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Partial antagonism of propofol anaesthesia by physostigmine in rats is associated with potentiation of fast (80–200 Hz) oscillations in the thalamus

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Editor's key points

- The thalamus is involved in anaesthesia.
- In this study, propofol anaesthesia in rats was antagonized by physostigmine.
- Increased thalamic activity was seen when physostigmine was given during propofol anaesthesia.
- Impaired thalamic function is associated with anaesthesia-induced unconsciousness.

Background. Positron emission tomography studies in human subjects show that propofol-induced unconsciousness in humans is associated with a reduction in thalamic blood flow, suggesting that anaesthesia is associated with impairment of thalamic function. A recent study showed that antagonism of propofol-induced unconsciousness by the anticholinesterase physostigmine is associated with a marked increase in thalamic blood flow, supporting the implication of the thalamus. The aim of the present study was to assess the role of the thalamus in the antagonistic effects of physostigmine during propofol anaesthesia using electrophysiological recordings in a rat model.

Methods. Local field potentials were recorded from the barrel cortex and ventroposteromedial thalamic nucleus in 10 chronically instrumented rats to measure spectral power in the gamma/high-gamma range (50–200 Hz). Propofol was given i.v. by target-controlled infusion at the lowest concentration required to abolish righting attempts. Physostigmine was given during anaesthesia to produce behavioural arousal without changing anaesthetic concentration.

Results. Compared with baseline, gamma/high-gamma power during anaesthesia was reduced by 31% in the cortex (P=0.006) and by 65% in the thalamus (P=0.006). Physostigmine given during anaesthesia increased gamma/high-gamma power in the thalamus by 60% (P=0.048) and caused behavioural arousal that correlated (P=0.0087) with the increase in power. Physostigmine caused no significant power change in the cortex.

Conclusions. We conclude that partial antagonism of propofol anaesthesia by physostigmine is associated with an increase in thalamic activity reflected in gamma/high-gamma (50–200 Hz) power. These findings are consistent with the view that anaesthetic-induced unconsciousness is associated with impairment of thalamic function.

Keywords: acetylcholine; anaesthesia; anticholinesterase; barrel cortex; electrophysiology; general anaesthetics; local field potentials; rat; thalamus

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The mechanisms by which general anaesthetics cause unconsciousness are not fully understood.[1 2] Positron emission tomography studies have shown that unconsciousness induced by propofol is associated with reductions in thalamic blood flow,[3 4] suggesting that the thalamus has a role in how anaesthetics impair consciousness.[1 2] A recent study has shown that antagonism of target-controlled infusion (TCI) of propofol anaesthesia by the anticholinesterase physostigmine in human subjects is associated with a marked increase in thalamic blood flow, further supporting the implication of the thalamus in the changes in the level of consciousness.[5] The aim of the present study was to use electrophysiological recordings in an animal model to assess the role of the thalamus in the antagonistic effects of physostigmine during propofol anaesthesia. Blood flow measures provide an indirect measure of neuronal activity, and assume intact coupling between metabolism and blood flow.[6] Electrophysiological recordings offer complementary evidence of changes in thalamic activity by providing a direct measure of neuronal activity.[6]

We have found previously in unpublished studies that physostigmine also antagonizes propofol anaesthesia in rats as revealed by spontaneous movements of the limbs and of the head, by spontaneous whisker movements, and by orientating with occasional biting after gentle touch of the snout with a swab. We now report the results of

recording thalamic and cortical local field potentials (LFPs) to measure the spectral power of spontaneous oscillations in the gamma (51–80 Hz) and high-gamma ranges (81–200 Hz)[7] during antagonism of TCI propofol anaesthesia by physostigmine in rats. These frequency ranges were chosen because these oscillations correlate tightly with changes in regional cerebral blood flow[8] and provide a very useful index of brain activation.[7] Furthermore, intracranial cortical recordings from human patients show that propofol attenuates power in the 50–200 Hz range,[9] and intracranial recordings from rats also show that isoflurane anaesthesia reduces power in the 70–140 Hz range.[10] We did not include the low gamma (30–50 Hz) range, which has limited usefulness to assess anaesthetic effects.[9 10] We predicted that the spectral power in the 50–200 Hz range would be reduced during anaesthesia with propofol for both the thalamus and the cortex, and that the arousal response induced by physostigmine would be accompanied by an increase in power in the thalamus.

Methods

All procedures adhered to the guidelines of the Canadian Council on Animal Care, and were approved by the Animal Ethics Boards of Concordia and McGill University. Male Long–Evans rats (300–350g, n=14) were acquired from Charles River Laboratories (Senneville, Quebec, Canada). They were housed individually, provided food and water *ad libitum,* and maintained on a 12 h reversed light cycle (lights on from 20:00 to 08:00 h).

Surgery

Recording microelectrodes were implanted stereotaxically under isoflurane anaesthesia in the ventroposteromedial nucleus (VPM; stereotaxic coordinates: P, −3.4; L, 2.5; V, 6.4 mm relative to bregma)[11] and in the barrel cortex (P, −2.0; L, 4.6; V, 2.0 mm). A catheter suitable for long-term use was also inserted in the right jugular vein. Full details of the procedures are described in the Supplementary material. After surgery, catheters were flushed daily with a dilute solution of gentamycin and heparin and 10 days were allowed for recovery. After completing the testing sessions, the animals were killed with a lethal dose of urethane and perfused via the left ventricle with heparinized saline followed by 10% neutral buffered formalin. The isolated brains were post-fixed in 4% paraformaldehyde for histological processing and confirmation of correct placement of the electrodes.

Design

There were two testing sessions, separated by at least 3 days. In the first session, physostigmine was injected as a reversal agent during anaesthesia. In the second session, normal saline was injected as a control reversal agent. Physostigmine was tested first to maximize the number of observations for the active drug in the event that an animal would not complete the entire experimental sequence. Each session consisted of four periods: baseline, anaesthesia, attempted reversal of anaesthesia with either physostigmine or a saline injection, and recovery. Five of the 10 animals had been previously exposed to physostigmine as part of a related study assessing the effects of physostigmine during inhaled (isoflurane) anaesthesia. Results from naïve rats were similar to those from rats that had been previously exposed to physostigmine, and data from both groups were therefore pooled. The results of the study with isoflurane will be reported separately.

After baseline recordings of LFPs, the propofol tubing was connected to the jugular vein catheter via one arm of a Y-connector (Interlink System, Baxter Healthcare Corporation, Deerfield, IL, USA). The infusion rate of propofol was adjusted by a Harvard 22 syringe pump controlled by the Stanpump software developed by Steven L. Shafer and colleagues (Department of Anesthesiology, Stanford University, CA, USA) using pharmacokinetic parameters derived by Knibbe and colleagues.[12] The initial target plasma concentration was set at 4 μg ml^{-1}. It was increased by 0.5 μg ml^{-1} every 2 min until the animal made no attempts to right itself when placed sequentially on its right and left sides. The range of final target plasma concentrations was 7.0–10.0 μg ml^{-1}. With this level of anaesthesia, the animals made no spontaneous movements and did not react when their snout was gently touched. Recording of the LFPs during anaesthesia was initiated 6–8 min after reaching the final target plasma concentration. After these recordings, physostigmine (0.4 mg kg^{-1}) mixed with glycopyrrolate (0.08 mg kg^{-1}), or an equivalent volume of normal saline, was injected over 2 min via the other arm of the Y-connector. This dose was chosen because behavioural pilot tests with physostigmine had revealed a ceiling effect for doses of 0.30 mg kg^{-1}. Glycopyrrolate, a muscarinic blocker that does not cross the blood–brain barrier, was always given with physostigmine to prevent the peripheral muscarinic side-effects. The injection of physostigmine occurred [mean (SD)] 29.4 (5.4) min after reaching the final target plasma concentration. That of saline occurred 36.4 (6.4) min after reaching the final target plasma concentration (P<0.01, paired t-test). After the injection, the animals were observed for signs of arousal, and the following behaviours were immediately recorded on an itemized scoring sheet as absent, mild, or moderate in intensity (0, 1, or 2, respectively): spontaneous movements of the limbs, of the head, of the whiskers, and orientating after gentle touch of the snout with a swab. Overall arousal was ranked based on the following sum: orientating score+whisker movements score+head movements score+[(limb movements score)/2]. Reduced weight was given to the limb movement score as it sometimes included fasciculations, which could reflect an isolated muscle response to the anticholinesterase. The righting reflex was assessed, and LFPs were recorded 2–3 min after the appearance of the arousal response, or 10 min after the injection if the animal showed no signs of arousal. A final set of LFPs recordings was obtained during recovery from anaesthesia 10 min after the return of ambulation. Rectal temperature was maintained at 36.5–37.0°C with heat pads placed beneath the chamber.