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Rapid in vitro elimination of anesthetic doses of thiopental in the isolated guinea pig brain

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Abstract

Electrophysiological and metabolic activities in brain tissue preparations maintained in vitro may be influenced by the persistent effect of anesthetic drugs utilized during tissue dissection. In order to clarify this issue, we studied elimination kinetics of the barbiturate thiopental from the brain parenchyma in the isolated guinea pig brain maintained in vitro, arterially perfused with a protein-free saline solution [M. de Curtis, G. Biella, C. Buccellati, G. Folco, Simultaneous investigation of the neuronal and vascular compartments in the guinea pig brain isolated in vitro, Brain Res. Protoc. 3 (1998) 21–28]. At the onset of anesthesia induced by a single i.p. injection of 125 mg/kg thiopental, the brain concentration of the drug, measured by high-performance liquid chromatographic assay, was 44.22 ± 5.1 mg/L (mean ± S.E.; $n = 7$). After 30 min of arterial perfusion in vitro with a thiopental-free solution, the cerebral levels of the barbiturate decreased to 2.03 ± 0.56 mg/L ($n = 3$), and reached values close to zero within 1 h. No significant changes in thiopental elimination curve were observed when in vitro perfusion rate was either increased or decreased. The study demonstrates that thiopental is rapidly eliminated from the brain tissue with a mono-exponential kinetic. It can be concluded that barbiturate anesthesia utilized during brain dissection is not likely to influence activities recorded from the in vitro isolated brain preparation.

Keywords: HPLC; Isolated guinea pig brain; Thiopental

Barbiturates are potent anesthetic compounds that efficiently bind to plasma proteins, rapidly accumulate in the cerebral tissue and are slowly eliminated through liver metabolism. Whether brain extrusion through the blood–brain barrier is a limiting factor in barbiturate elimination is still not known. Theoretical estimates predict that, since brain uptake of barbiturates is fast and passive, their elimination from the brain to the plasma should also be rapid.

Thiopental (5-ethyl-5-(1-methylbutyl)-2-thiobarbiturate) is an ultra-short-acting barbiturate used as general anesthetic, that controls intracranial hypertension following trauma, prevents or reduces post-ichemic cerebral damage, and has anticonvulsant properties [29]. As for other barbiturates, thiopental is assumed to exert its pharmacological effect by enhancing inhibitory gamma-aminobutyric acid-mediated (GABAergic) synaptic transmission and by inhibiting excitatory glutamatergic transmission [6,21]. So far, studies of thiopental pharmaco-kinetics and pharmacodynamics performed in animal models [12,16] and in humans [29] demonstrated that this barbiturate reaches anesthetic concentration in the brain within 1–2 min from intravenous (i.v.) inoculation and it is slowly eliminated within 9–20 h. Even though indications on the brain elimination kinetic of thiopental (and other barbiturates) are available in the literature, the time course of its brain removal has never been directly evaluated. This piece of information is crucial to understand whether electrophysiological or metabolic activities recorded on acute in vitro preparations of brain tissue extracted from animals anesthetized with barbiturates (such as brain slices, isolated brain structures and the isolated whole brain) are influenced by the presence of the anesthetic.

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We studied brain elimination of thiopental in the isolated guinea pig brain preparation maintained in vitro by arterial perfusion [8,9,20,26]. The extraction and isolation of the guinea pig brain was performed under anesthesia with thiopental and the brain drug concentration was measured at different times after in vitro placement. Previous reports demonstrated that the vascular and neuronal compartments and the blood brain barrier are preserved in the isolated guinea pig brain preparation [8,18,19,24].

Brains were isolated from young adult Hartley guinea-pigs (150–200 g, Charles River Laboratories, Comerio, Italy) according to the standard technique described in detail elsewhere [8,9,26]. After barbiturate anesthesia induced by 125 mg/kg thiopental i.p., the animal was perfused for 3 min with a cold, complex saline solution (see the following) through the aorta to reduce brain temperature during the dissection. The brain was carefully removed and transferred to a perfusion chamber under hypothermic conditions (15 °C). A custom-made polyethylene cannula was inserted and secured to the basilar artery, and artificial perfusion of the whole brain was re-established at a rate of 5.5 ml/min. Faster (8.25 ml/min) and slower (4.38 ml/min) perfusion rates were also utilized. The peculiar arrangement of the Willis circle in the guinea pig, characterized by large diameter posterior comunicans arteries that originate from the basilar artery, allows an adequate brain perfusion via the basilar artery [8,9,26]. The dissection and isolation procedure lasted 6–8 min. The perfusate (composition NaCl 126 mM, KCl 3 mM, KH_2PO_4 1.2 mM, $MgSO_4$ 1.3 mM, $CaCl_2$ 2.4 mM, $NaHCO_3$ 26 mM, glucose 15 mM, 3% dextran MW 70,000) was oxygenated with a 95% O_2–5% CO_2 gas mixture (pH 7.3). Experiments were carried out on brains maintained in vitro for periods variable between 30 min and 5 h at 32 °C (Fig. 1). To test the viability of the brains, extracellular recordings were performed in different brain regions with glass micropipettes filled with 0.9% NaCl solution (2–5 MΩ input resistance). Bipolar stimulation of the lateral olfactory tract (LOT) was performed to evoke potentials in olfactory/limbic cortices [2]. The experimental protocol was reviewed and approved by the Committee on Animal Care and Use and by the Ethics Committee of the Istituto Nazionale Neurologico, in accordance with the International policy on care and use of laboratory animals.

After different periods in vitro (30 min to 5 h), isolated brains were stored at −80 °C. Thiopental measurements were performed on homogenized brain tissue. Brains of control animals were perfused for 3 min via the aorta with the cold saline solution and were collected immediately after dissection, without in vitro placement. Blood samples drawn immediately after the induction of anesthesia were centrifuged and plasma was stored at −20 °C for HPLC assay of thiopental concentration.

Calibration standards (0.1, 1, 10 and 100 mg/L) were prepared by adding the appropriate volumes of thiopental stock solution (1 mg/ml) to guinea pig drug-free serum or brain homogenates. Our method revealed a very linear response in the proposed range of thiopental concentrations. As internal standard to assess extraction reproducibility, a synthetic intermediate of the antiepileptic drug oxcarbazepine, CGP23827 (Novartis, Italy), was utilized. Samples extracted with organic buffer were treated in parallel with controls. Briefly, 300 μl of brain homogenate or serum spiked with thiopental as control (1, 10 and 100 μg), were added with 100 μl of internal standard, 1 ml of extraction buffer (60 mM $NaH_2PO_4{\cdot}H_2O$, 13 mM $Na_2HPO_4{\cdot}2H_2O$) and 6 ml of a dichlorometane:isopropanol mixture (97:3%, vol/vol). Samples were then agitated for 40 min on an orbital shaker and centrifuged at 2000 rpm at room temperature for 20 min. Upper aqueous phase was removed and samples were brought to dryness in N2 current. Dry pellets were suspended in 300 μl of methanol and injected in a Shimadzu chromatographic apparatus, composed by a pump (LC-10ADVP) with degasser (FLV-10ALVP), an automatic sampler (SL-10ADVP), a UV spectrophotometer (SPD-10AVP) and an integrator (SCL-10AVP). Chromatograms were acquired and analyzed on a PC equipped with Class VP 6.0 software (Shimadzu, Italy). HPLC conditions were as follows: mobile phase 30% acetonitrile and 70% phosphate buffer (10 mM KH_2PO_4, pH 6.00); flow 1.5 ml/min. Column was Reverse Phase C18 Merck LichroCart/Lichrosphere 100 RP-18 (250 mm × 4.6 mm). Peaks were detected at 290 nm. All chemicals were purchased at Merck (Milan, Italy).

Retention times, at chromatographic conditions used for this study, were 24 min for thiopental and 9.5 min for the internal standard. All determinations of brain samples were

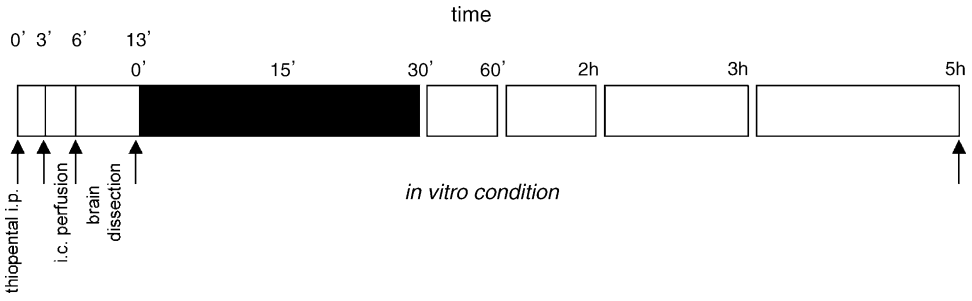

Fig. 1. Experimental protocol for measurements of thiopental brain concentration in the isolated guinea pig brain maintained in vitro. Times before and after in vitro placement are illustrated above the bar.