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The Influence of Fentanyl upon Cerebral High-energy Metabolites, Lactate, and Glucose during Severe Hypoxia in the Rat

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The effects of intravenous administration of high-dose fentanyl (100 $\mu g \cdot kg^{-1}$, loading dose followed by an infusion of 200 $\mu g \cdot kg^{-1} \cdot h^{-1}$) were compared with those of a barbiturate (pentobarbital sodium 25 $mg \cdot kg^{-1}$, intraperitoneal) or hypothermia (rectal temperature 32° C) on changes in cerebral cortical tissue levels of adenosine triphosphate (ATP), phosphocreatine (PCr), lactate, and glucose in severely hypoxemic rats (Pa_{O_2} 13–23 mmHg for 20 min) with unilateral (left side) carotid ligation (10–12 animals in each group). Ligation of the carotid artery alone produced no change in brain high-energy metabolites, lactate, or glucose. The control values on the ligated side (nitrous oxide, 70%, + normoxia group) for cortical ATP, PCr, lactate, and glucose were 2.86 ± 0.09 ($\mu mol \cdot g^{-1}$ wet weight, mean ± 1 SE), 3.83 ± 0.11, 1.68 ± 0.21, and 3.29 ± 0.47, respectively. Hypoxia (nitrous oxide, 70%, + hypoxia group) produced a significant ($P < 0.05$) decrease in ATP (1.83 ± 0.37) and PCr (1.93 ± 0.48) and an increase in lactate (15.8 ± 1.77) compared with the normoxic group, whereas brain glucose was not significantly changed (1.97 ± 0.65). Fentanyl (fentanyl + hypoxia group) did not prevent the deleterious effects of hypoxia on cortical high energy metabolites (ATP, 2.0 ± 0.27; PCr, 2.24 ± 0.3) or lactate (19.33 ± 3.16); however, fentanyl caused no alteration in high-energy cerebral metabolite concentrations in normoxic rats, nor did fentanyl produce a significant difference in brain tissue glucose or lactate. In contrast to the effects of fentanyl, pentobarbital (barbiturate + hypoxia group) or hypothermia (nitrous oxide + hypothermia + hypoxia group) prevented the significant decrease in ATP or PCr and attenuated, but did not eliminate, the increase in lactate. Though hypoxia produced changes on the unligated (right) side these tended to be similar to the ligated side though typically less severe. Blood glucose concentrations were determined on samples obtained before and after the experimental insult. The concentrations of glucose were variable, but there appeared to be no relationship between treatment or changes in brain metabolites and blood glucose. In summary, fentanyl in doses that have previously been shown to reduce cerebral oxygen metabolism by 35% did not prevent or retard the loss of cerebral high-energy metabolites or the production of lactate during 20 min of severe hypoxia. This was in distinct contrast to the effects of mild hypothermia or an anesthetic dose of pentobarbital. (Key words: Anesthetics, intravenous: fentanyl. Brain: hypoxia; metabolism. Hypnotics: barbiturates. Hypothermia.)

ADVANCES in the care of critically ill patients have resulted in increased survival following cardiopulmonary resuscitation and stroke. However, dysfunction of the central nervous system following an hypoxic–ischemic episode is still a major complication in these patients,[1] and development of effective methods to prevent injuries to the nervous tissue due to hypoxia or ischemia is still an ongoing effort. Hypothermia has long been known to protect against cerebral hypoxia or ischemia by preventing or minimizing the depletion of brain high-energy metabolites during the insult.[2–5] Barbiturates, as well as some inhalational anesthetic agents, have been shown to limit aspects of cerebral tissue damage caused by hypoxia or ischemia in some animals, as well as in certain clinical situations.[6–12] It has been suggested that the decrease in cerebral metabolic rate for oxygen ($CMRO_2$) during hypothermia or deep anesthesia is a major factor in some forms of cerebral protection against hypoxia–ischemia.

In previous studies of the effects of fentanyl on cerebral circulation and metabolism, we showed that fentanyl in a dose of 100 $\mu g \cdot kg^{-1}$ followed by an infusion of 200 $\mu g \cdot kg^{-1} \cdot h^{-1}$ caused cerebral blood flow (CBF) and $CMRO_2$ to decrease by about 35% compared with animals receiving nitrous oxide.[13] The present study was designed to investigate whether the decrease in $CMRO_2$ caused by fentanyl provides protection when compared with the effects of a barbiturate or hypothermia against high-energy metabolite depletion in brain tissue during hypoxemia in rats with unilateral carotid ligation.

Materials and Methods

The study was approved by the institutional animal care and use committee. Male Wistar rats weighing 250–300 g had free access to food and water prior to each study. They were anesthetized with halothane 2–3% in oxygen. Following tracheostomy mechanical ventilation using a small animal ventilator was begun. During surgical preparation anesthesia was maintained with halothane, 0.5–1.5% in nitrous oxide (N_2O), 70%, and oxygen (O_2),

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TABLE 1. Physiologic Variables Measured at the End of the Hypoxic Period in the Various Experimental Groups

Group	MABP (mmHg)	Pa_{O_2} (mmHg)	Pa_{CO_2} (mmHg)	*p*Ha
1 (N_2O + normoxia)	124 ± 5.1	122 ± 5.9	39.9 ± 0.8	7.39 ± 0.02
2 (Fentanyl + normoxia)	102 ± 2.4*	128 ± 3.2	37.6 ± 2.0	7.36 ± 0.02
3 (N_2O + hypoxia)	113 ± 4.4*	20.2 ± 0.6*	31.0 ± 2.0*	7.46 ± 0.03
4 (N_2O + hypothermia + hypoxia)	110 ± 3.3*	17.6 ± 0.8*	28.9 ± 1.5*	7.53 ± 0.02*
5 (Barbiturate + hypoxia)	109 ± 4.3	20.6 ± 0.4*	30.2 ± 1.7*	7.53 ± 0.02*
6 (Fentanyl + hypoxia)	106 ± 3.2*	21.4 ± 0.3*	31.4 ± 1.7*	7.46 ± 0.03

Values are given as mean ± SE.

* Significantly different from group 1, $P < 0.05$.

30%. The left carotid artery was separated from the sympathetic trunk and divided between silk ligatures. The femoral vessels were cannulated for monitoring arterial blood gases and *p*H, continuous arterial pressure monitoring, iv drug infusion, and blood transfusion. The head was then immobilized in a stereotaxic head holder, the skull exposed through a midline incision in the scalp, and a plastic funnel was fixed to the skull. Bilateral frontooccipital platinum needle electrodes were inserted in the scalp for continuous monitoring of the electroencephalogram (EEG). A rectal temperature probe was inserted and the temperature was controlled at the desired level by means of a heat lamp servomechanism. The animals were then paralyzed with *d*-tubocurarine, 1 $mg \cdot kg^{-1}$ iv, and ventilation was adjusted to maintain a Pa_{CO_2} of 35–40 mmHg. Upon completion of surgical preparation the halothane was discontinued and the animals ventilated with 70% N_2O and 30% O_2 for at least 30 min prior to the onset of the experiment.

The animals were randomly assigned to six different groups, 12 animals in each group. Group 1 animals served as normoxic controls, and were ventilated with N_2O, 70%, and O_2 throughout the experiment. Group 2 animals received a loading dose of fentanyl, 100 $\mu g \cdot kg^{-1}$ iv followed by infusion of fentanyl at a rate of 200 $\mu g \cdot kg^{-1} \cdot h^{-1}$. In addition, N_2O was replaced by 70% nitrogen (N_2) for the period of the experiment. This group was not made hypoxic. Animals in the remaining groups were exposed to 20 min of severe hypoxemia(Pa_{O_2} 13–23 mmHg) induced by partially replacing O_2 with N_2 during the experiment. These hypoxic groups were treated as follows: group 3 animals received no additional treatment. In group 4 the body temperature was decreased to 32° C by surface cooling prior to the onset of hypoxemia and then maintained at that temperature for the duration of the experiment.[13] Group 5 animals received pentobarbital, 25 $mg \cdot kg^{-1}$, intraperitoneal, 15 min prior to the onset of hypoxia. Group 6 received fentanyl 100 $\mu g \cdot kg^{-1}$ iv immediately before the start of hypoxia, followed by an infusion of fentanyl 200 $\mu g \cdot kg^{-1} \cdot h^{-1}$ during the experiment. In the last two groups (5 and 6), N_2O was replaced by N_2 for the period of the experiment. The dose and infusion rate for fentanyl were determined during our previous study.[14]

During the hypoxic period mean arterial blood pressure (MABP) was maintained at approximately 100 mmHg and acidosis was corrected by means of a slow infusion of phenylephrine and sodium bicarbonate, respectively, as needed. Arterial blood gases and *p*H were measured before the onset of hypoxia, at 5-min intervals during hypoxia, and at the end of experiment. In many animals a blood sample was obtained before and at the end of the experimental insult. The blood was dripped into liquid N_2 and the frozen blood stored for later glucose analysis. At the end of the 20-min hypoxic period in the last four groups and the corresponding interval in the first two groups, the brain was frozen *in situ* by pouring liquid N_2 into the plastic funnel.[15] Mechanical ventilation was continued during freezing and arterial pressure was maintained for 1–2 min. The brains were chiseled out and stored in liquid N_2 until samples of cortical brain tissue were taken separately from the cerebral hemispheres for microfluorometric analysis of brain adenosine triphosphate (ATP), phosphocreatine (PCr), lactate, and glucose. Blood samples were analyzed for glucose using the same techniques.

Statistical analysis of the changes in ATP, PCr, lactate, and glucose for the treatment groups in ligated and unligated hemispheres was performed using two-way analysis of variance (ANOVA) for repeated measures. Comparisons between treatment groups and the N_2O + normoxia or N_2O + hypoxia groups and between the ligated and unligated sides of the brain were made using the least significant difference technique.[16] Comparisons between groups were only made if the overall *F* value was statistically significant. Statistical testing of other variables was done using one-way ANOVA. $P < 0.05$ was considered to be statistically significant.

Results

The final data set had 10–12 animals in each group. Table 1 summarizes the physiologic variables in the six groups. MABP was decreased in groups 2–6 compared

TABLE 2. Cerebral Cortical Tissue Concentration of ATP, PCr, Lactate, and Glucose from Left and Right Hemisphere*

	ATP		PCr		Lactate		Glucose	
Hemisphere Group	Left	Right	Left	Right	Left	Right	Left	Right
1 (N_2O + normoxia)	2.86 ± 0.09	2.84 ± 0.09	3.83 ± 0.11	3.73 ± 0.15	1.68 ± 0.21	1.62 ± 0.18	3.29 ± 0.47	3.25 ± 0.48
2 (Fentanyl + normoxia)	2.81 ± 0.06†	2.95 ± 0.07	3.97 ± 0.13†	3.93 ± 0.17†	1.44 ± 0.30†	1.48 ± 0.36†	4.00 ± 0.30	3.72 ± 0.24
3 (N_2O + hypoxia)	1.83 ± 0.37‡	2.42 ± 0.30	1.93 ± 0.48‡	2.46 ± 0.30‡	15.8 ± 1.77‡	12.94 ± 0.91‡	1.97 ± 0.65	2.19 ± 0.54
4 (N_2O + hypothermia + hypoxia)	2.99 ± 0.09†	3.04 ± 0.06	3.78 ± 0.20†	3.78 ± 0.18†	6.35 ± 0.50†‡	5.60 ± 0.28†	3.91 ± 0.51	4.02 ± 0.54
5 (Barbiturate + hypoxia)	2.55 ± 0.20†	3.75 ± 0.72†	3.29 ± 0.27†	3.42 ± 0.22	10.29 ± 1.37†‡	8.90 ± 1.28‡	4.78 ± 0.4†	4.54 ± 0.29†
6 (Fentanyl + hypoxia)	2.00 ± 0.27‡	2.48 ± 0.21	2.24 ± 0.30‡	2.82 ± 0.32	19.33 ± 3.16‡	12.36 ± 0.88‡	3.33 ± 0.66	3.20 ± 0.60

Values are given as $\mu mol \cdot g^{-1}$ wet weight (mean ± SE) with 10–12 animals per group.

* Left hemisphere was ipsilateral to carotid ligation.

† Significantly different from group 3, $P < 0.05$.

‡ Significantly different from group 1, $P < 0.05$.

with the N_2O + normoxia group (group 1). Pa_{CO_2} was lower and pH was significantly higher in most of the hypoxic groups compared to the normoxic groups (1 and 2). There were no significant differences in physiologic variables between the various hypoxic groups. Blood glucose values obtained before (9 ± 0.5 $\mu M \cdot g^{-1}$ wet weight, mean for all groups combined ±1 SE,) and at the end of the insult were similar, and there was no relationship to experimental treatment or to the changes in brain metabolites. The values for blood glucose obtained in this study were similar to those reported by Agardh *et al.* who also studied rats receiving nitrous oxide.[17]

Table 2 shows the values for ATP, PCr, lactate, and glucose from the cortical tissue of both ligated and unligated hemispheres obtained at the end of the hypoxic period. Compared to group 1 (N_2O + normoxia), administration of fentanyl did not cause any change in the levels of the high-energy phosphates, lactate or glucose (group 2, fentanyl + normoxia). Hypoxia caused significant depletion of the high-energy phosphate compounds and accumulation of lactate in group 3, (N_2O + hypoxia). The decrease in PCr and the increase in lactate involved both hemispheres while ATP decreased only in the left cortex (side of the carotid ligation). Hypothermia (group 4) completely preserved high-energy phosphate concentrations and significantly attenuated the production of lactate. Administration of pentobarbital (group 5) maintained ATP and PCr levels. On the ligated side it also significantly decreased the accumulation of lactate in comparison with group 3 (N_2O + hypoxia), although the values for lactate were significantly higher than with N_2O + normoxia (group 1). Administration of fentanyl (group 6, fentanyl + hypoxia) did not prevent the depletion of high-energy phosphates or the accumulation of lactate caused by hypoxia. This is evidenced by significant decreases in both ATP and PCr on the ligated side compared to group 1 (N_2O + normoxia). Neither hypoxia nor treatment had any effect on the brain glucose levels, except for a significant elevation in group 5; however, this did not appear to have any effect on the changes in brain metabolites or lactate.

There were ligated (left) side *versus* unligated (right) side differences in the values of ATP for animals in groups 3 (N_2O + hypoxia), 5 (barbiturates + hypoxia), and 6 (fentanyl + hypoxia), and of PCr and lactate for groups 3 and 6. There were no side to side differences with respect to glucose.

Representative samples of the EEG are shown in figure 1. After fentanyl (group 6) and pentobarbital (group 5) the EEG showed marked slowing compared to the animals receiving N_2O. In both group 3 (N_2O + hypoxia) and group 6 (fentanyl + hypoxia group) the EEG developed much lower amplitudes and lower frequencies within 2–5 min after the beginning of hypoxia, and at the end of the hypoxic period the EEG in many of these animals was virtually isoelectric. In contrast, those animals made hypothermic and or who were treated with pentobarbital had appreciable preservation of EEG activity during and at the end of hypoxia.

Discussion

We selected the Levine model[18] of unilateral carotid ligation and hypoxia for this study because the degree of hypoxia alone necessary to produce brain damage is so severe that it often affects the cardiovascular system causing arrhythmias and hypotension. Previous studies with the same model showed that acute occlusion of one carotid artery decreased CBF by only 10% on the side of occlusion and that this reduction in CBF did not cause any change in the levels of high-energy phosphate and lactate levels between the two (ligated and unligated) hemispheres. During hypoxia these earlier studies demonstrated that CBF increases twofold on the side with the carotid artery occlusion and fivefold on the side with the nonoccluded carotid artery.[19] Thus, unilateral carotid ligation does not

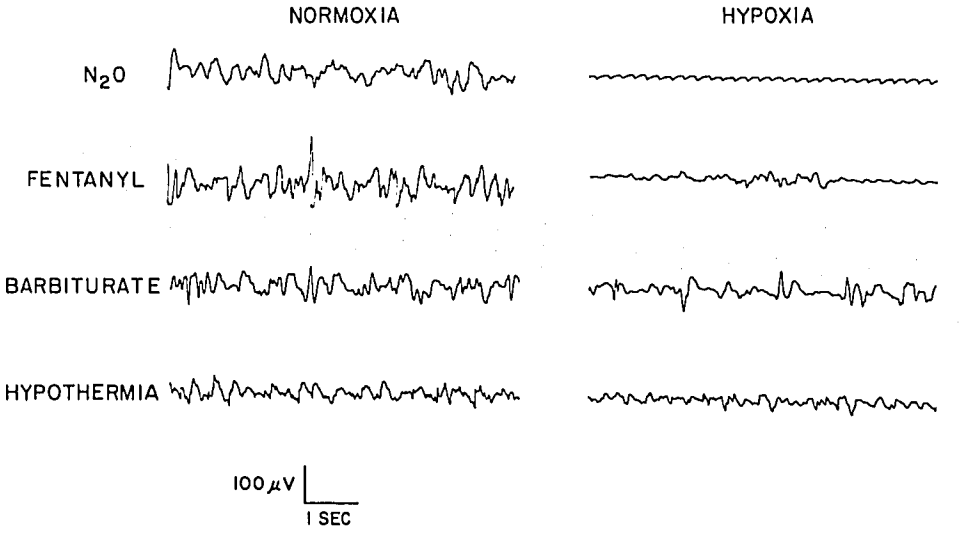

FIG. 1. Representative electroencephalograms showing examples from the four treatment groups prior to and toward the end of the hypoxic period. The segments shown during hypoxia represent the maximal change noted.

primarily cause ischemia, but it reduces the compensatory increase in CBF during hypoxia.

It has been hypothesized that reduction in $CMRO_2$ is important in protecting brain tissue from damage caused by hypoxia–ischemia. The relationship between a decrease in $CMRO_2$ and brain protection has been demonstrated in various animal models. The use of barbiturates, though not effective in preventing brain tissue damage during complete ischemia, has shown good results in models of incomplete cerebral ischemia and hypoxia.[6,7,20,21] Recent studies in humans have confirmed the animal studies by showing that administration of barbiturates in a dose producing a sustained burst suppression pattern in the EEG improved the neuropsychiatric outcome in patients undergoing cardiopulmonary bypass for open ventricle cardiac surgery.[12] However, barbiturates in doses that are effective against hypoxic brain damage often cause pronounced hypotension and have an extended duration of action that requires vigorous cardiovascular support and delayed tracheal extubation.

In a previous study we demonstrated that the iv administration of high doses of fentanyl produced a significant dose-dependent depression in the $CMRO_2$.[13] The decrease in $CMRO_2$ reached a maximum of about 35% at 100 $\mu g \cdot kg^{-1}$, a finding recently confirmed by Baughman *et al.*[22] The absence of profound hypotension during administration of high doses of fentanyl during clinical anesthesia and the available means to completely reverse the effects of this drug at the conclusion of surgery suggested some possible advantages when compared to barbiturates. However, our results indicate that whereas hypothermia or barbiturates preserved high-energy phosphate levels and attenuated the accumulation of lactate in brain cortical tissue during hypoxia, fentanyl at a dose that decreased $CMRO_2$ by 35% had no such effect. In our prior study attempts to accomplish larger decreases in $CMRO_2$ by increasing the dose of fentanyl to 200 $\mu g \cdot kg^{-1}$ and 400 $\mu g \cdot kg^{-1}$ resulted in seizure activity on EEG accompanied by a marked increase in $CMRO_2$ in the animals with seizures.[13] Thus, in rodents at least, the maximal fentanyl dose that can be used is limited. However, inspection of that data suggested that even in those animals without seizures there was no further fall in $CMRO_2$ after 100 $\mu g \cdot kg^{-1}$.

The degree of hypothermia and the dose of pentobarbital used in this study was purposely limited in an attempt to approximate the metabolic reduction that would be achieved by fentanyl. With respect to pentobarbital, 25 $mg \cdot kg^{-1}$ ip tends to produce unconsciousness and often surgical levels of anesthesia in rats. This should produce about a 30% reduction in CBF and $CMRO_2$. In addition, seven of ten EEG records in our study showed slowing only, although the other three had some degree of burst suppression. With respect to temperature, it has previously been shown that hypothermia in the rat decreases $CMRO_2$ by 5%/° C.[14] Thus, 32° C should reduce $CMRO_2$ by approximately 25%. In addition, Hagerdal *et al.*[20] have shown that in the rat model of unilateral carotid ligation and hypoxia this degree of temperature fall prevents ATP and PCr loss and attenuates the increase in lactate.

The reason for the failure of fentanyl to prevent loss of cerebral high-energy phosphates and a decrease in lactate accumulation is not known. Possibly, the degree of metabolic depression produced was not adequate to prevent deleterious changes. It is also possible that other factors may be involved and that depression of metabolic rate alone is not sufficient for brain preservation as has also been suggested by the differing effects of isoflurane and halothane on preserving the EEG during carotid endarterectomy.[23,24] It is, however, important to note that

fentanyl in normoxic animals had no deleterious effects on cerebral high-energy phosphate compounds or lactate levels, and in hypoxic animals the effects of fentanyl were no worse than the effects of hypoxia alone. Nehls *et al.*[25] used a model of ischemia (in contrast to our model of hypoxemia) and demonstrated that a combination of nitrous oxide and fentanyl did not exacerbate neurologic injury, although it did not prevent injury compared to thiopental. Thus, although fentanyl may not improve metabolic status after hypoxia or neurologic status after ischemia, it does not exacerbate the injury.

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