Changes in extracellular brain ascorbate in rat striatum in response to administration of non-volatile anaesthetic agents

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Summary
We used constant potential in vivo voltammetry to measure changes in striatal ascorbate in the rat in response to i.p. administration of several non-volatile anaesthetics. Propofol and pentobarbitone, which enhance GABA-mediated neuronal inhibitions, decreased ascorbate current to 27.0 (SEM 7.4) % and 46 (6) % of control, respectively. Chloral hydrate, diazepam alone or with ketamine, and fentanyl–fluanisone with midazolam produced no changes in ascorbate current. (Br. J. Anaesth. 1997; 78: 588–590).

Key words

In vivo voltammetry has been used extensively to monitor extracellular ascorbate in the brain. The extracellular concentration of ascorbate is 416±66 μmol litre⁻¹.¹ There is evidence from several different approaches that changes in neuronal activity are accompanied by changes in the extracellular concentration of ascorbate. There is extensive evidence that increased activity of glutamatergic pathways is accompanied by release of ascorbate.²

In this study we have used in vivo voltammetry to monitor changes in extracellular ascorbate in the rat striatum in response to i.p. administration of various non-volatile anaesthetic agents in order to test the hypothesis that extracellular ascorbate could be used as an index of the level of neuronal activity.

Methods and results
A carbon paste electrode was implanted stereotactically in the right striatum of male Sprague–Dawley rats (200 and 300 g) under anaesthesia. Extracellular brain ascorbate was measured using constant potential voltammetry.³ Experiments were performed 24 h after electrode implantation. Anaesthesia was assessed using palpebral, corneal and withdrawal reflexes. Muscle tone was evaluated by assessing resistance to flexion or extension of the limbs.

Results are given as mean (SEM); n = number of observations. Data were compared by Students’s t test using absolute values.

Basal ascorbate current values were 0.89 (0.80) nA (n = 21) (range 0.3–1.3 nA). After i.p. injection of saline there was a rapid increase in ascorbate, which returned to control values within 5–10 min (fig. 1A).

Two rats were given propofol 25 mg kg⁻¹ i.p. The rats were deeply sedated rather than fully anaesthetized for 45 (2.5) min. Transition from sedation to full recovery was slow. Sedation was accompanied by a decrease to 73.0 (7.4) % (P = 0.038, n = 8) of basal ascorbate current (fig. 1B) which showed slow recovery and reached basal values only when animals had recovered fully and resumed their normal behaviour.

Sodium pentobarbitone 40 mg kg⁻¹ was given i.p. to three rats. Rats were deeply anaesthetized for 91 (4) min and emerged from anaesthesia very slowly. During anaesthesia the ascorbate current decreased gradually to 54 (6.1) % from a baseline current of 0.84 (0.23) nA (P = 0.0034, n = 6) (fig. 1C). The decrease in ascorbate produced by sodium pentobarbitone was significantly greater than that produced by propofol (P = 0.05, Mann–Whitney U test).

Chloral hydrate 400 mg kg⁻¹ was given i.p. to two rats. Duration of anaesthesia was 107 (7) min, from which rats emerged rapidly and with little warning. There was no change in ascorbate current throughout the period of anaesthesia (fig. 1D). Fluctuations related to motor activity reappeared when animals were recovered fully and resumed their normal behaviour.

Diazepam 5 mg kg⁻¹ was administered i.p. to two rats. The animals remained still and sedated for 20–30 min. Basal ascorbate current remained constant throughout the experiment.

A mixture of fentanyl 0.25 mg kg⁻¹–fluanisone 0.8 mg kg⁻¹ and midazolam 0.4 mg kg⁻¹ produced anaesthesia of 35 (5) min duration with no change in ascorbate current throughout the period of anaesthesia.

Ketamine 100 mg kg⁻¹ mixed with diazepam 5 mg kg⁻¹ was given i.p. to three rats. Duration of anaesthesia was 28 (1.8) min (n = 5). There were no significant changes in ascorbate current during ketamine–diazepam anaesthesia.

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Comment

The results of our experiments, designed to test the hypothesis that extracellular ascorbate could be used as an index of the level of neuronal activity, showed only a partial correlation between reduction in ascorbate current and depth of anaesthesia. Although all drugs produced a significant reduction in behavioural activity and reflex responses, only propofol and pentobarbitone produced a reduction in ascorbate current. These differences are probably a result of different mechanisms of action, although all non-volatile anaesthetic agents reduce the cerebral metabolic demand for oxygen and depress electrophysiological activity. Most anaesthetics either enhance inhibition or depress excitation by interacting with receptors for GABA, glutamate or opioid peptides. A strong correlation exists between drug potency as modulators of the GABA<sub>A</sub> receptor and anaesthetic potency in vivo.4

Propofol and pentobarbitone are lipophilic compounds and act on the GABA<sub>A</sub> receptor at a site distinct from that of benzodiazepines, enhancing GABA-mediated transmission.5 Unlike barbiturates, the benzodiazepines diazepam and midazolam do not directly gate the Cl<sup>−</sup> channels of GABA<sub>A</sub> receptors but require GABA to express their effects. Voltammetric measurement of ascorbate in a previous study showed that an anxiolytic dose (3 mg kg<sup>−1</sup>) of diazepam had no effect on basal ascorbate current but reduced the stimulated increase in ascorbate.6 This suggests a different origin for basal and stimulated ascorbate.

Fentanyl mimics the action of opioid peptides acting selectively on μ opioid receptors, which blunt or eliminate responses to noxious stimuli. In microdialysis experiments, fentanyl was found to produce no change in the concentration of glutamate in striatal dialysate (unpublished).

The dissociative anaesthetic ketamine acts on glutamatergic NMDA receptors producing a state of sedation, immobility, amnesia and marked analgesia.

The results of this study suggest that anaesthetic agents which enhance GABA-mediated inhibition of neuronal activity produced a reduction in ascorbate. Although chloral hydrate shares the same mechanism of action as pentobarbitone and propofol, the increase in regional cerebral blood flow and dopamine implies that it produced some neuronal activation.

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References


Figure 1  Ascorbate current recorded from the striatum of a freely moving rat using voltammetry at carbon paste electrodes. A: Effects of saline (S) injection i.p.; B: effects of propofol (P) 25 mg kg<sup>−1</sup> i.p.; C: effects of pentobarbitone (Pent.) 50 mg kg<sup>−1</sup> i.p.; D: effects of chloral hydrate (CH) anaesthesia 400 mg kg<sup>−1</sup> i.p. Arrows indicate i.p. injection. Dark bars indicate anaesthesia; white bars indicate spontaneous activity.

