Visual evoked potentials and nitrous oxide-induced neuronal depression: role for benzodiazepine receptors

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Summary
We have examined the role of benzodiazepine receptors in nitrous oxide-induced neuronal depression in rats. The changes in neuronal excitability induced by nitrous oxide and the benzodiazepine inverse agonist, Ro15-4513, were monitored by measurement of visual evoked potentials (VEP). Administration of Ro15-4513 10 mg kg\(^{-1}\) i.p., in rats breathing air, did not affect the amplitude or latency of VEP. However, the same concentrations of Ro15-4513 antagonized nitrous oxide-induced depression of VEP amplitudes. We conclude that antagonism of nitrous oxide-induced depression by Ro15-4513 indicates that at least part of the decreased neuronal excitability caused by nitrous oxide could be ascribed to interactions with the GABA\(_A\) receptor complex. (Br. J. Anaesth. 1996;77:522–525)

Key words

The high correlation between the degree of lipophilicity and potency of anaesthetic agents, ranging from inert gases such as xenon to alcohols of varying phyllicity and potency of anaesthetic agents, ranging from inert gases such as xenon to alcohols of varying length, has always been interpreted as evidence that general anaesthesia occurs secondary to non-specific disruption of lipids of neuronal cell membranes. Although the solubility relationships provide a framework for any proposed theory of anaesthesia, the mechanism of action of anaesthetics and corresponding membrane disturbances remain intriguing. Recent studies have focused on membrane proteins as a site of anaesthetic action. In this respect two transmitter receptors, namely glutamate and \(\gamma\)-aminobutyric acid (GABA), were considered frequently as mediators of anaesthetic drugs.

GABA is the major inhibitory neurotransmitter in the mammalian brain and thus a prime candidate for a mechanism of the depressant effects of anaesthetic agents. The increase in Cl\(^-\) conductance caused by GABA can be modulated by benzodiazepines, ethanol and various inhalation anaesthetics. Galindo showed that halothane potentiated the inhibitory action of GABA in the cuneate nucleus. These results have been confirmed recently by Jones, Brooks and Harrison, who showed enhancement of GABA-activated Cl\(^-\) currents in cultured rat hippocampal neurones by enflurane, halothane and isoflurane.

Glutamate-activated receptors are other obvious targets for general anaesthetics. The NMDA receptor, when activated, causes a Ca\(^{2+}\) influx modulating the excitability of a neurone. Ethanol-reduced, NMDA-induced Ca\(^{2+}\) currents in cultured hippocampal neurones share this property with other general anaesthetics. As many ion channels have been shown to be affected differently by anaesthetics, it appears that an interaction between a particular channel and an anaesthetic cannot provide a basis for a general mechanism of anaesthesia. Ethanol, for example, potentiates GABA binding and inhibits NMDA receptors, making more pathways possible for the clinically observed effects. An imidazobenzodiazepine inverse agonist, Ro15-4513 (ethyl-8-azido-5,6-dihydro-5-methyl-6-oxo-4H-imidazo [1,5-a] [1,4] benzodiazepine-3-carboxylate) however, selectively blocks ethanol-stimulated Cl\(^-\) uptake and many of the behavioural effects.

Mice rendered tolerant to nitrous oxide also exhibit cross-tolerance to ethanol hypnosis, suggesting a similarity in mode of action between these two drugs. This study was undertaken to see if nitrous oxide-induced neuronal depression can be altered by a benzodiazepine inverse agonist. Changes in neuronal excitability induced by nitrous oxide and Ro15-4513 were measured in this study by visual evoked potentials (VEP). This method was used previously for monitoring the central effects of nitrous oxide in humans and animals. All of these studies showed that VEP is a reliable method for measurement of nitrous oxide-induced neuronal depression.

Materials and methods
We used male adult albino Wistar rats, weighing 250–300 g. Each rat was placed in a separate cage...
and provided with food and water ad libitum. The holding room was maintained at approximately 22°C, 50% humidity and a 12-h light–dark cycle (light on at 08:00). The animals were allowed at least 7 days for adaptation to these circumstances before surgery. Before electrode placement, the rats were anaesthetized with Hypnorme 0.15 ml 100 g⁻¹ (Hypnorme = fluanison 10 mg and fentanyl 0.2 mg ml⁻¹).

Individual stainless steel screw electrodes were implanted surgically over the right and left visual cortices at points 7 mm posterior to the bregma and 3 mm lateral to the midline. A reference electrode was placed in the frontal sinus. The electrodes were soldered to a miniature socket, which was fixed to the skull with dental cement. After a 7-day postoperative recovery period, each rat was habituated to the recording procedure. The experiments and habituation were performed with rats moving freely and housed in an exposure chamber which was maintained at a temperature of 22°C. The exposure chamber was made of glass surrounded on all sides by mirrors except for the top where the flash-lamp was placed. This allowed complete illumination of the exposure chamber in all directions. During habituation, the electrode leads were connected and a flash stimulus was induced once every 7 s for 10 min. Such treatment was repeated daily over the following 3–4 days, until VEP discharges stabilized. Previous studies have shown that under these conditions, VEP discharges stabilize after several days. During the experiments the flashlight was triggered by a Grass S44 stimulator at a frequency of 0.14 Hz. Brain responses were amplified with a Grass model 79 B, connected to an analogue-to-digital converter (Lab Master, Scientific solutions Inc., OH, USA), which was triggered by the Grass S44 stimulator after every flash. A computer connected to the analogue-to-digital converter performed the averaging of 25 VEP over an 800-ms epoch after every flash and printed the results. Potentials less than −500 μV or more than +500 μV were defined as artefacts induced by movement and rejected automatically. With few rejections per measurement a typical recording of one averaged VEP lasted for approximately 3 min. VEP discharges were recorded in animals breathing air before exposure to 70% nitrous oxide–30% oxygen.

A concentration of 70% nitrous oxide was selected because in many studies of nitrous oxide-induced dependence phenomena in animals or nitrous oxide-induced changes in evoked potentials in humans, the same or a similar concentration of nitrous oxide was used. In addition, a concentration of 70% nitrous oxide is used frequently during clinical anaesthesia. While exposed to nitrous oxide for 60 min, i.p. administration of drug (Ro15-4513 10 mg kg⁻¹) or vehicle (Tween 0.3%, volume 1 ml) was given. VEP were measured at intervals of 15 min for the following 45 min. During the recording session the animal was observed closely to ensure that recording was performed while the eyes were open. The flashlight itself was usually enough to alert the animal. Nitrous oxide, oxygen and carbon dioxide were monitored during the experiment with a Datex Capnomac Ultima (Datex Instrumentarium Corp., Helsinki, Finland). Nitrous oxide was withdrawn by exposing the animals to air. Tween was obtained from Sigma Chemical Co (St Louis, MO, USA) while Ro15-4513 was obtained from Hoffmann-La Roche. A VEP during the first 500 ms normally includes three positive–negative component complexes. Peak-to-peak amplitudes and peak latency were determined. Peak-to-peak amplitudes obtained during exposure to nitrous oxide were compared with values recorded while the rats were breathing air. Data are presented as mean (SEM); n = number of animals tested. Significance of differences in mean values was examined by analysis of variance (ANOVA) (P < 0.05).

Results

An example of a visual evoked potential response in one rat, in air, is shown in figure 1. The typical evoked potential is characterized by six waves, labelled P1 to N3. As the baseline may vary throughout the experiment we looked at top-to-top amplitudes. Although all top-to-top amplitudes showed a decrease when the rat was exposed to nitrous oxide, only potentials with a latency of 100 ms or more were studied because of their stability. Therefore, only amplitudes P2-N2, N2-P3 and P3-N3 were studied.

To assess the effects of Ro15-4513 without the interference of nitrous oxide, a group of eight rats, while breathing air, was given Ro15-4513 10 mg kg⁻¹ in 0.3% Tween i.p. Top-to-top amplitudes are shown in figure 2. After obtaining control measurements, an i.p. injection was given (arrow in fig. 2) and the first

![Figure 1](Image_53x69_to_222x197)  
Figure 1  An example of a visual evoked potential (VEP) in one rat while the animal was breathing air. Amplitude peaks are marked P1 to N3.

![Figure 2](Image_303x68_to_531x242)  
Figure 2  Top-to-top values P2-N2 (□), N2-P3 (△) and P3-N3 (○) were not influenced by injection of Ro15-4513 10 mg kg⁻¹ (arrow) while the animals breathed air (n = 8).
VEP after injection was measured 15 min later. No significant changes in top-to-top amplitudes were observed within 45 min after administration of Ro15-4513 and no change in general behaviour was observed.

Figure 3A and 3B depicts top-to-top amplitudes of 20 rats showing an expected depression of all three amplitudes after 60 min of exposure to nitrous oxide compared with values obtained in air (control values). While breathing nitrous oxide, 10 animals were given the solvent 0.3% Tween 1 ml i.p. (arrow in fig. 3A) while others received Ro15-4513 (fig. 3B). In the following 45 min VEP were measured at 15-min intervals in both groups. Animals given Tween showed no normalization of amplitudes towards values obtained in air, except for P2-N2. As this top-to-top amplitude proved to be sensitive to handling or i.p. injection of solvent, or both, it was not evaluated further. After administration of Ro15-4513, however, top-to-top values for N2-P3 and P3-N3 increased significantly compared with values obtained after 60 min exposure to nitrous oxide. Additionally, all observed amplitudes recovered to values that were not significantly different from baseline. Administration of nitrous oxide and i.p. injection of the solvent Tween or Ro15-4513 had no influence on latency (not shown).

An additional observation was that after injection of Ro15-4513, animals showed increased activity caused by rotational behaviour, which did not occur in rats while breathing air but was common in rats exposed to nitrous oxide. Therefore, although aroused, the animals did not behave normally until exposure to nitrous oxide was discontinued.

**Discussion**

The mechanism of action of inhalation anaesthetic agents remains obscure, although there is substantial evidence that at least part of the effects may be explained by potentiation of the GABAergic system. The role of the GABAergic system on the effects produced by inhalation of nitrous oxide has, to our knowledge, never been explored. When involvement of the GABAergic system is investigated, and particularly when the benzodiazepine site of the GABA receptor complex is of interest, a benzodiazepine antagonist or inverse agonist, or both, may be used. Using an antagonist such as flumazenil (Ro15-1788) may give results easier to interpret compared with inverse agonists. Unfortunately, flumazenil has some intrinsic activities. This drug decreased the minimum alveolar concentration of isoflurane in dogs when given i.v., suggesting partial agonist activity. In addition, flumazenil was unable to alter methoxyflurane-induced sleep time in mice.
but antagonized the reduction in sleep time produced by Ro15-4513. This combined with unfavourable pharmacokinetics made a study with flumazenil, in freely moving rats, unsuitable. Our control experiments showed that Ro15-4513 did not have any effect on the VEP when given at a concentration of 10 mg kg\(^{-1}\) (fig. 2). The same concentration of Ro15 4513, however, reversed the changes in VEP induced by nitrous oxide (fig. 3B). The fact that the animals persisted in their unusual rotational behaviour suggests that this reversal in nitrous oxide-induced anaesthesia was only partial, although with regard to VEP, complete reversal was obtained. Higher concentrations of Ro15-4513 may have produced further improvement towards normal behaviour but would make interpretation less obvious as higher concentrations have epileptogenic properties.

In summary, our results indicated that neuronal depression induced by nitrous oxide was sensitive to antagonism by Ro15-4513. In this respect nitrous oxide resembles alcohol and other inhalation anaesthetics. Although the precise interaction between nitrous oxide and the GABA receptor is not clear, our results indicate that at least part of the decreased neuronal excitability caused by nitrous oxide could be ascribed to interactions with a binding site on the GABA\(_A\) receptor complex.

Acknowledgement

We thank Dr Eran Geller and Dr Joze Rupreht for their interest and helpful comments during the experiments. Dr Bert Van Duijn is acknowledged for his interest, encouragement and critical reading of the manuscript.

References