.[2–4] In one of these studies, we found that the increase in CMR_{O_2} with nitrous oxide was attenuated by prior administration of thiamylal in dogs.[2] This study measured, however, CMR_{O_2} in the cerebral hemispheres, particularly the cortical areas, and did not allow evaluation of the combined effects of nitrous oxide and barbiturate in any other brain structures. The 2-[^{14}C]-deoxyglucose (2-[^{14}C]DG) method, introduced by Sokoloff *et al.*,[5] provides a quantitative measurement of local cerebral glucose utilization (LCGU) in the various brain structures and, hence, a mapping of the metabolic activity of the brain during different functional states. Using this method, the present study was designed to examine the metabolic effects of nitrous oxide and pentobarbital either alone or in combination in the different brain structures in rats. It was found that LCGU in various brain structures is higher during the combined use of nitrous oxide and pentobarbital anesthesia than that measured during pentobarbital anesthesia alone, in the presence of an active electroencephalogram (EEG).

Methods

The experiments were performed on 27 male Wistar rats, weighing 270–380 g. Anesthesia was induced with 3.5% halothane and 67% nitrous oxide and maintained with 1% halothane and 67% nitrous oxide during the 30-min operative procedure. The rats were tracheostomized, paralyzed with *d*-tubocurarine chloride (1.5 mg/kg iv), and mechanically ventilated (Harvard® pump). Both femoral arteries were cannulated, one for blood pressure recording and the other for the determination of arterial blood gases, *p*H, plasma glucose, and 2-[^{14}C]DG concentrations. Both femoral veins were cannulated for the administration of the drugs and 2-[^{14}C]DG. Rectal temperature was kept at 37 ± 0.1° C by external means in all the groups. A bipolar EEG was recorded from the frontoparietal area throughout the experiment. Heparin was given intravenously in a dose of 100 units.

The rats were divided into five groups. Halothane was discontinued upon completion of the surgical procedure in all the groups. In five rats (Group 1), anesthesia was maintained with 67% nitrous oxide throughout the study, and 1 h later, 2-[^{14}C]DG was administered for determination of LCGU. In six rats (Group 2), pentobarbital, 30 mg/kg, was administered intravenously over a period of 30 s, 1 h after discontinuation of halothane. One minute after the pentobarbital administration, nitrous oxide was discontinued and a 10-min period was allowed before the administration of 2-[^{14}C]DG and then LCGU was determined. In a series of separate experiments, rats given the same dose of pentobarbital through a tail catheter were found to be anesthetized for 60–90 min. In six rats (Group 3), the procedure was identical to that in Group 2, except that

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TABLE 1. Physiologic Variables

	Group				
	1 N_2O (n = 5)	2 PB (30 mg/kg) (n = 6)	3 PB + N_2O (30 mg/kg) (n = 6)	4 PB (125 mg/kg) (n = 5)	5 PB + N_2O (125 mg/kg) (n = 5)
MAP (mmHg)	132 ± 3	111 ± 2*	117 ± 8*	77 ± 3*	77 ± 3*
Pa_{O_2} (mmHg)	113 ± 11	139 ± 12	134 ± 10	134 ± 10	117 ± 3
Pa_{CO_2} (mmHg)	39 ± 2	37 ± 2	37 ± 1	36 ± 2	37 ± 1
*p*H	7.44 ± 0.02	7.44 ± 0.02	7.41 ± 0.01	7.37 ± 0.02*	7.37 ± 0.02*
Temperature (° C)	37 ± 0.1	37 ± 0.1	37 ± 0.1	37 ± 0.1	37 ± 0.1

The values are mean ± SE.

* Significantly different from Group 1.

nitrous oxide was continued throughout the experiments. In five rats (Group 4), pentobarbital, 125 mg/kg, was given intraperitoneally 1 h after discontinuation of halothane. One minute after pentobarbital administration, nitrous oxide was discontinued and a 10-min period was allowed before the administration of 2-[^{14}C]DG and then LCGU was determined. This dose of pentobarbital was selected through a series of separate experiments where several different doses of pentobarbital, administered either intravenously or intraperitoneally, were tested. The selected dose (125 mg/kg) was proven appropriate to produce a nearly flat EEG for 45–60 min. With this dose, however, it was necessary to administer phenylephrine intravenously (2–6 $\mu g \cdot kg^{-1} \cdot min^{-1}$) to maintain mean arterial pressure (MAP) above 70 mmHg. In five rats (Group 5), the procedure was identical to that in Group 4, except that nitrous oxide was continued throughout the experiments.

Quantitative measurement of LCGU has been described by Sokoloff *et al.*[5] Briefly, 2-[^{14}C]DG (New England Nuclear, specific activity, 51-52 mCi/mmol), 150 μCi/kg in 0.5 ml saline, was infused intravenously over a 30-s period. Fifteen arterial blood samples were obtained over a 45-min period, being frequently sampled during the early period so as not to miss the peak of the arterial curves. The samples were centrifuged, and the plasma was separated and frozen at −40° C for later determination of 2-[^{14}C]DG and glucose concentrations. Immediately after the final arterial blood sample was obtained at 45 min, the rat was decapitated and the brain was removed quickly and frozen in freon chilled with liquid nitrogen to −60° C. Following serial sectioning (20 μm in thickness), the brain tissue sections were exposed to x-ray film (Kodak SB-5) for 7–14 days, along with a set of calibrated [^{14}C]methylmethacrylate standards (The Radiochemical Center, Amersham). Densitometric measurements of autoradiograms were made with the use of a transmission densitometer (Sakura PDA 25) with an aperture of 0.5 mm. Plasma glucose concentrations were determined fluorometrically with the use of the hexokinase method. The plasma 2-[^{14}C]DG concentrations were determined by pipetting 20 μl plasma into scintillation vials containing 1 ml of a mixture of Soluen® and isopropanol (1:1 v/v). To the vials 7.5 ml of a mixture of Instagel® and 0.1 N HCl (9:1, v/v) then was added. The samples were counted in a Packard liquid scintillation counter, using the external standard method. LCGU was calculated according to the operational equation described by Sokoloff *et al.*[5]

Statistical differences were tested by one-way analysis of variance for repeated measures, with the least significant difference test for multiple comparisons. $P < 0.05$ was considered statistically significant.

Results

The physiologic variables in the five experimental groups are given in table 1. Mean arterial pressure (MAP) was significantly higher in Group 1 than any of the other groups that received pentobarbital. In all groups the mean Pa_{O_2} was above 113 mmHg and the mean Pa_{CO_2} was kept between 36 and 39 mmHg. The mean *p*H in Groups 4 and 5 was significantly lower than that of Group 1 but was within physiologic range. Table 2 shows the mean LCGU in 22 different structures in the five experimental groups. EEGs obtained before and 45 min after the administration of 2-[^{14}C]DG in each group are shown in figure 1.

In Group 1, the EEG consisted of 20–25 Hz wave of 10–20 μV intermingled with a 6–7 Hz wave of about 30 μV. The mean LCGU varied from 25 to 97 $\mu mol \cdot 100\ g^{-1} \cdot min^{-1}$, depending on the brain structures examined. Among the gray matter, the LCGU was high in the auditory system, above 80 $\mu mol \cdot 100^{-1} \cdot min^{-1}$ but was low in the periventricular, cerebellar gray, substantia nigra, hypothalamus, reticular formation, and pontine gray, at a level below 60 $\mu mol \cdot 100\ g^{-1} \cdot min^{-1}$. LCGU was also low in the white matter, the corpus callosum, being less than half of the level observed in the gray matter.

TABLE 2. Local Cerebral Glucose Utilization during Anesthesia

Structures	Group 1 N_2O (n = 5)	2* PB (30 mg/kg) (n = 6)	3 PB + N_2O (30 mg/kg) (n = 6)	4† PB (125 mg/kg) (n = 5)	5‡ PB + N_2O (125 mg/kg) (n = 5)
Cerebral association area					
Frontal cortex	77 ± 8	45 ± 6	62 ± 5§¶	28 ± 3¶	32 ± 2
Parietal cortex	64 ± 5	38 ± 4	51 ± 5§¶	25 ± 4	31 ± 2
Auditory system					
Cortex	95 ± 10	53 ± 5	71 ± 7§¶	33 ± 4¶	35 ± 2
Medial geniculate	80 ± 9	40 ± 4	54 ± 7§	35 ± 4	40 ± 3
Inferior colliculus	97 ± 9	69 ± 5	93 ± 7¶	40 ± 5¶	46 ± 3
Visual system					
Cortex	75 ± 7	41 ± 5	59 ± 4§¶	29 ± 3	30 ± 2
Lateral geniculate	71 ± 6	42 ± 4	54 ± 4§¶	32 ± 4	38 ± 2
Superior colliculus	64 ± 6	46 ± 5	75 ± 5¶	33 ± 4	41 ± 2
Sensorimotor system					
Cortex	67 ± 5	42 ± 4	61 ± 4¶	26 ± 4¶	31 ± 2
Thalamus					
Ventrolateral	77 ± 7	41 ± 4	59 ± 4§¶	38 ± 5	42 ± 2
Dorsomedial	87 ± 9	42 ± 4	63 ± 3§¶	37 ± 5	46 ± 3
Periventricular gray	46 ± 4	31 ± 5	42 ± 3	29 ± 5	34 ± 3
Cerebellar gray	37 ± 2	27 ± 3	37 ± 4¶	25 ± 3	31 ± 2
Extrapyramidal system					
Caudate-putamen	80 ± 7	42 ± 4	74 ± 7¶	30 ± 4	35 ± 3
Substantia nigra	57 ± 5	42 ± 4	48 ± 3	44 ± 5	55 ± 5
Limbic system					
Septal nucleus	36 ± 4	24 ± 3	35 ± 3¶	23 ± 4	30 ± 3
Amygdala	55 ± 6	39 ± 4	47 ± 3	30 ± 4	35 ± 2
Hypothalamus	45 ± 5	32 ± 3	44 ± 3¶	29 ± 4	34 ± 2
Hippocampus	67 ± 7	52 ± 6	67 ± 5¶	38 ± 4	45 ± 2
Reticular formation	50 ± 3	29 ± 4	45 ± 3¶	28 ± 5	34 ± 3
Pontine gray	49 ± 4	33 ± 4	42 ± 3	30 ± 4	34 ± 3
Myelinated fiber tract					
Corpus callosum	25 ± 3	15 ± 2	25 ± 3¶	18 ± 3	21 ± 1

The values are mean ± SE ($\mu mol \cdot 100\ g^{-1} \cdot min^{-1}$).

* Significantly different from Group 1 except hippocampus and substantia nigra.

† Significantly different from Group 1 except substantia nigra.

‡ Significantly different from Group 1 except cerebellar gray, substantia nigra and corpus callosum.

§ Significantly different from Group 1.

¶ Significantly different from Group 2.

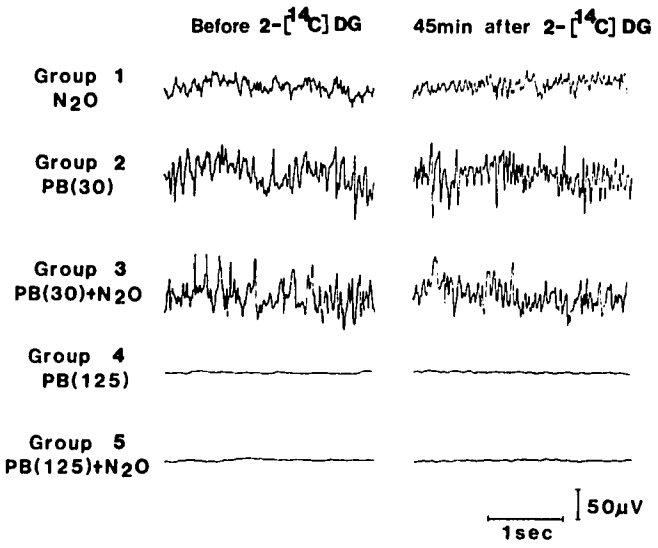

FIG. 1. EEGs obtained immediately before and 45 min after the administration of 2-[^{14}C]deoxyglucose in each group.

In Group 2, the EEG showed 4–6 Hz wave superimposed by 10–15 Hz wave. The mean LCGU was 22–52% lower than that of Group 1 in the different structures examined. The average cortical LCGU of the auditory, visual, sensorimotor, frontal, and parietal cortices was 42% lower than that in Group 1. The structures with higher LCGU in Group 1 were more depressed, hence, heterogeneity of the LCGU pattern was less than that of Group 1.

In Group 3 there were no apparent differences in the EEG compared with that in Group 2. In 17 of the 22 brain structures examined, the mean LCGU was significantly higher than that in Group 2. Even in the five other structures, the directional change was the same. A pronounced increase, more than 50%, was observed in the superior colliculus, dorsomedial thalamus, reticular formation, caudate-putamen, and corpus callosum. When compared with Group 1, the mean LCGU in only eight structures (frontal, parietal, visual,

auditory cortices, medial geniculate, lateral geniculate, ventrolateral, and dorsomedial thalamus) was significantly lower.

In Group 4, the EEG became nearly flat with the high dose of pentobarbital. When compared with Group 1, the decrease in LCGU was least in the substantia nigra (by 23%) while greatest in the auditory cortex (by 65%). The average LCGU in the cortices measured, namely the auditory, visual, sensorimotor, frontal and parietal cortices, was 62% lower than that in Group 1. The mean LCGUs in this group were significantly lower than those of Group 2 in only four structures (inferior colliculus, auditory, sensorimotor, and frontal cortices). In other structures, the reduction in LCGU was not statistically significant.

In Group 5, the EEG remained nearly flat, as in Group 4. Although the values were numerically higher, the LCGUs in all structures examined in Group 5 were not significantly different from the values observed in Group 4.

Discussion

The present study demonstrates that nitrous oxide has a metabolic stimulative effect on many brain structures in the presence of an active EEG during pentobarbital anesthesia. The LCGU values of the nitrous oxide group in the present study are similar to those reported by Ingvar *et al.*[6] and Ingvar and Siesjö.[7] Our LCGU values for the pentobarbital group are comparable to those reported by Sokoloff's group,[8] despite the difference in dose used and the limited number of brain structures examined in their study. Reported values of LCGU in awake rats vary from laboratory to laboratory and even in the same laboratory on different experimental occasions.[5-10] For example, LCGU in the parietal cortex, auditory cortex, caudate-putamen, and hippocampus in awake rats range from 63–107, 72–157, 70–111, and 48–79 $\mu mol \cdot 100\ g^{-1} \cdot min^{-1}$, respectively. These wide variations in LCGUs may be due to different magnitudes of stress (immobilization, artificial ventilation, painful stimuli) in the awake state, even though meticulous care, including local anesthetic infiltration around the surgical wounds, adrenalectomy, and preexperimental training of the rat have been practiced. Thus, we are not confident of the LCGU values reported for awake rats and will focus our discussion on the interaction between nitrous oxide and pentobarbital either in the presence or absence of an active EEG.

In a previous study by Theye and Michenfelder[1] and subsequently by us,[2,3] nitrous oxide was found to increase hemispheric CMR_{O_2} in dogs. Recently, Pelligrino *et al.*[4] reported a pronounced increase in cortical CMR_{O_2} with nitrous oxide in goats. Region specific increases in LCGU with nitrous oxide alone has also been recently reported for the brain[7] and spinal cord[8] in rats. Since glucose utilization is stoichiometrically related to oxygen consumption in the brain, these findings on nitrous oxide seem consistent among different species. The notable findings in the present study is that LCGUs were higher during combined use of nitrous oxide and pentobarbital than those of pentobarbital alone in the presence of an active EEG. This suggests the possibility of metabolic stimulation of nitrous oxide or interference of nitrous oxide with pentobarbital. If the latter is the case, nitrous oxide must have counteracted to the effect of pentobarbital, because pentobarbital is a metabolic depressant. In any event, nitrous oxide, when it was used with pentobarbital, produced a metabolically stimulated state as compared with that with pentobarbital alone. The increase in LCGU by nitrous oxide varied, depending on the structure and was most pronounced in the caudate-putamen. Looking at specific structures such as the midbrain reticular formation and periventricular gray, which have been assumed to be related to the site of action for anesthesia and analgesia, the change in LCGU was in the same direction as the change in other structures. With barbiturates, depression of the reticular formation is believed by some to be the functional mechanism for their anesthetic actions. The remarkable decrease in LCGU in the reticular formation with pentobarbital observed in the present study is presumed to be a reflection of the depression of neuronal activity of the reticular formation. The situation is different during combined use of nitrous oxide and pentobarbital, since LCGUs were higher in many brain structures, including the reticular formation, than those during pentobarbital anesthesia alone. This result suggests that the mechanism by which nitrous oxide and pentobarbital affect the central nervous system may be different.

Clinical experience suggests that the combined use of nitrous oxide and pentobarbital produces deeper anesthesia than when nitrous oxide or pentobarbital anesthesia are used alone. If this is the case in our circumstance, the results indicate that an increase in the depth of anesthesia with a combination of these two anesthetics does not necessarily produce depression of glucose metabolism. The stimulative effect of nitrous oxide was observed only when cortical function was present as judged by an active EEG. Nitrous oxide's stimulative effect on LCGU was no longer observed when the dose of pentobarbital was increased to produce a nearly flat EEG. It was proposed by Michenfelder and Theye[11] that anesthetics alter cerebral metabolism only by altering neural function. When one applies their hypothesis to the present study, the results may indicate that at least in the cortex nitrous oxide increases that component of

cerebral metabolic activity that is related to functional activity, while it does not affect those metabolic processes subserving cellular integrity. However, we can not entirely exclude the possibility that differences in LCGUs between groups with high-dose pentobarbital plus nitrous oxide and with high-dose pentobarbital alone might have been undetected because of limited sensitivity of the methods used.

Pentobarbital, as expected, decreased LCGU, but there were no significant differences in LCGU between two dose groups of pentobarbital studied except in the frontal, sensorimotor, auditory cortices, and the inferior colliculus. This suggests that near maximum decrease in glucose utilization in subcortical structures was produced by a smaller dose of pentobarbital than in the cortical structures.

In summary, the expected deepening of anesthetic levels with the combined use of nitrous oxide and pentobarbital is not accompanied by depression of LCGU. Nitrous oxide appears to act as a cerebral metabolic stimulant in the presence of cortical function (EEG) during pentobarbital anesthesia.

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