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·Report·

Cortico-centric effects of general anesthetics on cerebrocortical evoked potentials

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ABSTRACT

Despite their ubiquitous use for rendering patients unconscious for surgery, our understanding of how general anesthetics cause hypnosis remains rudimentary at best. Recent years have seen increased interest in “top-down” cortico-centric theories of anesthetic action. The aim of this study was to explore this by investigating direct cortical effects of anesthetics on cerebrocortical evoked potentials in isolated mouse brain slices. Evoked potentials were elicited in cortical layer IV by electrical stimulation of the underlying white matter. The effects of three anesthetics (ketamine, etomidate, and isoflurane) on the amplitude, latency, and slope of short-latency evoked potentials were quantified. The N2/P3/N4 potentials – which represent the early cortical response – were enhanced by etomidate (increased P3-N4 slope, P <0.01), maintained by ketamine, and reduced by isoflurane (lower N2/P3 amplitude, P <0.01). These effects closely resemble those seen *in vivo* for the same drugs and point to a cortical mechanism independent of effects on subcortical structures such as the thalamus.

Keywords: anesthesia; evoked potentials; cortical slice; thalamus

INTRODUCTION

Whether general anesthetics principally affect cerebrocortical or subcortical structures to induce hypnosis is a matter of ongoing debate and speculation. The "thalamic gate" theory proposes that unconsciousness occurs when anesthetics deactivate the thalamus, preventing sensory information from reaching the cortex[1]. This is the so-called "bottom-up" theory of anesthetic action. Studies on the effect of anesthetics on sensory-evoked potentials have given some credence to this theory, showing that the morphology of the early cortical evoked potentials (reflecting primary cortical responses to sensory input) is often altered during anesthesia[2,3]. A subcortical basis is further supported by the apparent mechanistic overlap between anesthesia and natural sleep, which is thought to be driven largely by brainstem arousal mechanisms[4]. However, there are exceptions that call into question the thalamic gate explanation as a unifying mechanism. Most notable is ketamine, which not only preserves sensory transmission to the cortex[5], but may even enhance cortical responsiveness to incoming sensory information[6] and maintains an activated EEG. Alternative explanations that accommodate anomalies such as ketamine invoke the cerebral cortex as the primary site of anesthetic action — the so-called "top-down" theories. Top-down cortico-centric proposals are based on the idea that anesthetics disrupt the cortical processing of incoming sensory information[7-10], particularly impairing its capacity to represent complex information and/or integrate information across distributed cortical regions[11].

The isolated cerebrocortical slice preparation provides an ideal model for testing the direct effects of anesthetic drugs on the cerebral cortex. In particular, somatosensory and visual cortical evoked potentials closely resembling those obtained *in vivo*[12] can be

generated in isolated cortical slices by directly stimulating the subcortical white matter[13,14], without any influence from the thalamus and other subcortical structures. White matter stimulation in the slice taps into reciprocal efferent and afferent thalamocortical pathways[13] from excitatory layer IV cortical neurons, from which classic sensory-evoked potentials can be readily recorded[15]. Accordingly, the *in vitro* slice preparation is a useful model for investigating and interpreting the mechanisms of *in vivo* evoked potentials[13,14]. In this study, we took advantage of this to investigate the direct effects of general anesthetics on cortical evoked potentials. In keeping with the cortico-centric theories of anesthetic action, a recent study showed that the suppression of auditory evoked potentials by isoflurane is primarily through its direct effects on intracortical pathways[10]. The aim of this study was to expand these findings to include anesthetics with diverse molecular mechanisms of action, namely ketamine (primarily an N-methyl-*D*-aspartate antagonist) and etomidate (primarily a type-A γ-aminobutyric acid agonist).

MATERIALS AND METHODS

Slice Preparation

The animal procedures were approved by the Waikato Animal Ethics Committee. Coronal brain slices (400 μm) were prepared between bregma –2 to –5 mm from adult wild-type mice (C57 and 129SV) following CO_2 anesthesia. Once dissected, the brain was immediately placed in carbogenated (95% O_2, 5% CO_2) ice-cold normal artificial cerebrospinal fluid (aCSF) composed of (in mmol/L) 125 NaCl, 26 $NaHCO_3$, 1.25 NaH_2PO_4, 2.5 KCl, 1 $MgCl_2$, 2 $CaCl_2$, and 10 *D*-glucose. Slices were prepared using a vibratome (Campden Instruments, Loughborough, UK) and held for at least 1 h before commencing recording. For recording, the slice was transferred to a recording bath (Kerr Tissue Recording System, Kerr Scientific Instruments, Christchurch, New Zealand) perfused with aCSF at 3–5 mL/min. The slices were maintained submerged at room temperature (20–22°C).

Recording Parameters

The slices were stimulated with constant-current 100 μs square-wave pulses delivered at 0.2 Hz (DS3, Digitimer Ltd, Welwyn Garden City, UK) *via* a bipolar tungsten electrode (75 μm, 0.5 mm separation) positioned in the subcortical white matter. The current amplitude was adjusted to elicit a submaximal response and ranged from 300–900 μA. Alternatively, in three instances (all etomidate experiments), a constant-voltage stimulus (1–2 V) was used. The results derived from these cases were entirely consistent with those obtained otherwise and were therefore included in the final analysis. The evoked potentials were recorded with a tungsten electrode (25 μm) in layer IV of the cortex, positioned radially from the stimulation site. An Ag/AgCl disc electrode served as the bath reference. The recording was filtered at either 0.1 Hz or 1.0 Hz (high-pass) and 1 kHz (low-pass) with a gain of 1000 (Model 3000 Differential Amplifier, A-M systems Inc., Sequim, WA) and digitally sampled at 10 kHz (Power 1401, CED, Cambridge, UK). The evoked responses are reported without averaging unless otherwise stated.

Experimental Protocols

Three anesthetics were tested, ketamine (16 μmol/L, 10 slices from 3 animals), etomidate (24 μmol/L, 11 slices from 6 animals) and isoflurane (1 minimum alveolar concentration (MAC) equivalent, 9 slices from 3 animals). In the etomidate and ketamine experiments, the recording bath was perfused using a passive gravity-feed method, with the appropriate amount of each drug added directly to the pre-carbogenated perfusate. Isoflurane could not be delivered using this method because of its volatility and was instead perfused *via* syringe-pump at 5 mL/min (see details below).

In each trial, a baseline of at least 10 min was recorded, followed by 20-min drug perfusion and 20-min washout. These time periods formed the delineations for data averaging and statistical analyses. Control experiments (13 slices from 4 animals) consisted of uninterrupted 50-min recordings with equivalent time delineations used for statistical comparison. On five occasions, the same slice was used for investigating drug effects after a 50-min control recording period.

Determination of Isoflurane Concentration

In pilot experiments, we determined that adding 0.01 mL isoflurane to 50 mL of pre-carbogenated aCSF in a sealed syringe, perfused *via* syringe pump at 5 mL/min, generated a slice bath isoflurane concentration very close to 1 rat MAC. This was confirmed using two methods. First, following the above perfusion regime, bath aCSF samples

were extracted and isoflurane concentration measured every 4 min during a 20-min perfusion period using HPLC analysis. The isoflurane levels peaked after ~8 min and remained stable throughout the perfusion period, during which the average concentration was 480 μmol/L, ~1 rat MAC[16-18]. Second, using the same perfusion protocol, the headspace above the perfusion bath was sealed and the headspace gas above the bath was recirculated through an S5 entropy monitor (Planar Systems Inc., Beaverton, USA). The volume percent of isoflurane in the headspace reached a stable equilibrium value of 1.2% after ~10 min. Furthermore, the anticonvulsant effect of isoflurane at this dose on neocortical slice seizures is known to be equivalent to that for 1 rat MAC of isoflurane[17], confirming the dose calculation.

Data Analysis

Individual evoked potentials were analyzed according to the scheme shown in Figure 1. The P3 wave was used as the basis for analysis because the P1 wave was either not always present or could not be reliably resolved from the stimulus artefact. The amplitude and latency of the P3 wave was derived from the voltage and time differences from the N2 trough to the P3 peak. The P3-N4 slope was calculated as the maximum slope between the P3 peak and the N4

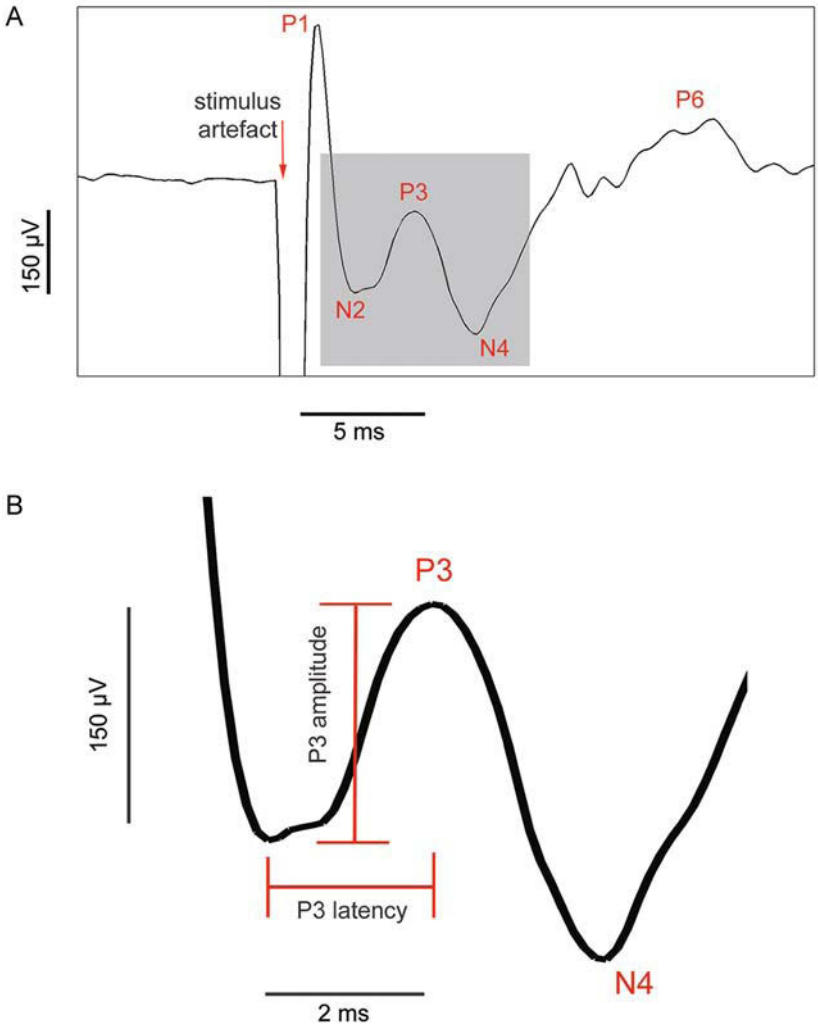

Fig. 1. A recorded evoked potential illustrating the component waves and the method of quantification. (A) A compressed time view of the entire evoked response; (B) An enlarged and expanded view of the grey section showing the method of P3 wave quantification. See text for details.

trough. That is, the voltage differential was calculated for all sequential data-points between the P3 peak and N4 trough and the maximum negative differential was recorded as the maximum slope for that evoked response. Each parameter was then averaged within the time windows specified above, giving "baseline", "drug", and "washout" periods for statistical analysis. The three periods were compared for each agent using the non-parametric Friedman test for repeated measures ANOVA. The percentage change in each parameter ("baseline" *versus* "drug") was also compared across agents using the Kruskal-Wallis test. Results are presented as mean (SEM) and a *P*-value <0.05 was considered statistically significant.

RESULTS AND DISCUSSION

The effects of each intervention on P3 amplitude, P3 latency, and P3-N4 slope are summarized in Figure 2.

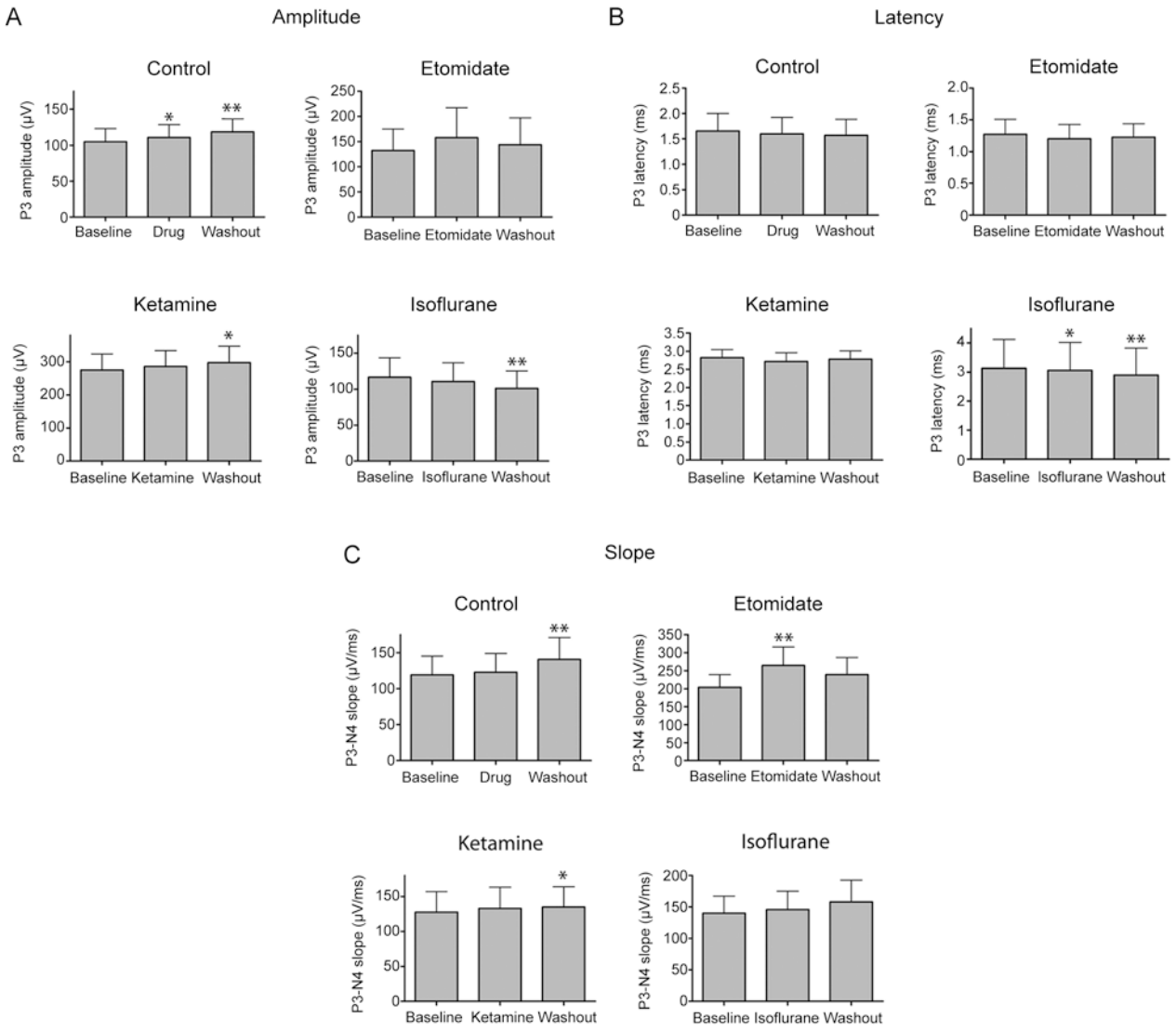

Fig. 2. Histograms comparing the effects of controls, etomidate, ketamine, and isoflurane on: (A) P3 amplitude; (B) P3 latency, and (C) maximum slope of the P3-N4 wave. In each case the data were divided into baseline (before drug delivery), drug exposure, and drug washout periods. For the control recordings, the equivalent time-periods are shown. ****P* **<0.05, *****P* **<0.01.

Control Findings

In the control experiments, subtle but significant time effects were seen as increases in the P3-N4 slope (140 μV/ms *versus* 119 μV/ms, P <0.01) and P3 amplitude (119 μV *vs* 105 μV, P <0.05) during the washout period. The P3 latency also trended downward in the control slices (P = 0.052).

Anesthetic Findings

These changes in the control group were closely mirrored in the ketamine group, suggesting a negligible drug effect in this case. That is, the P3-N4 slope increased (135 μV/ms *vs* 128 μV/ms, P <0.05), as did P3 amplitude (297 μV *vs* 275 μV, P <0.05). A time trend in the P3-N4 slope also appeared to book-end the etomidate experiments (Fig. 2C), but with a marked elevation of the P3-N4 slope above baseline superimposed during the drug delivery period (265 μV/ms *vs* 204 μV/ms, P <0.01). Thus, etomidate enhanced the P3-N4 slope over and above that attributable to the effect of time alone. Isoflurane was the only anesthetic tested that reduced the P3 amplitude, an effect that was strongest during the washout period (101 μV *vs* 117 μV, P <0.01). This was unlikely to be a time effect because the P3 amplitude increased over time in the control slices. Isoflurane also reduced the P3 latency, which was again most evident during washout (2.9 ms *vs* 3.1 ms, P <0.01). These results were mirrored by the percentage changes in evoked potential parameters across agents. Thus, P3 amplitude decreased in the isoflurane group (P <0.05 *vs* control and etomidate, Dunn's multiple comparisons test); while the P3-N4 slope increased in the etomidate group (P <0.05 *vs* control and isoflurane, Dunn's multiple comparisons test).

Effect of aCSF Perfusion Rate

When changing aCSF solutions (for drug delivery and/or washout), small perturbations to the aCSF delivery characteristics can occur, resulting in slight changes in the flow rate and/or fluid level in the bath. To rule out the possibility that this could account for the drug-correlated changes in P3-N4 slope, we tested the effect of deliberately altering each on the evoked response. The slope index was robust against such effects, even with a 3-fold change in flow rate, which far exceeded that occurring in this study (Fig. 3).

Results from *In Vivo* Studies

The aim of this study was to investigate whether the anesthetic effects on evoked potentials *in vivo*, which are

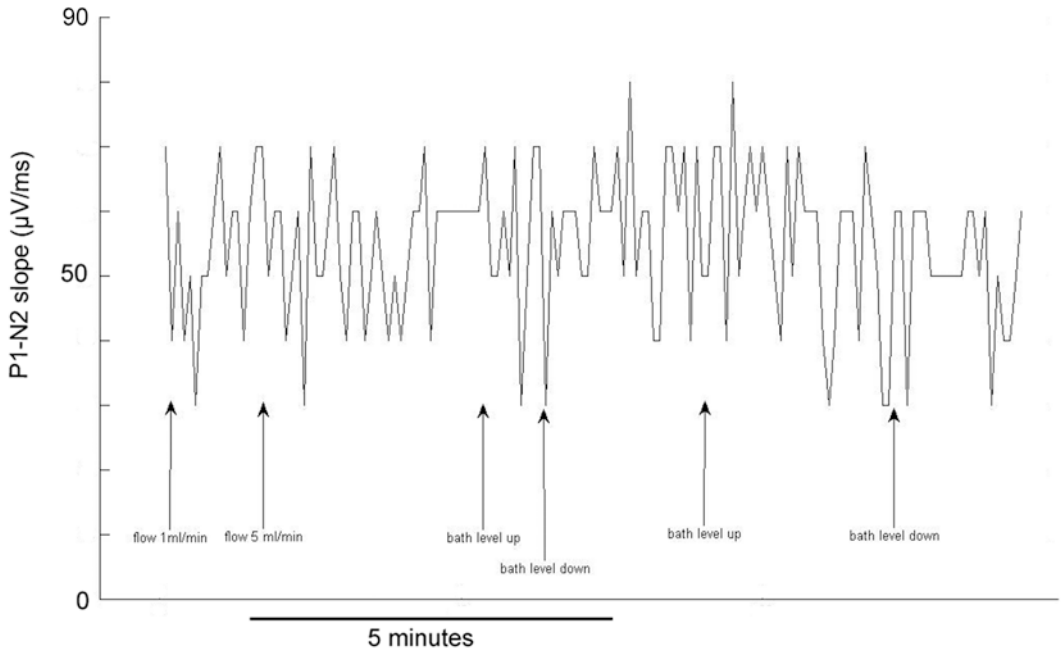

Fig. 3. Time-course of P3-N4 slope during deliberate perturbations to aCSF fluid flow rate and perfusion bath fluid level.

generally attributed to a "bottom-up" subcortical (thalamic) mechanism, could be reproduced in isolated cortical slices, thereby implicating a "top-down" cerebrocortical action. *In vivo* studies have shown that primary somatosensory and auditory cortical sensory evoked potentials are variably affected by anesthetics. For example, the amplitude of the N20-P23 wave (representing the early primary cortical response) is consistently increased during etomidate anesthesia[2,19]. Evoked potential slope is not typically measured in clinical studies; however, an increase in population event amplitude is usually accompanied by an increase in event slope. Clinical studies have shown mixed results for ketamine, with either maintenance[5] or enhancement[20] of N20-P23 amplitude. The effects of etomidate and ketamine contrast with the volatile anesthetics, tending to reduce the amplitude and increase the latency of cortical evoked responses[3,21].

Comparison with *In Vitro* Data from This Study

The cortical slice findings in our study largely paralleled the above *in vivo* data. Etomidate enhanced the cortical evoked response, seen as an increase in the P3-N4 slope. While the group analysis of P3 amplitude did not reveal a significant etomidate effect, a number of individual slices exhibited strong increases in P3 amplitude corresponding to the period of drug delivery (Fig. 4) — an effect not observed during perfusion with the other anesthetics tested. Similar to the clinical studies, ketamine also increased the P3 amplitude and P3-N4 slope, but to a lesser degree. While both effects could be attributed to an effect of time, at the very least we can conclude that ketamine maintained the evoked potential morphology in the slice model. Isoflurane was the only anesthetic tested that caused a reduction in P3 amplitude. Together, these findings suggest that anesthetic effects on cortical evoked potentials are due largely to top-down cortical effects, supporting the cortico-centric view of anesthetic action.

Mechanistic Considerations for Anesthetic Hypnosis

We would point out that our study does not provide direct insight into how cortex-targeted anesthetic effects translate into clinical endpoints such as hypnosis. Each agent uniquely altered the early cortical response — which in the case of ketamine and etomidate may even involve the facilitation of cortical responsiveness. This indicates that disruption of primary cortical sensory processing is not a unifying explanation for anesthetic impairment of consciousness. The implication is that higher-order cortical processes are affected, such as the representation of complex information and/or the integration of information across distributed cortical regions[9,11].

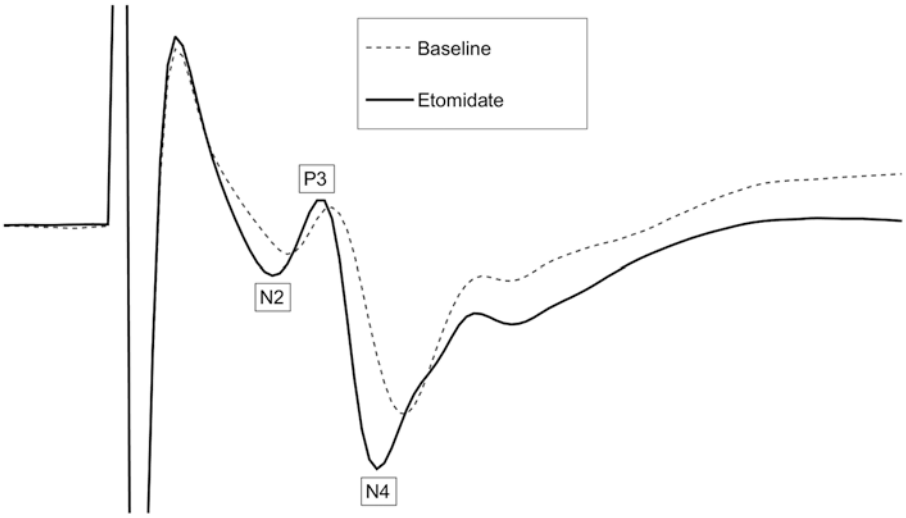

Fig. 4. Traces from a single experiment showing the change in evoked potential morphology induced by etomidate. In this example, an increase in P3 amplitude is evident along with an increase in P3-N4 slope. The traces are averages of 50 and 100 evoked potentials during baseline (grey dashed line) and drug-exposure (black solid line).

Anesthetic Dosage

Without directly quantifying the drug levels in slice tissue, there is no absolutely reliable method to ensure equipotent, clinically relevant hypnotic dosing of anesthetic drugs in slices. Our choice of dosage regimens for etomidate and ketamine was based largely on the known diffusion characteristics of etomidate into cortical slice tissue. When recording from the middle of a 400-μm slice, the etomidate concentration reaches only 17%–50% of its aCSF level after 10-min perfusion at a constant aCSF concentration[22]. The perfusion system and protocol we used achieved a constant aCSF etomidate concentration in the bath of 6 μg/mL for ~10 min (data not shown), which would achieve a tissue concentration of ~1–3 μg/mL. In reality, the maximum concentration reaching the tissue would have been slightly higher than this, taking into account the drug wash-in and wash-out periods. Deep anesthesia with etomidate requires an effect site concentration of ~3.3 μg/mL in rodents[23]. Thus, the etomidate perfusion regime we used probably delivered the equivalent of a deep hypnotic dose. Because there are no experimental data on the diffusion of ketamine in cortical slices, a concentration of 4 μg/mL was chosen based on the relative clinical hypnotic potencies of ketamine and etomidate.

Data Consistency and Exclusions

It is evident from Figure 1 that evoked potential morphology varies somewhat from one preparation to another. This is probably due to small variations in recording electrode placement[13]. Despite the variant waveform shapes, the P3-N4 complex previously described[13] was clearly discernable in all but 3 cases. These recordings (2 etomidate and 1 control) were excluded from the analysis. Two further recordings were excluded due to preparation disturbances that prevented completion of the experimental protocol.

Conclusion

We conclude that the effects of three different categories of general anesthetic drugs on primary evoked potentials in isolated cortical slices closely replicate the effects of these drugs seen in clinical somatosensory evoked potential studies. This suggests that the anesthetic effects on evoked potential morphology are largely independent of any actions on subcortical structures.

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