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Supplemental Material

Anesthesia with Isoflurane Increases Amyloid Pathology in Mice Models of Alzheimer's Disease

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SUPPLEMENTARY MATERIAL

Genotyping by PCR

The genotype of each animal was confirmed as described below [1]. Genomic DNA was extracted from mouse-tail by using the High Pure PCR template preparation kit according to the manufacturer's instructions (Roche, Barcelona, Spain); 150 ng of DNA were used for PCR reaction.

For genotyping AβPP mice, the PCR conditions were as follows: the genomic DNA was denatured for 3 min at 94°C and then 35 cycles were started: 1 min at 94°C, 1 min at 63°C, and 1 min at 72°C, followed by a final extension of 5 min at 72°C. PCR was performed in a final volume of 50 μL containing 2 units of Taq DNA polymerase, 0.2 mM dNTP, 3 mM $MgCl_2$, 5 mM Tris–HCl (pH 8.0), 0.5 μL of DMSO, 0.7 mM of PrP-sense, and 0.08 mM PrP-antisense primers with a specific APP-sense primer at 0.3 mM. The sequences of the primers used were:

PrP antisense: 5'-GTGGATACCCCCTCCCCCAG-CCTAGACC-3'

PrP sense: 5'-CCTCTTTGTGACTATGTGGACT-GATGTCGG-3'

APP sense: 5'-CCGAGATCTCTGAAGTGAAGA-TGGATG-3'

The PCR reaction products were analyzed by electrophoresis on a 1.8% agarose gel stained with ethidium bromide for visualization of DNA bands. DNA molecular weight markers (Roche, Spain) were used to provide a size reference. In AβPP genotyping, all mice (both transgenic and non-transgenic) produce a 750-bp DNA fragment that represents a segment of the endogenous PrP gene, which serves as a positive control for DNA quality. Only AβPP transgenic mice produce a 400-bp DNA fragment specific for AβPP insert.

Cardiovascular, respiratory and body temperature monitoring

The temperature was maintained in normal limits with an electric blanket. Ten mice ($n = 5$ WT, $n = 5$ $A\beta PP_{swe}$) were anaesthetized as above and their ECGs were recorded using two hypodermic needles placed subcutaneously in their extremities. Electrodes were connected to a differential amplifier (Tektronix, 26A2). The ECG and the muscular events due to ventilation were recorded during one minute, at 10, 20, and 30 min of anesthesia. The signal was digitized at 5 kHz using a Digidata 1200 (Axon Instruments) and stored on a computer using Axotape software (Axon Instruments).

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Ten additional mice ($n = 5$ WT, $n = 5$ AβPP$_{\rm swe}$) were used for non-invasive measurements of blood pressure and O_2 saturation under isoflurane anesthesia for 20 min, in the same conditions described above, using a tail-cuff recording device (model LE 160M, Panlab, Barcelona, Spain) and monitor display (LE 5001, Panlab, Barcelona, Spain). As described above, the temperature was carefully controlled, since this is critical for accurate blood pressure measurements. The recordings of the blood pressure were performed three times, separated by one min each, after a period of 10 min of equilibration.

The blood O_2 saturation was measured in 10 mice ($n = 5$ WT, $n = 5$ AβPP$_{\rm swe}$), treated with isoflurane, 2%, plus O_2, 98%, with a MouseOx pulsi-oxymeter (Starr Life Sciences Corp, Panlab, Barcelona, Spain).

The analyses of the cardiovascular and respiratory data obtained in WT and AβPP$_{\rm swe}$ was performed by student's t-test.

Reference

[1] Carro E, Trejo JL, Gomez-Isla T, LeRoith D, Torres-Aleman I (2002) Serum insulin-like growth factor I regulates brain amyloid-beta levels. *Nat Med* **8**, 1390-1397.