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Are fentanyl and remifentanil safe opioids for rat brain mitochondrial bioenergetics?

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ABSTRACT

Fentanyl and remifentanil are potent opioid widely used in routine anesthesia procedures. This study evaluates and compares the effects of fentanyl/remifentanil in isolated brain mitochondria bioenergetic status. Fentanyl and remifentanil in clinical concentrations does not interfere with rat brain isolated mitochondria. Do not withstand, fentanyl concentrations >4 μg/mL, induces an impairment of the respiratory chain characterized by a decrease in respiratory control ratio, state 3 and uncoupled respiration. Additionally, membrane potential collapses and ADP/O were reduced. Remifentanil follows the same profile but with effects at higher concentrations (>10 μg/mL). High concentrations of fentanyl and remifentanil interfere with mitochondrial electron chain (complexes III, IV) and on mitochondrial phosphorylation unit (complex V). Mitochondrial permeability transition pore was not induced by both fentanyl and remifentanil in tested concentrations. These data provide the first indication that fentanyl and remifentanil (μg/mL range) alters mitochondrial metabolism. Fentanyl showed a stronger inhibitory effect on mitochondrial bioenergetics.

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1. Introduction

Synthetic opioids belong to the most potent analgesics and are widely used in anesthesia and analgesia procedures. The opioid fentanyl, a semisynthetic agonist with strong affinity for the mu opioid receptor site, has been used since the 1960s as an analgesic (Pasero, 2005). More recently a fentanyl-derived compound remifentanil (Fig. 1) was clinically available, differing from the other opioids agonists, because it is rapidly hydrolyzed and metabolized by nonspecific red-cell and tissue esterases (Feldman et al., 1991; Bürkle et al., 1996; Duthe, 1998). This implies that remifentanil have a shorter half-life, predictable pharmacokinetics and a close concentration–effect relationship (Glass et al., 1993; Duthe, 1998), properties which allows a safer use by continuous infusion. Fentanyl and renifentanyl have shown a high degree of side effects in clinical practice (Duthe, 1998; Pasero, 2005). However, pharmacokinetic data show that remifentanil has more advantage in clinical practice when compared with other opioids including fentanyl and alfentanyl (Michelsen and Hug, 1996; Duthe, 1998).

Mitochondrial bioenergetics has been proposed to be involved in cellular effects of anesthetics and analgesics (Muravchick and Levy, 2006; Nouette-Gaulain et al., 2007). We have shown unusual excitatory effects of fentanyl upon rat electroencephalogram (EEG) during anesthesia, theses responses being inconsistent with the classical concept of EEG depression as an indicator of deepening anesthesia may be related with an increase in oxygen consumption (Antunes et al., 2003).

In vitro effects of anesthetics and analgesics on the energetic metabolism, like impairment of oxygen consumption, transmembrane electric potential, oxidative phosphorylation and decrease on ATPase activity, have been described on mitochondria isolated from brain, kidney, liver and heart (Branca et al, 1995; Stevanato et al., 2002; Devin et al. 2006). However, the extent to which they alter and the mechanisms implicated is not yet understood (Muravchick and Levy, 2006). The biochemical mechanisms underlying the energetic metabolism response to fentanyl, remifetanil and other opioids needs further investigation (Bürkle et al., 1996; Muravchick and Levy, 2006).

The purpose of our study was to investigate and compare the effects of fentanyl and remifentanil on the rat brain isolated mitochondria bioenergetics.

2. Materials and methods

2.1. Chemicals

Fentanyl (B. Braun, Queluz de Baixo, Portugal). Remifentanil (Ultiva®, GlaxoSmithKline-Produtos Farmacêuticos Lda., Algés, Portugal). Protease type VIII (Subtilisin Carlsberg), from Sigma (St. Louis, MO). Digitonin from Calbiochem (Merck Biosciences Ltd.,

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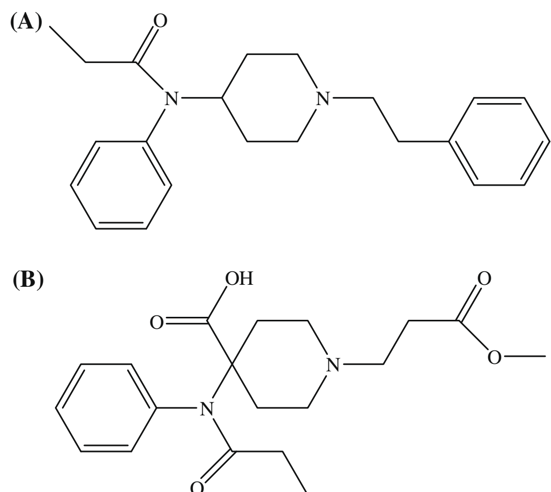

Fig. 1. Chemical structures of opioids, fentanyl (A) and remifentanil (B).

Nottingham). All the other chemicals were of the highest grade of purity commercially available.

2.2. Animals

Male Wistar rats (Harlan Ibérica, Barcelona, Spain) weighing between 250 and 300 g were used. The animals were kept in a room with controlled temperature (21 °C) and humidity (55%). Lights were on a 12/12 h cycle, with lights off at 17:00 h. Water and rodent pellets (4RF25-GLP Mucedola, SRL) were provided ad libitum. The animals were housed in Makrolon type IV cages, provided with corn-cob bedding material (Probiológica, Lisbon, Portugal), tissue nesting material and a cardboard tube. All procedures were carried out under personal and project licenses approved by the national regulatory office (Direcção Geral de Veterinária – DGV). Animals were sacrificed by cervical displacement followed by decapitation.

2.3. Isolation of brain mitochondria

Rat brain mitochondria were isolated by the method of Rosenthal et al. (1987), with slight modifications, using 0.02% digitonin to free mitochondria from the synaptosomal fraction. After rat sacrifice, the whole brain**,** minus the cerebellum**,** was rapidly removed, washed, minced and homogenized with a motor-driven Teflon Potter-Elvjhem homogenizer in the presence of 10 mL ice-cold (4 °C) isolation medium (225 mM mannitol, 75 mM sucrose, 5 mM Hepes, 1 mM EGTA, 1 mg/mL bovine serum albumin, pH 7.4) containing 5 mg of the bacterial protease. Single brain homogenates were brought to 30 mL and then centrifuged at 2000 × *g* for 3 min. The pellet, including the fluffy synaptosomal layer, was resuspended in 10 mL of the isolation medium containing 0.02% digitonin and centrifuged at 12,000 × *g* for 8 min. The brown mitochondrial pellet without the synaptosomal layer was then resuspended in 10 mL of medium and recentrifuged at 12,000 × *g* for 10 min. The mitochondrial pellet was resuspended in 300 μL of resupension medium (225 mM mannitol, 75 mM sucrose, 5 mM Hepes, pH 7.4). Mitochondrial protein was determined by the biuret method using bovine serum albumin as standard (Gornall et al., 1949).

2.4. Mitochondrial respiration

Oxygen consumption of isolated mitochondria was monitored polarographically with a Clark-type oxygen electrode, using Hansatech Oxygraph Measurement System (Hansatech, Norfolk, UK), at 25 °C. Fentanyl and remifentanil freshly prepared and pH adjusted (pH 7.2) was incubated for 10 min at 25 °C in 1 mL of reaction medium (100 mM sucrose, 100 mM KCl, 2 mM KH_2PO_4, 5 mM Hepes, 10 μM EGTA, pH 7.4), supplemented previously with mitochondria (1 mg) and 2 μM rotenone. State 4 respiration was initiated with 5 mM succinate and state 3 was established after addition of 100 μM ADP. Respiration rates were calculated assuming an oxygen concentration of 240 nmol O_2/mL in the experimental medium at 25 °C, when the medium was air-equilibrated at 760 torr (Reynafarge et al., 1985). Respiratory rates, respiratory control ratio (RCR = state 3/state 4) and ADP/O (number of ADP molecules added to the medium per oxygen atom consumed during phosphorylation) were calculated according to Chance and Williams (1956). Appropriate dilutions were made with deionized water just prior to addition to the assay medium. The maximum deionized water added in there action medium never exceeded 2%.

2.5. Mitochondrial membrane potential ($\Delta\Psi_m$) measurements

Mitochondrial membrane potential ($\Delta\Psi_m$) was estimated with a ion-selective electrode of TPP^+ (tetraphenylphosphonium) prepared according to Kamo et al. (1979) to measure the transmembrane distribution of TPP^+ as previously established (Moreno and Madeira, 1991) using an Ag/AgCl-saturated electrode as reference. Reactions were carried out in an open vessel with magnetic stirring at 25 °C and performed with 1 mg mitochondrial protein in 1 mL of reaction medium (100 mM sucrose, 100 mM KCl, 2 mM KH_2PO_4, 5 mM Hepes, 10 μM EGTA, pH 7.4), supplemented with 2 μM rotenone and 3 μM TPP^+. Fentanyl and remifentanil were added to the reaction medium after protein addition and incubated 10 min before starting the reactions. The experiments were started by adding 5 mM succinate and, after a steady state distribution of TPP^+ (around 2 min of recording), 100 nmol/mg protein ADP was added. $\Delta\Psi_m$ was expressed in mV and was estimated from the decrease of TPP^+ concentration in the reaction medium, as described elsewhere (Santos et al., 2002).

2.6. Enzymatic activities

ATP sinthase activity was measured by the ADP phosphorilated. Mitochondria (0.5 mg) was pre-incubated with fentanil or remifentanil for 10 min in a respiratory medium, then succinte (5 mM) was added, followed by ADP (100 μM). The reaction was stopped 3 min later by the addition of 100 μL of perchloric acid (0.3 M). Each mitochondrial suspension was rapidly centrifuged at 14,000 rpm for 6 min and the supernatants were neutralized with 10 M KOH in 5 M Tris and centrifuged at 14,000 rpm for 5 min. The resulting supernatants were assayed for ATP by separation in a reverse-phase high performance liquid chromatography. The chromatography apparatus was a Beckman-System Gold, consisting of a 126 model Binary Pump and a photodiode array detector controlled by a computer. The detection wavelength was 254 nm, and the column was a Lichrospher 100 RP-18 (5 mm) from Merck (Darmstadt, Germany). An isocratic elution with 100 mM phosphate buffer (KH_2PO_4), pH 6.5 and methanol 1% was performed with a flow rate of 1 mL/min. The time required for each analysis was 15 min.

Succinate dehydrogenase activity was measured polarographically (Singer, 1974) at 25 °C in 1 mL of standard reaction medium supplemented with 5 mM succinate, 2 μM rotenone, 0.1 μg antimycin A, 1 mM KCN, 0.03% Triton X-100 at 25 °C, and 0.5 mg protein of broken mitochondria, with two cycles of freezing and thawing. The reaction was initiated by the addition of 1 mM PMS, used as an artificial electron acceptor.

Succinate cytochrome *c* reductase activity was measured spectrophotometrically (Tisdale, 1967) at 25 °C, following the reduction

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