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Effects of Sufentanil on Regional Cerebral Glucose Utilization in Rats

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Sufentanil, a narcotic five to ten times more potent than fentanyl, reduces cortical cerebral blood flow and oxygen consumption in rats, with seizure activity occurring in some animals. However, the effects of sufentanil on blood flow and metabolism in subcortical structures have not been defined clearly. The present study examines the effects of intravenous sufentanil (40 or 160 μg/kg) on regional cerebral glucose utilization (r-CMRgl) in paralyzed, mechanically ventilated rats using 2-deoxy-D-[^{14}C]glucose and autoradiography. Regional cerebral glucose utilization was decreased in all cortical areas examined in rats receiving either dose of sufentanil; the larger dose of sufentanil (160 μg/kg) decreased r-CMRgl in cortical structures 20–45% below control values. Two subcortical structures, the caudate nucleus and the ventral thalamic nucleus, manifested a 39–54% decrease in r-CMRgl at each dose of sufentanil. Limbic system structures responded differently. Sufentanil 40 μg/kg produced focal areas of markedly increased r-CMRgl in the amygdala of two of six rats; sufentanil 160 μg/kg produced marked increases in r-CMRgl in focal areas of hippocampus (four of eight rats) and amygdala (seven of eight rats). EEG activation suggestive of seizure activity was evident in the two low-dose sufentanil and six of the seven high-dose sufentanil rats that had focally increased r-CMRgl in the amygdala. Sufentanil causes a selective increase in r-CMRgl in subcortical limbic nuclei, particularly the amygdala, in the rat. EEG patterns of seizure activity may reflect subcortical, rather than cortical activation. (Key words: Analgesics: sufentanil. Anesthetics, intravenous: sufentanil. Brain: convulsions; electroencephalography; limbic system activation; metabolism, glucose. Measurement techniques: autoradiography.

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SUFENTANIL, a narcotic that is five to ten times more potent than fentanyl, is being investigated in high-dose narcotic anesthetic techniques because it appears to provide satisfactory surgical conditions, more hemodynamic stability, and a greater therapeutic ratio than fentanyl.[1] Sufentanil has been shown to decrease cortical cerebral blood flow and oxygen consumption in rats, while seizure activity at greater doses has been noted.[2] Because subcortical areas may be affected by drugs differently than the cortex, we studied the effects of sufentanil on regional cerebral glucose utilization (r-CMRgl) in rats.

Materials and Methods

Male Wistar rats weighing between 200–420 g were used. Food was withheld from all animals for approximately 16 h before the experiment to ensure steady state plasma glucose concentrations; free access to water was maintained. The animals were anesthetized with halothane (2% inspired) and nitrous oxide (70%) in oxygen. Following tracheotomy, they were paralyzed with curare (1.8 mg/kg), connected to a small animal ventilator, and ventilated mechanically. The halothane concentration was decreased to 0.3% inspired. Polyethylene catheters were placed bilaterally in the femoral arteries and veins for anaerobic sampling of arterial blood, blood pressure recording, and infusion of drugs and blood. The animals were placed prone, and bilateral parieto-occipital needle electrodes were inserted into the connective tissue of the skull for continuous EEG recording. The halothane was discontinued at the completion of the surgical preparation.

Blood pressure was measured continuously with a transducer and recorded on a polygraph; mean arterial pressure was maintained greater than 100 mmHg with intermittent transfusions of fresh rat blood. Temperature was measured rectally and maintained at 37° C with a servo-controlled heat lamp. The ventilator was adjusted to maintain an arterial blood carbon dioxide tension (Pa_{CO_2}) between 35–40 mmHg. The arterial blood oxygen tension (Pa_{O_2}) was maintained between 100–160 mmHg.

The sufentanil administration began 30 min after the halothane was discontinued. Sufentanil powder (preservative free), provided by Janssen Pharmaceutica, Inc. (Piscataway, New Jersey), was dissolved in saline to

produce a 500 μg/ml stock solution. This was diluted further to a concentration of 50 μg/ml before use. Two doses of sufentanil were studied; 40 μg/kg (S40), which in a previous study produced significant decreases in both cortical CBF and $CMRO_2$ along with continuous slow wave EEG activity, and 160 μg/kg (S160), which produced seizure activity in many animals.[2] The maximum volume of sufentanil received by any animal did not exceed 5 ml. Each of these two intravenous loading doses of sufentanil was followed by an infusion of twice the initially injected dose per hour. These infusion rates were derived from previous studies in which the EEG patterns noted with the loading doses were maintained most consistently.[2] Nitrous oxide was replaced by nitrogen (70%) at the beginning of sufentanil administration. Control animals were maintained on nitrous oxide and received no narcotic.

Fifty minutes after the halothane was discontinued, measurement of r-CMRgl was begun; 35 μCi of 2-deoxy-D-[^{14}C] glucose (^{14}C-DG, New England Nuclear, Boston, Massachusetts) was injected into a femoral vein and the cannula flushed by 0.5 ml of normal saline. Eighteen 50 μl arterial blood samples were collected over the next 45 min, with 11 of the samples being collected during the first 10-min postisotope injection. The samples were centrifuged immediately, 10-μl aliquots of plasma from each sample were placed into vials containing 15 ml ACS-II (Amersham, Arlington Hts., Illinois), and counted in a liquid scintillation counter. The counting efficiency was calculated from the internal standard ratios using ^{14}C-toluene (New England Nuclear). Plasma from samples drawn at 0 min and subsequent 5-min intervals was analyzed for glucose concentration with a Beckman® glucose analyzer.

At the end of the 45-min period, the animals were decapitated and their brains quickly dissected out and frozen in freon. The brains later were stored in liquid nitrogen or dry ice until they were sectioned in a cryostat (−18 to −20° C). Serial coronal sections 20 μm in thickness from different levels of the brain were obtained for analysis. The sections were mounted on glass slides, and, along with a set of calibrated ^{14}C-methyl methacrylate standards, were placed in contact with a single-emulsion x-ray film (Kodak® SB-5) for 10 days. The films were developed, and the density of various cortical and subcortical areas was determined using a micro-densitometer (Gamma Scientific, San Diego, California; aperture 0.25 mm). Multiple densitometric measurements (minimum of six) were made in each of three or more sections of each cortical and subcortical structure with an attempt to sample representative areas of each structure. All data were collected on line with a PDP 11/10® computer. Final values for regional glucose uptake were calculated according to the formula developed by Sokoloff *et al.*[3] Glucose utilization for a particular structure was estimated by using the mean of the individual measurements within each structure. Comparison between treatment groups was made using analysis of variance and appropriate range tests. Variability of glucose utilization within each structure was estimated by obtaining the standard deviation of multiple samplings from that structure. Analysis of variance was used to test uniformity of variance between treatment groups.

TABLE 1. Physiologic Conditions during Determinations of Regional Cerebral Glucose Utilization

	Control (N = 7)	S40 (N = 6)	S160 (N = 8)
Pa_{O_2} (mmHg)	132 ± 6.3	125 ± 2.5	133 ± 4.2
Pa_{CO_2} (mmHg)	35 ± 1.4	36 ± 1.2	36 ± 1.1
pH_a	7.39 ± 0.02	7.36 ± 0.02	7.36 ± 0.02
Mean arterial blood Pressure (mmHg)	104 ± 2.1	105 ± 2.7	105 ± 2.6
Plasma glucose (mg/dl)	186 ± 18.8	204 ± 20.3	165 ± 15.2

Each value is the mean ± 1 SEM.

Results

Physiologic conditions during r-CMRgl determinations were comparable in the control and sufentanil 40 μg/kg (S40) and sufentanil 160 μg/kg (S160) groups (table 1). Plasma glucose measurements ranged from 110 to 122 mg/dl in the animal with the lowest blood glucose levels to 270–304 mg/dl in the animal with the highest blood glucose levels. All plasma glucose measurements were less than 330 mg/dl in each animal, the level at which glucose concentrations may interfere with the accuracy of ^{14}C-DG measurements.[3]

Densitometry was performed on 22 different structures in each animal. Figure 1 shows selected autoradiographic sections from animals receiving nitrous oxide, S40, and S160. Autoradiographs from animals given

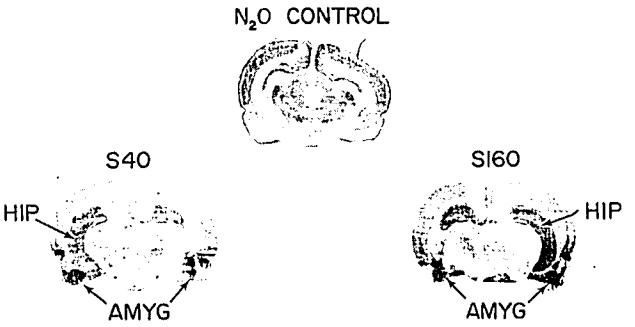

FIG. 1. Selected autoradiographic sections from rats receiving nitrous oxide, sufentanil 40 μg/kg (S40), and sufentanil 160 μg/kg (S160). Amygdala (AMYG) and hippocampus (HIP) are indicated.

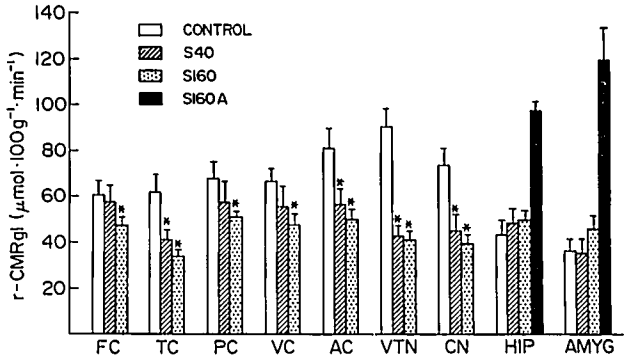

FIG. 2. The effects of two doses of sufentanil on regional glucose utilization (r-CMRgl) in various cortical and subcortical structures compared with nitrous oxide controls. Each dose is the mean ± 1 SEM of six to eight rats. Black histogram bars are selected readings from areas of greatest density within the named structure; they were not used in the statistical calculations. Abbreviations of brain areas are given in the text. Asterisks indicate a value significantly different from control ($P < 0.01$), using two-way analysis of variance.

either dose of sufentanil showed a qualitative generalized decrease in density, suggesting a decrease in r-CMRgl in all cortical and many subcortical areas compared with nitrous oxide controls. This is borne out by the quantitative data. As shown in figure 2, control r-CMRgl values in cortical areas ranged from 61 ± 7 to 81 ± 8 $\mu mol \cdot 100\ g^{-1} \cdot min^{-1}$ (mean ± SEM). Both doses of sufentanil produced quantitative decreases in r-CMRgl in frontal (FC), temporal (TC), parietal (PC), visual (VC), and auditory (AC) cortices. This decrease ranged from 5 to 45% below control, with the greatest per cent decrease noted in the temporal (45% decrease with S160) and auditory (37% decrease with S160) cortices. The decrease in r-CMRgl from control levels was statistically significant ($P < 0.01$) with S40 in the temporal and auditory cortices and with S160 in all cortical structures examined.

With respect to subcortical nuclei, the ventral thalamic nucleus (VTN) and caudate nucleus (CN) each manifested statistically significant decreases in r-CMRgl ($P < 0.01$) at both doses of sufentanil (fig. 2). There was no significant decrease in r-CMRgl in the hippocampus (HIP) of the six rats receiving S40, nor was there a significant decrease in the amygdala (AMYG) in four of the six rats receiving S40.

Though the data are not shown, two other subcortical structures, the medial and lateral geniculate nuclei, showed dose-dependent decreases in r-CMRgl of 55–63%. Other subcortical and brainstem structures examined included the hypothalamus, periventricular nucleus, pons, superior colliculus, reticular formation, and periaqueductal gray matter. Each of these structures also showed dose-dependent decreases in r-CMRgl, ranging from 12 (periaqueductal gray) to 43% (pons) with S40 and 28 (hypothalamus) to 48% (pons) with S160. The inferior colliculus and septal nucleus showed no change in r-CMRgl with either dose of sufentanil. White matter structures, the corpus collosum, internal capsule, and cerebellar white matter showed dose-dependent decreases in r-CMRgl of 16 (cerebellar white) to 39% (internal capsule) with S40 and 41 (cerebellar white) to 45% (internal capsule) with S160.

In two of the six animals receiving S40, there were areas of markedly increased density within the amygdala (fig. 1), but because of the focal nature of these areas and their presence in only two of six rats, densitometric readings of the total amygdala resulted in no significant increase in r-CMRgl (fig. 2). Marked increases in density also were noted in focal areas of both the amygdala (seven of eight rats) and hippocampus (four of eight rats) with S160 (fig. 1). In contrast, no areas of increased density were seen in nitrous oxide controls. The foci of this markedly increased density encompassed discrete areas within each structure, though anatomically different areas were activated in each animal. This resulted in no significant increase in the calculated r-CMRgl for the structure as a whole (fig. 2). However, these focal areas of increased density resulted in a marked increase in the variability of the multiple densitometric measurements within the amygdala and hippocampus in each animal; this variability in the amygdala of S160 animals was significantly greater than for controls ($P < 0.01$). Selective measurement of the areas of peak density revealed that r-CMRgl increased 123% above control in the hippocampus and 230% above control in the amygdala (fig. 2, S160A).

EEG recordings were evaluated continuously from the beginning of the sufentanil administration until the injection of ^{14}C-DG, during, and then every 5 min after the injection of the ^{14}C-DG. Figure 3 shows representative EEGs from control, S40, and S160 animals. EEGs for S40 animals generally showed spikes and slow waves in all animals. However, EEGs suggestive of seizure activity as indicated by multiple large amplitude waves of 200–300 μV were noted in two of the S40 animals. EEGs suggestive of seizure activity also were noted in seven of eight rats receiving S160. This activity was of similar amplitude to that of EEGs compatible with seizure activity in the S40 animals and ranged from single episodes that lasted approximately 10 s to multiple episodes of 25 s duration.

There was a distinct association between EEGs suggestive of seizure activity and increased ^{14}C-DG in limbic structures. Ten animals demonstrated focal increases in limbic structure r-CMRgl, and EEG evidence of seizure activity was noted in nine of the 10 animals. However, the relationship between the occurrence of the seizures and the CMRgl measurement is complex. In the S40 animals, both animals that seized showed increased CMRgl in the amygdala. However, in one animal the seizure occurred just prior to the onset of the CMRgl evaluation, while in the other, seizures

occurred both before and after the injection of ^{14}C-DG. In the S160 animals, six of the seven animals with seizures on EEG showed focal increases in amygdaloid r-CMRgl. The other animal with seizure activity showed increased hippocampal r-CMRgl. In addition, one animal had no EEG evidence of seizures but did show increased amygdaloid r-CMRgl. However, in only one of the seven S160 animals with increased limbic r-CMRgl and seizures did the seizures occur during the r-CMRgl measurement. In the other animals, EEG evidence of seizures occurred prior to the ^{14}C-DG injection.

Discussion

The results of this study showed a dose-dependent decrease in r-CMRgl produced by both doses of sufentanil in all cortical structures examined. Such a cortical effect is consistent with our findings that large doses of sufentanil caused a decrease in both cortical cerebral blood flow (CBF) and oxygen consumption ($CMRO_2$) compared with nitrous oxide controls using the modified Kety-Schmidt washout technique.[2] Similar findings of decreased cortical CBF and $CMRO_2$ have been noted with fentanyl.[4] Our values for r-CMRgl in control animals were generally less than those reported by Sokoloff *et al.*[3] for awake animals restrained by plaster casts; however, investigators who have used paralyzed rats mechanically ventilated with 60–70% nitrous oxide report values comparable to those cited here.[5,6] In addition, measurements of r-CMRgl by Ingvar and Siesjö[7] in awake, caged rats ranged from 17 to 49% less than the control values reportedby Sokoloff but were similar to our current values.

With the exception of the amygdala and hippocampus, all subcortical structures generally manifested a decrease in r-CMRgl with increasing doses of sufentanil, a finding that is consistent with similar work using fentanyl.[5] Though focal areas of the amygdala and hippocampus showed large increases in glucose utilization, these were not reflected in measurements of total r-CMRgl in the respective structures. Evidence of focal increases in glucose utilization in the rat hippocampus following intravenous lidocaine have been noted previously by Ingvar and Shapiro[6]; however, these focal increases were not as pronounced as those noted in our studies.

The focal increase in limbic system glucose utilization following sufentanil administration was observed in 10 animals; nine of these animals manifested EEG seizure activity, despite generalized cortical depression of glucose utilization. All other animals that the received narcotic at either dose showed continuous spiking activity on EEG but no evidence of frank seizures. Cortical depression of glucose utilization also was noted in these animals. In the nine animals that did show EEG evidence of seizures, there was no difference in the decreases in cortical r-CMRgl between the seven animals that showed

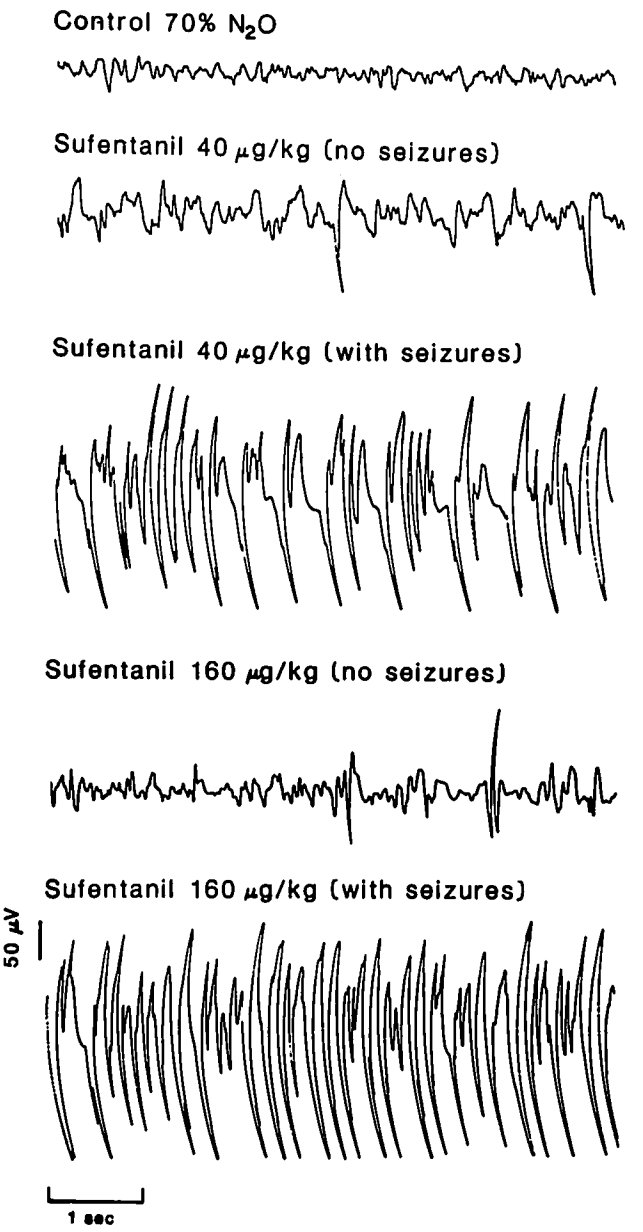

FIG. 3. Representative parieto-occipital EEG tracings from rats ventilated with nitrous oxide–oxygen or given various doses of sufentanil. Two of six animals receiving S40 experienced seizures, while seven of eight animals that received S160 had seizures.

seizures before ^{14}C-DG infusion and the two animals that showed seizures both before and during the r-CMRgl determination. In addition, there was no difference between these two groups of animals with respect to the increases in r-CMRgl noted in limbic structures. These findings suggest that the EEG recorded at the cranium may reflect subcortical activity in some situations. That Keykhah *et al.*[2] found epileptoid activity at high doses of sufentanil, despite decreases in cerebral blood flow and $CMRO_2$, using a method that measures primarily cortical activity, is further evidence to support this hypothesis. Since activation of the EEG by cortical

neurons without any increase in cortical r-CMRgl is unlikely, the seizure patterns noted are most probably the result of amygdala activation in the presence of a depressed cortex. Simultaneous measurements of the EEG from scalp and cortical or subcortical electrodes may be useful in defining this phenomenon further, since significant differences between the electrical activity recorded from the scalp and that recorded from the cortex or subcortical areas exist.[8]

The amygdala and hippocampus have been noted to be important seizure foci for local anesthetics.[9,10] Electrophysiologic and metabolic changes consistent with seizure activity in limbic structures also have been observed with ketamine and enflurane anesthesia.[11,12] However, the reasons for the limbic system to have increased susceptibility to seizures have not been defined clearly. Various hypotheses have been advanced, based on work done with local anesthetics, which suggested selective perfusion, uptake, or distribution to these structures as possible mechanisms.[9] Whether this limbic system activation is a nonspecific effect of sufentanil *versus* a selective interaction with opiate receptors in limbic structuresis not clear. Studies of endorphin- and enkephalin-induced neuronal activation, as measured by subcortical microelectrodes, showed that limbic system neuronal structures were most susceptible to excitation, while most neurons in cortical and thalamic structures were inhibited.[13] These findings may support the concept of selective action of opiates at specific receptors rather than a nonspecific action.

Limbic structures have been demonstrated to be major sites of opiate analgesic action.[14] However, animal studies have suggested that enkephalin-induced analgesia and enkephalin-induced seizures are mediated by anatomically and pharmacologically different receptors.[14] The possibility of a relationship between selective activation of seizure-producing receptors by sufentanil and the increased glucose utilization in limbic structures that may result from seizure activity must be considered. The increase in EEG activity and hippocampal involvement with larger doses of sufentanil may be associated with greater intensity of electrical stimulation and subsequently more widespread propagation of EEG changes.

With respect to the use of the deoxyglucose method for this study, for satisfactory quantitation of r-CMRgl, the metabolic rate for glucose utilization of the brain must be in a steady state from the time of deoxyglucose injection to the time of the animal's death. The occurrence of seizures precludes the assumption of a steady state, thus the brain's metabolic requirement for glucose may not be reflected entirely by ^{14}C-DG uptake. However, relative changes in r-CMRgl from normal may be determined, keeping in mind that such measurements may not reflect precise cerebral metabolic rates for glucose, but rather a range of metabolic activity over time.[15] Note, however, that the rate of deoxyglucose uptake is greatest just after the injection, so that the final measurements of glucose uptake primarily reflect the initial portion of the experimental period.

We conclude that high doses of sufentanil cause a selective increase in glucose metabolic rate in subcortical limbic nuclei in the rat, particularly the amygdala. This increase in r-CMRgl in animals that manifest EEG patterns of seizure activity may reflect subcortical activation in the presence of cortical metabolic depression.

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