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ORIGINAL ARTICLE

Effect of fentanyl on ischemic depolarization and ischemic neuronal damage of hippocampal CA1 in the gerbil

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Abstract
Purpose Temporary brain ischemia occurring during surgery under general anesthesia may induce the death of neuronal cells and cause severe neurological deficits. On the other hand, it is not clear whether μ-opioid receptor agonists promote ischemic brain injury. It is known that duration of ischemic depolarization affects the degree of neuronal damage. However, the effects of fentanyl during brain ischemia on ischemic depolarization have not been investigated. Therefore, in the current study, the effects of fentanyl on ischemic neuronal damage and ischemic depolarization were quantitatively evaluated.
Methods Forty-two male gerbils were randomly assigned to a saline-administered group (control group, $n = 21$) and a fentanyl-administered group (fentanyl group, $n = 21$). Fentanyl at 50 μg/kg was first administered over a 10-min period and then 50 μg/kg/h was administered continuously for the fentanyl group. Forebrain ischemia was initiated by occlusion of bilateral common carotid arteries and sustained for 3, 5, or 7 min ($n = 7$ in each group). Direct-current potentials were measured in bilateral CA1 regions, in which histological evaluation was performed 5 days later.
Results There were no significant differences in onset time, duration of ischemic depolarization, and percentage of neuronal damage between the two groups with any ischemic duration. In the relationships between ischemic time and neuronal damage and those between duration of ischemic depolarization and neuronal damage, there was no significant difference in the percentage of neuronal damage between the two groups.
Conclusion Fentanyl at a clinically relevant dose does not affect ischemic depolarization and ischemic neuronal damage.

Keywords Fentanyl · Brain ischemia · Neuronal damage · Ischemic depolarization · μ-Opioid receptor

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Introduction

During general anesthesia, it is not rare for anesthesiologists to experience various medical situations that can lead to temporary brain ischemia. Temporary brain ischemia, such as that induced by temporary clipping during the operation for a brain aneurysm, may cause neuronal damage in the brain.

The μ-opioid receptor agonist fentanyl, which was released in 1965 in Europe, has been used with inhalational and intravenous anesthetic agents for analgesia during surgery under general anesthesia. Although several studies have been conducted, a definite conclusion regarding the effect of fentanyl on ischemic neuronal damage has not been obtained. Morimoto et al. [1], Soonthon-Brant et al. [2], and Kofke et al. [3] observed a beneficial effect, no effect, and an adverse effect of fentanyl on ischemic neuronal damage, respectively.

It is known that onset time of ischemic depolarization and duration of ischemic depolarization affect the degree of neuronal damage [4, 5]. To the best of our knowledge,

however, the effects of fentanyl on ischemic depolarization have not been evaluated.

The objective of the present study was to quantitatively evaluate the effect of fentanyl on ischemic brain neuronal damage by initiating different durations of ischemia (3, 5, and 7 min) in gerbils. The correlation between ischemic duration and degree of damage of hippocampal CA1 pyramidal neurons was depicted using logistic regression curves (probit curves), and then the ischemic duration that would induce 50% of neuronal damage (P50) was obtained. In addition, forebrain ischemia was induced in gerbils for durations of 3, 5, and 7 min, whereupon ischemic depolarization in the hippocampal CA1 region was observed. After that, the effects of fentanyl on ischemic depolarization were evaluated by extracellular recording in the CA1 region.

Materials and methods

Animals

Forty-two male Mongolian gerbils (Charles River Japan, Yokohama, Japan), weighing 63.1 ± 2.0 g, were used. The animals were on 6-h food deprivation before the experiments. All experiments were performed in accordance with the National Institutes of Health Animal Care Guidelines and were approved by the Animal Research Control Committee of Okayama University Medical School.

Direct current potential and histological outcome

All animals were anesthetized before surgery with halothane (1–2%) in 30% oxygen and 70% nitrogen under spontaneous respiration. Polyethylene catheters (PE-10) were inserted into the right femoral artery for continuous monitoring of mean arterial blood pressure (mABP) and blood sampling and into the right femoral vein for administration of saline or fentanyl. Arterial blood samples were obtained before the administration of saline or fentanyl and brain ischemia and immediately after reperfusion and end of fentanyl administration.

Arterial blood gas, glucose, and hemoglobin were then analyzed (i-STAT 300F; i-STAT Corporation, East Windsor, NJ, USA). The bilateral common carotid arteries were exposed, and a ring (silicon tube, 0.3 mm in diameter) was loosely placed around each artery. After securing the head in a stereotaxic apparatus (Narishige, Tokyo, Japan), a monopolar lead electroencephalogram (EEG; S1516, Nihon Koden, Tokyo, Japan) was monitored using needle electrodes placed subcutaneously in the right frontal region. A reference electrode was placed in the left ear and a laser Doppler flow probe (ALF2100; Advance, Tokyo, Japan) was placed on the right parietal cortex to continuously monitor regional cerebral blood flow (CBF). Rate of change in CBF was utilized because it has been reported that a laser-Doppler flow meter provides accurate information only on changes in CBF [6].

Two borosilicate glass electrodes (tip diameter, <5 μm) were then placed in the CA1 regions of the bilateral hippocampus in accordance with the brain atlas (2 mm caudal to the bregma, 1.5 mm bilateral from the sagittal line, and 1 mm below the cortical surface) [7] for measurement of direct current (DC) potentials (MEZ-8300; Nihonkoden, Tokyo, Japan). After surgery, the halothane concentration was reduced to 1%.

The animals were randomly assigned to either a saline-administered group (control group, $n = 21$) or a fentanyl-administered group (fentanyl group, $n = 21$). In the fentanyl group, 50 μg/kg fentanyl (14.3 μg/ml, in 0.9% saline) was first administered over a 10-min period (1.2 ml/h), and then fentanyl was administered at 50 μg/kg/h (0.2 ml/h) for 90 min. The plasma concentration of fentanyl was estimated using BeConSim developed by one of the authors (Kenichi Masui) [8] with pharmacokinetic parameters for a three-compartment model in rodents (30–40 g mice) reported by Kalvass et al. [9]. Because the brain equilibration half-life of fentanyl in mice is 5 min [9], forebrain ischemia was initiated 20 min after the start of continuous infusion. In the control group, an equivalent amount of saline was administered. In both groups, 1% halothane administration was continued until closure of the incision.

Forebrain ischemia was initiated by occlusion of the bilateral common carotid arteries for a predetermined duration (3, 5, or 7 min; $n = 7$ for each duration in each group). Initiation of ischemia and initiation of reperfusion were confirmed by a sudden decrease and rapid increase in CBF, respectively. Changes in DC potentials, CBF, and EEG were recorded with the use of an analog/digital system (PowerLab; ADInstruments, Sydney, NSW, Australia). Changes in DC potentials were assessed by measuring onset time (from the initiation of ischemia to sudden negative shift of DC potentials) and duration of ischemic depolarization (from sudden negative shift of DC potentials to 80% recovery from maximal DC deflection).

Brain surface temperature was maintained at 37.0° ± 0.5°C with a gentle flow (1.6–2.0 ml/min) of warmed saline (38.0° ± 0.5°C) into a polyethylene cylinder (5 mm in height, 13 mm in inner diameter) that had been placed on the skull surface. Rectal temperature was maintained at 37.0° ± 0.5°C using a heated-water blanket and infrared lamp. These temperatures were continuously measured and controlled from 30 min before the initiation of ischemia until 90 min after initiation of reperfusion to avoid the influence of temperature on ischemia, because it had been previously reported that any chance of neuronal death

induced by postischemic hyperthermia could be eliminated by maintaining normothermia for a duration of 85 min after initiation of reperfusion [10].

After a 5-day survival period, all animals were anesthetized with 4% halothane in oxygen and perfused with heparinized physiological saline (20 U/ml) and 4% formaldehyde with buffer solution (pH 7.4). The areas in which DC potential had been recorded were marked by using a 27-gauge needle with blue ink.

After brain removal and paraffin embedding, tissue including the bilateral hippocampal CA1 regions (area marked with blue ink) was sectioned coronally (5 μm in thickness). The sections were stained with hematoxylin and eosin. The areas in which DC potential had been recorded were enlarged to 400×, and the numbers of both damaged and intact pyramidal neurons in bilateral hippocampal CA1 regions were counted. In the current study, pyramidal neurons showing aggregated chromatin in the nucleus, shrinkage, or eosinophilic staining in the cytoplasm were considered to be injured. The number of injured pyramidal neurons in the bilateral hippocampal CA1 regions was counted by an observer who was blinded to this study. The percentages of neuronal damage in the two groups were calculated as damaged neurons/total neurons × 100 in the visual field.

Statistical analysis

Values obtained from the experiments are expressed as means ± SD.

The changes in EEG were evaluated through a power spectrum analysis for every 1 Hz of the frequency band ranging from 0 to 30 Hz.

Physiological parameters were analyzed by repeated-measures analysis of variance (ANOVA) followed by Fisher's protected least significant difference for multiple comparisons. Parameters for ischemic depolarization and neuronal damage were analyzed by two-factor factorial ANOVA (groups vs. ischemic time). In all statistical tests, a level of $P < 0.05$ was considered to be significant.

Dose–reaction curves for evaluating acute drug toxicity in toxicology are usually expressed by the use of probit curves. In the current study, the relationships of neuronal damage with ischemic duration and duration of ischemic depolarization were determined by logistic regression curves (probit curves) as dose–reaction curves. Ischemic duration or duration of ischemic depolarization was represented on the *x*-axis and neuronal damage was represented on the *y*-axis. The *y*-axis was converted to probit transformation, and regression lines in the two groups were drawn. At the same time, 95% confidence intervals were also drawn. Finally, the *y*-axis was returned to percent change. These regression curves were drawn by using data-analysis software (Microcal Origin 8; Microcal Software, Northampton, MA, USA). A probit curve, which expresses the probability of occurrence, is used to search for the median lethal dose in toxicology. Therefore, in this study, ischemic durations and durations of ischemic depolarization necessary for causing 50% neuronal damage in both groups were determined from logistic regression curves.

Results

As shown in Fig. 1, the predicted plasma concentrations of fentanyl using BeConSim with mouse parameters were 8.1 ng/ml at the initiation of ischemia and 6.6 ng/ml at the end of fentanyl administration.

Table 1 shows physiological variables obtained before administration of fentanyl (baseline), immediately before initiation of brain ischemia, during ischemia, after reperfusion (mABP, CBF; 5 min after reperfusion) and after end of fentanyl administration. Although respiratory rate was significantly decreased following administration of fentanyl before ischemia (fentanyl vs. control, $P = 0.0003$; baseline vs. before ischemia, $P < 0.0001$) and after reperfusion (fentanyl vs. control, $P < 0.0001$; baseline vs. after reperfusion, $P < 0.0001$) and after administration of fentanyl (fentanyl vs. control, $P < 0.0001$; baseline vs. after administration, $P < 0.0001$; before ischemia vs. after administration, $P = 0.03$; after reperfusion vs. after administration, $P = 0.01$), there were no statistically significant differences in other parameters between the control group and fentanyl group.

Figure 2 shows results of power spectrum analysis of EEG measured immediately before initiation of forebrain ischemia. In the fentanyl group, the amplitude in 0–1 Hz ($P = 0.0065$) and 1–2 Hz ($P = 0.0001$) was significantly

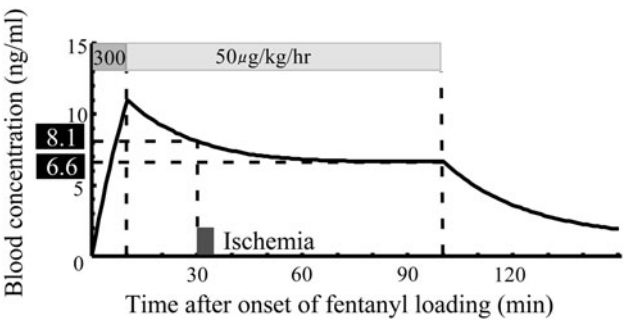

Fig. 1 Changes in estimated fentanyl plasma concentration. Rates of fentanyl administration were 300 μg/kg/h for the first 10 min and 50 μg/kg/h for the next 90 min. Plasma concentration of fentanyl was estimated using BeConSim developed by one of the authors (Kenichi Masui) with pharmacokinetic parameters for a three-compartment mouse model. Predicted plasma concentrations of fentanyl were estimated to be 8.1 ng/ml upon initiation of ischemia and 6.6 ng/ml upon completion of fentanyl administration

Table 1 Physiological variables before administration (baseline), before ischemia, during ischemia, and after reperfusion and end of administration

	pH	$PaCO_2$ (mmHg)	PaO_2 (mmHg)	Respiratory rate (times/min)	Hb (g/dl)	Glucose (mg/dl)	mABP (mmHg)	Percent change in CBF
Control								
Baseline	7.38 ± 0.03	44.9 ± 3.53	98.4 ± 9.26	118 ± 12.1	16.7 ± 0.22	123 ± 14.6	83.5 ± 8.46	100
Before ischemia	7.36 ± 0.02	44.1 ± 1.45	100 ± 6.08	117 ± 11.8	16.6 ± 0.26	121 ± 10.8	80.8 ± 8.02	96.3 ± 11.4
During ischemia							107 ± 9.38$^{\#,\#\#,\#\#\#,\#\#\#\#}$	8.90 ± 5.82$^{\#\#,\#\#\#,\#\#\#\#}$
After reperfusion[a]	7.32 ± 0.03	44.1 ± 4.19	102 ± 12.7		16.4 ± 0.26	125 ± 17.0	80.2 ± 8.31	112 ± 32.2$^{\#\#}$
After administration	7.31 ± 0.03	42.3 ± 4.17	102 ± 14.2	116 ± 14.4	16.1 ± 0.63	125 ± 16.7	81.6 ± 6.04	49.5 ± 19.2$^{\#\#,\#\#\#}$
Fentanyl								
Baseline	7.36 ± 0.02	45.0 ± 4.18	104 ± 9.71	120 ± 12.3	16.6 ± 0.50	127 ± 12.1	87.3 ± 6.06	100
Before ischemia	7.34 ± 0.02	45.3 ± 3.00	100 ± 6.98	101 ± 11.1$^{*,\#}$	16.6 ± 0.7	125 ± 16.8	83.7 ± 7.23	99.7 ± 7.90
During ischemia							109 ± 7.62$^{\#,\#\#,\#\#\#,\#\#\#\#}$	7.94 ± 3.92$^{\#\#,\#\#\#,\#\#\#\#}$
After reperfusion[a]	7.31 ± 0.03	44.6 ± 4.03	99.0 ± 9.63		16.4 ± 0.70	130 ± 15.7	76.0 ± 5.09$^{\#,\#\#}$	108 ± 29.9$^{\#\#}$
After administration	7.30 ± 0.03	43.2 ± 2.38	101 ± 7.66	93.5 ± 12.1$^{*,\#,\#\#}$	16.4 ± 0.64	120 ± 21.4	81.2 ± 6.96$^{\#,\#\#\#}$	50.6 ± 12.4$^{\#\#,\#\#\#}$

Values are presented as mean ± SD

Hb, hemoglobin; mABP, mean arterial blood pressure; percent change in CBF, % change in cerebral blood flow compared with that before administration of saline or fentanyl (before infusion = 100)

[a] mABP, CBF: 5 min after reperfusion

* $P < 0.05$ compared with the control group

# $P < 0.05$ compared with that before administration (baseline in each group)

$P < 0.05$ compared with that before ischemia in each group

$P < 0.05$ compared with that after reperfusion in each group

$P < 0.05$ compared with that after administration in each group

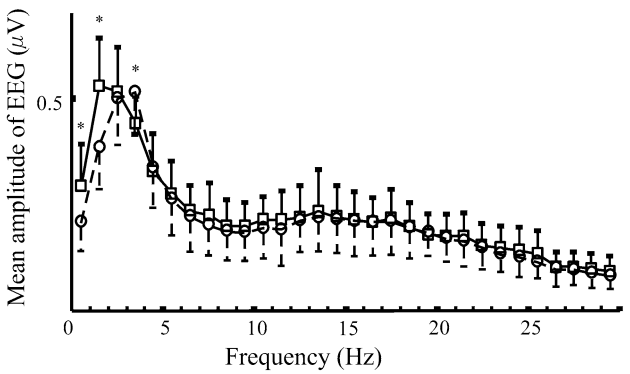

Fig. 2 Power spectrum analysis of EEG immediately before initiation of brain ischemia. Data are expressed as mean ± SD. Mean amplitude for every 1 Hz of the frequency band ranging from 0 to 30 Hz was calculated. The mean amplitude of EEG in the fentanyl group (*squares*) showed significant increase in δ wave (0–2 Hz) and decrease in 3–4 Hz compared with those in the control group (*circles*) (*$P < 0.05$)

increased and the amplitude in 3–4 Hz ($P = 0.004$) was decreased compared to those in the control group.

The variables of DC potential in each experimental group are summarized in Table 2. Significant differences in onset time between the two groups were not observed with any ischemic duration ($P = 0.98$, two-factor factorial ANOVA), and there were no significant differences in duration of ischemic depolarization between the two groups with any ischemic duration ($P = 0.93$, two-factor factorial ANOVA).

Percentages of damaged neurons in the hippocampal CA1 region are shown in Fig. 3. Significant differences in percentage of damaged neurons between the two groups were not observed with any ischemic duration ($P = 0.95$, two-factor factorial ANOVA).

The logistic regression curves with 95% confidence intervals (CIs) seen in Fig. 4 show significant correlations between ischemic time and percentages of damaged neurons

Table 2 Variables of DC potential (onset time and duration of ischemic depolarization) in the CA1 region in each experimental group

Values are expressed as mean ± SD

Onset time: from the initiation of ischemia to sudden negative shift of DC potentials

Duration of ischemic depolarization: from sudden negative shift of DC potentials to 80% recovery from maximal DC deflection

	Onset time (min)	Duration of ischemic depolarization (min)
3 min of ischemia		
Control	1.79 ± 0.14	2.97 ± 0.39
Fentanyl	1.78 ± 0.15	2.93 ± 0.45
5 min of ischemia		
Control	1.76 ± 0.20	5.89 ± 0.62
Fentanyl	1.76 ± 0.15	5.93 ± 0.51
7 min of ischemia		
Control	1.78 ± 0.08	8.48 ± 0.71
Fentanyl	1.79 ± 0.10	8.45 ± 0.73

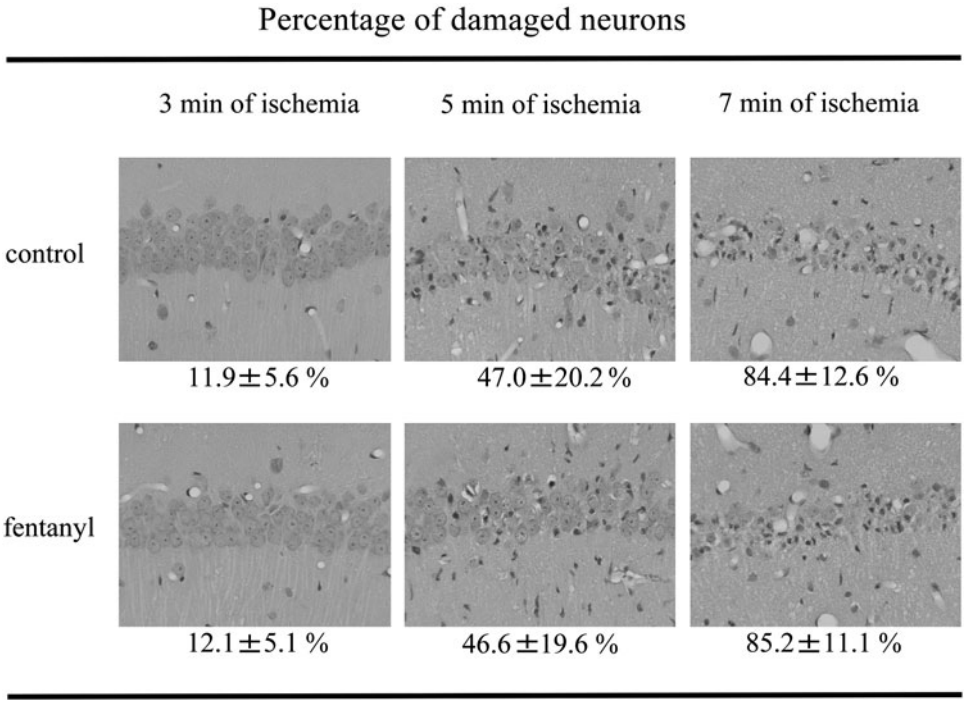

Fig. 3 Percentages of damaged neurons in the hippocampal CA1 region. In both the control group and fentanyl group with 3 min of ischemia, the layers of CA1 pyramidal neurons were well preserved. The cytoplasmic volume of each neuron was maintained, and there were only a few pyramidal neurons showing aggregated chromatin in the nucleus or eosinophilic staining in the cytoplasm. In both groups with 5 min of ischemia, the layers were destroyed and normal neurons and damaged neurons showing aggregated chromatin in the nucleus, shrinkage, or eosinophilic staining in the cytoplasm were mixed. In both groups with 7 min of ischemia, layers of pyramidal neurons were not observed. There were only a few normal neurons without aggregated chromatin in the nucleus, shrinkage, or eosinophilic staining in the cytoplasm. The number of damaged pyramidal neurons and normal pyramidal neurons were counted, and the percentage of neuronal damage in each subgroup was calculated as damaged neurons/total neurons × 100 in the visual field in which DC potential had been recorded. Significant differences in percentage of damaged neurons between the two groups were not observed with any ischemic duration

(control, $r^2 = 0.82$, $P < 0.001$; fentanyl, $r^2 = 0.84$, $P < 0.001$). The percentages of damaged neurons in which 95% confidence intervals overlap from end to end indicate that there is no significant difference between the two groups in ischemic times necessary for causing identical neuronal damage. P_{50} values in the control and fentanyl groups were estimated to be 5.11 min (95% CI, 4.87–5.35 min) and 5.08 min (95% CI, 4.86–5.32 min), respectively.

In Fig. 5, other logistic regression curves with 95% confidence intervals show significant correlations between duration of ischemic depolarization and percentages of damaged neurons (control, $r^2 = 0.75$, $P < 0.001$; fentanyl, $r^2 = 0.79$, $P < 0.001$). The percentages of damaged neurons in which 95% confidence intervals overlap from end to end indicate that there were no significant differences between the two groups in duration of ischemic

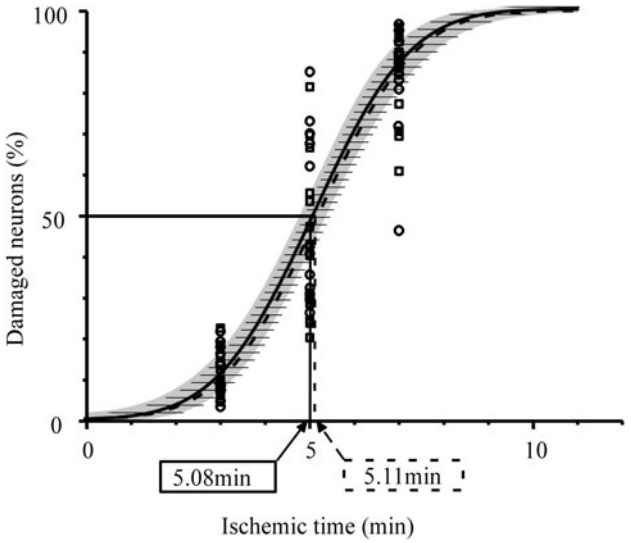

Fig. 4 Relationships between ischemic time and percentages of damaged neurons. *Circles*, percentages of damaged neurons in the control group; squares, those in the fentanyl group. Logistic regression curves (probit curves) (control, *dotted line*; fentanyl, *solid line*) with 95% confidence intervals (control, *parallel lines area*; fentanyl, *gray area*) show close relationships between ischemic time and percentages of damaged neurons (control: $r^2 = 0.82$, $P < 0.001$; fentanyl: $r^2 = 0.84$, $P < 0.001$). The percentages of damaged neurons in which 95% confidence intervals overlap from end to end indicate that there is no significant difference between the groups in the ischemic times necessary to cause identical neuronal damage. P_{50} values in the control and fentanyl groups were calculated to be 5.11 and 5.08 min, respectively

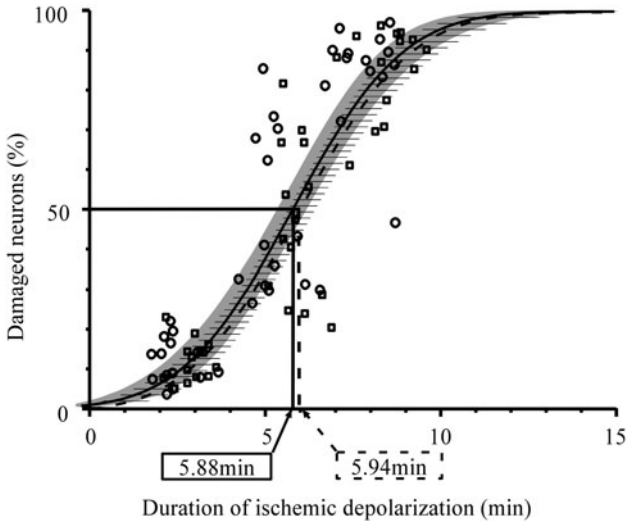

Fig. 5 Relationships between duration of ischemic depolarization and percentages of damaged neurons. Percentages of damaged neurons in the control group are shown by *circles* and those in the fentanyl group are shown by *squares*. Logistic regression curves (probit curves) (control, *dotted line*; fentanyl, *solid line*) with 95% confidence intervals (control, *parallel lines area*; fentanyl, *gray area*) show close relationships between duration of ischemic depolarization and percentages of damaged neurons (control: $r^2 = 0.75$, $P < 0.001$; fentanyl: $r^2 = 0.79$, $P < 0.001$). The duration of ischemic depolarization in which 95% confidence intervals overlap from end to end indicates that there is no significant difference between the two groups in percentages of damaged neurons. The duration of ischemic depolarization necessary to cause 50% neuronal damage was calculated to be 5.94 min in the control group and 5.88 min in the fentanyl group

depolarization necessary for causing identical neuronal damage. The durations of ischemic depolarization necessary to cause 50% neuronal damage were estimated to be 5.94 min (95% CI, 5.52–6.38 min) in the control group and 5.88 min (95% CI, 5.51–6.27 min) in the fentanyl group.

Discussion

As shown in Fig. 4, neuronal damage in the CA1 region increased with prolongation of ischemia in the control group. The logistic regression curve (probit curve) demonstrated an extremely high correlation ($r^2 = 0.82$, $P < 0.0001$), indicating that ischemic time is a crucial factor in neuronal damage. Duration of ischemia causing damage in 50% of CA1 neurons (P_{50}) was calculated to be 5.11 min. The logistic regression curve in the fentanyl group (probit curve) also demonstrated an extremely high correlation ($r^2 = 0.84$, $P < 0.0001$) and almost overlapped that in the control group. P_{50} in the fentanyl group was calculated to be 5.08 min, which is very close to the value in the control group. These findings indicate that the fentanyl dose used in this study does not affect neuronal damage, regardless of the duration of ischemia.

When neurons lose membrane potential because of ischemia, the intracellular calcium concentration increases by 300 fold, resulting in secondary neuronal damage [11]. Accumulation of intracellular calcium inhibits mitochondrial adenosine diphosphate phosphorylation [12] and protein synthesis as a result of the disaggregation of ribosomes [13], and further activates many enzymes, including phospholipase, protease, and protein kinase, leading to accumulation of arachidonic acid [14], collapse of cytoskeletal elements [15], and release of neurotransmitters [12]. As already mentioned, because ischemic depolarization triggers a cascade of neuronal damage following the loss of neuronal ion homeostasis, the onset time and the duration of ischemic depolarization have strong correlations with the degree of subsequent neuronal damage [4, 5]. In this study, the onset time and the duration of ischemic depolarization with each ischemic time of 3, 5, and 7 min were almost the same in the control and fentanyl groups (see Table 2). Logistic regression curves also suggested that fentanyl had little effect on neuronal damage for any duration of ischemic depolarization and that fentanyl did not modify the duration of ischemic depolarization that results in 50% neuronal damage (Fig. 5). These findings

suggested that fentanyl had little effect on the loss of ion homeostasis and on the cascade of neuronal damage during membrane depolarization.

As mentioned in the Introduction, results of past studies on the effect of fentanyl on ischemic neuronal damage have not been consistent. Morimoto et al. [1] evaluated damage of hippocampal pyramidal cells in rats 4 days after occlusion of bilateral common carotid arteries with hypotension for 10 min following administration of fentanyl (960 μg/kg/h for 30 min after bolus administration of 400 μg/kg); they observed a beneficial effect of fentanyl on neuronal damage. Soonthon-Brant et al. [2], who evaluated infarct volume in rats 7 days after right middle cerebral artery occlusion with occlusion of bilateral common carotid arteries for 90 min following administration of fentanyl (50 μg/kg/h for 20 min after 50 μg/kg for 10 min), observed no effect of fentanyl on neuronal damage. Kofke et al. [3] evaluated brain damage in rats 18 h after occlusion of bilateral common carotid arteries with hypotension for 12 min following administration of fentanyl (1,920 μg/kg/h for 15 min after bolus administration of 800 μg/kg) and observed a detrimental effect of fentanyl on neuronal damage. In those studies, the doses of fentanyl before initiation of ischemia were 13 fold larger, the same, and 19 fold larger, respectively, than the dose used in our study. Although the present study was designed to elucidate the effect of fentanyl on ischemic neuronal damage by different durations of ischemia instead of different concentrations of fentanyl, the effect of fentanyl on brain ischemia could be affected by its concentration.

Neuronal excitability is reduced by activation of μ-opioid receptors [16]. As μ-opioid receptors are present only on inhibitory interneurons in the hippocampal CA1 region and are not present on CA1 and CA3 pyramidal neurons [17], it is possible that administration of fentanyl increases excitability of CA1 pyramidal neurons by suppression of the excitation of inhibitory interneurons. On the other hand, μ-opioid receptors are abundant in pyramidal neurons in the cerebral cortex [18]. These neurons send excitatory inputs to CA1 pyramidal cells through connected fibers [19–21] and the CA3 pyramidal cell-mediated Shaffer collateral pathway [22]. Therefore, it is also possible that administration of fentanyl decreases excitability of CA1 pyramidal neurons by suppression of the excitation of cortical neurons. In the present study, administration of fentanyl shifted the power spectrum of EEG toward a slower frequency (Fig. 2), implying that activity of the cerebral cortex is suppressed. However, administration of fentanyl had little effect on onset time and duration of ischemic depolarization in the hippocampal CA1 region. It is assumed that this phenomenon is the consequence of competing effects of inhibitory input and excitatory input on CA1 pyramidal neurons. The effect of fentanyl on membrane depolarization and ischemic damage could be determined by the balance between suppression of the inhibitory pathway and suppression of the excitatory pathway. Therefore, the effect of fentanyl on neuronal damage in the CA1 region may differ with changes in its concentration. It has been shown that fentanyl increases the excitability of cortical pyramidal cells at a plasma concentration higher than 120 ng/ml in rats [23]. Therefore, the foregoing studies suggest that a large dose of fentanyl may upset the balance between inhibitory input and excitatory input and have an adverse effect on ischemic neuronal damage.

As shown in Fig. 1, the predicted plasma concentrations of fentanyl in this study were 8.1 ng/ml at the initiation of ischemia and 6.6 ng/ml at the end of fentanyl administration. It is possible that the drug sensitivity in humans differs from that in rodents. As shown in Table 3, the plasma concentrations of fentanyl at half-maximal slowing of the EEG were 6.9–9.8 ng/ml in humans (spectral edge frequency) [24] and 10.1 ng/ml in rodents (power of delta wave) [25]. In addition, the ranges of EC_{50} at which fentanyl suppresses body motion in response to noxious stimuli have been reported to be 2.8–3.9 ng/ml in humans [26, 27] and 2.2–2.8 ng/ml in rodents [9, 28], and the fentanyl EC_{50} values at which fentanyl inhibits increase in pulse rate because of noxious stimuli have been reported to be 52 ng/ml in humans [29] and 45 ng/ml in rodents [28]. These reports indicate that humans and rodents are similar in their fentanyl sensitivity to noxious stimuli and EEG. Plasma concentrations of fentanyl are generally considered to be in the range of 4–10 ng/ml for major surgeries [30]. Given that our estimated plasma concentration of fentanyl was 6.6–8.1 ng/ml in this study, it can be considered that the results of our study reflect the effects of fentanyl on brain ischemia as observed in major surgeries.

There are some limitations in this study. First, in the present study, 1% halothane was administered to animals in both the control group and the fentanyl group because the

Table 3 Comparison of fentanyl sensitivities in rodents and humans

	EC_{50} (ng/ml)	
	Rodent	Human
Analgesia upon receiving noxious stimuli	2.2[a]–2.8	2.8–3.9
Increase in delta-wave amplitude in EEG	10.1	6.9–9.8
Inhibition of cardiovascular responses caused by noxious stimuli	45[a]	52

EC_{50}, plasma concentration resulting in 50% of the maximum drug effect

[a] The value of EC_{50} was calculated from the value of ED_{50} (dose resulting in 50% of the maximum drug effect) by BeConSim (pharmacokinetic analysis software) with parameters shown in a three-compartment mouse model

animals had to be maintained without surgical stress. As it is known that volatile anesthetics have neuroprotective effects [31], halothane may mask the effects of fentanyl on brain ischemia. Second, in this study, the percentage of damaged pyramidal cells in the hippocampal CA1 region was assessed at 5 days after ischemia, because it has been reported that histological change of these cells was completed within 4 days after ischemia [32]. The neurological effect and the long-term effect of fentanyl on brain ischemia were not evaluated in this study.

In summary, the effects of fentanyl administered at a dose to maintain an optimal plasma concentration of balanced anesthesia during major surgery were quantitatively evaluated 5 days after temporary forebrain ischemia in gerbils. The duration of ischemic depolarization with each ischemic time of 3, 5, and 7 min was almost the same in the control and fentanyl groups. The degree of neuronal damage caused by ischemia with durations of 3, 5, and 7 min was hardly affected by fentanyl administration. Logistic regression curves also suggested that fentanyl had little effect on neuronal damage for any duration of ischemia or ischemic depolarization and that fentanyl did not modify the duration of ischemia and ischemic depolarization which results in 50% neuronal damage. These results indicate that ischemic neuronal damage occurring under balanced anesthesia with a clinical dose of fentanyl is hardly affected by fentanyl. Therefore, fentanyl optimally administered during surgery may be safe for temporary brain ischemia such as that induced by temporary clipping during the operation for a brain aneurysm.

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