*Br. J. Pharmac.* (1982), **75**, 353–357

# THE ACTION OF GENERAL ANAESTHETICS ON ACETYLCHOLINE-INDUCED INHIBITION IN THE CENTRAL NERVOUS SYSTEM OF *Helix*

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**1** The effects of general anaesthetics, thiopentone, etomidate, minaxolone and ketamine were studied on identified voltage-clamped neurones of *Helix aspersa*.

**2** At concentrations of 0.1–0.5 mM, thiopentone, etomidate and minaxolone had no effect on the resting conductance of identified cells, D1 and D2. Ketamine at a concentration of 0.1–0.5 mM depolarized and excited the cells.

**3** All four anaesthetics tested depressed a chloride-dependent inhibitory response to acetylcholine (ACh) in cells D1 and D2 at concentrations of 0.1–0.5 mM in a dose-dependent and reversible manner with no change in the reversal potential of the response.

**4** These results show that general anaesthetics can block the transmitter-evoked chloride-mediated increases in membrane conductance in *Helix* neurones.

## Introduction

Previous work in *Helix* has shown that the barbiturate, thiopentone, is unselective in its actions in that both sodium-dependent excitatory events and chloride-dependent inhibitory events are depressed by the anaesthetic. In addition, potassium-dependent inhibitory events were potentiated by the same concentrations of thiopentone (Judge, Norman & Walker, 1979). Similar effects of barbiturates on inhibitory and excitatory responses, mediated by chloride and sodium ions respectively, to applied transmitters have been observed in *Aplysia* (Cote & Wilson, 1980). All of these effects are mediated at the postsynaptic membrane.

It is difficult to compare these findings with results from studies in vertebrate systems because the ionic mechanisms of postsynaptic responses in vertebrates are mostly unknown. However, potentiation of postsynaptic inhibition by barbiturates has been shown in the vertebrate CNS (Nicoll, 1972; 1975) and it is well established that excitatory responses to transmitters are depressed by barbiturates (Crawford, 1970; Ransom & Barker, 1975; Richards & Smaje, 1976; Nicoll, 1978). Depression of inhibitory postsynaptic responses by barbiturates has not been reported in vertebrate systems.

In view of the lack of reports on depression of inhibitory responses in vertebrates, we have investigated the effects of other general anaesthetic agents on the chloride-dependent inhibitory events in *Helix* neurones. The chloride-dependent change which follows activation of acetylcholine receptors in identified neurones of *Helix* was selected for this study.

A preliminary account of this investigation has already been published (Judge & Norman, 1980).

## Methods

Specimens of *Helix aspersa* (from Gerrard & Haig Ltd, East Preston) were used. The suboesophageal ganglia were removed from the animal and the brain placed on a small sheet of glass and secured firmly with elastic bands. The outer connective tissue sheath was dissected away with fine forceps to expose the cell bodies. Cells D1 and D2 in the left parietal ganglion were used for this study (Judge, Kerkut & Walker, 1976). The preparation was then placed in a 'Perspex bath' (volume 2 ml) where it was perfused continuously at a constant rate of 60 ml/h with Ringer solution (composition mM: NaCl 80, KCl 4, $CaCl_2$ 8, $MgCl_2$ 5 and Tris buffer 5; pH = 7.8). For low chloride Ringer solution, chloride was replaced with acetate.

The temperature was maintained at 20–22°C. Standard intracellular recording techniques were employed including the use of a voltage clamp (based on Dagan Corporation 8500 with modifications). Double-barrelled glass microelectrodes filled with 1 M KAc (resistance 10–12 MΩ) were used, one barrel for recording voltage and the other for passing

0007-1188/82/020353-05 $01.00

and recording current. Signals were displayed on a Tektronix 5111 oscilloscope and permanent records obtained by the use of a polaroid camera. Each experiment was performed at least 5 times.

Acetylcholine (ACh) was applied iontophoretically (20–100 nA; 1 s) onto the cells through a microelectrode containing a 1 M solution of ACh bromide (pH 5) using a microiontophoresis programmer (WP Instruments). Application was programmed using a Digitimer (D4050). The iontophoretic pulses were applied at a constant frequency throughout the experiment at a rate which did not result in desensitization.

General anaesthetics in solution at pH 7.8 were perfused over the preparation at a constant rate of 60 ml/h. Sodium thiopentone (May & Baker Ltd), etomidate (Janssen), minaxolone (Glaxo) and ketamine hydrochloride (Parke-Davis) were tested.

## Results

Cells D1 and D2 are large, silent neurones in the caudal region of the left parietal ganglion of *Helix aspersa* (Judge *et al.*, 1976). Iontophoretic application of ACh (20–100 nA current, 1 s duration) evoked inhibitory responses in the cells. The cells were clamped at the resting potential of approximately −60 mV and commanded to various membrane potentials of between −40 and −90 mV. At each membrane potential the holding current was measured and a passive I–V relationship for the cell obtained. ACh was applied (20–60 nA, 1 s) in a 3 min cycle to avoid desensitization of the response. ACh caused a large increase in conductance (Figure 1).

The responses to ACh can be seen in Figure 1 which shows oscilloscope recordings of ACh currents at varying membrane potentials between −90 and −50 mV. The relationship of the ACh peak current to membrane potential is shown in Figure 2. The line crosses the membrane potential axis at the reversal potential of the response (−65 to −70 mV). At membrane potentials more negative than −68 mV the response is seen as an inward clamp current. The response to ACh was unaffected by changes in external potassium concentration. When the extracellular chloride concentration was decreased from 115 mM to 33 mM, the reversal potential of the ACh current shifted from −65 mV to −50 mV. Therefore the ACh current was believed to be carried mainly by chloride ions.

When the perfusing fluid was changed from Ringer

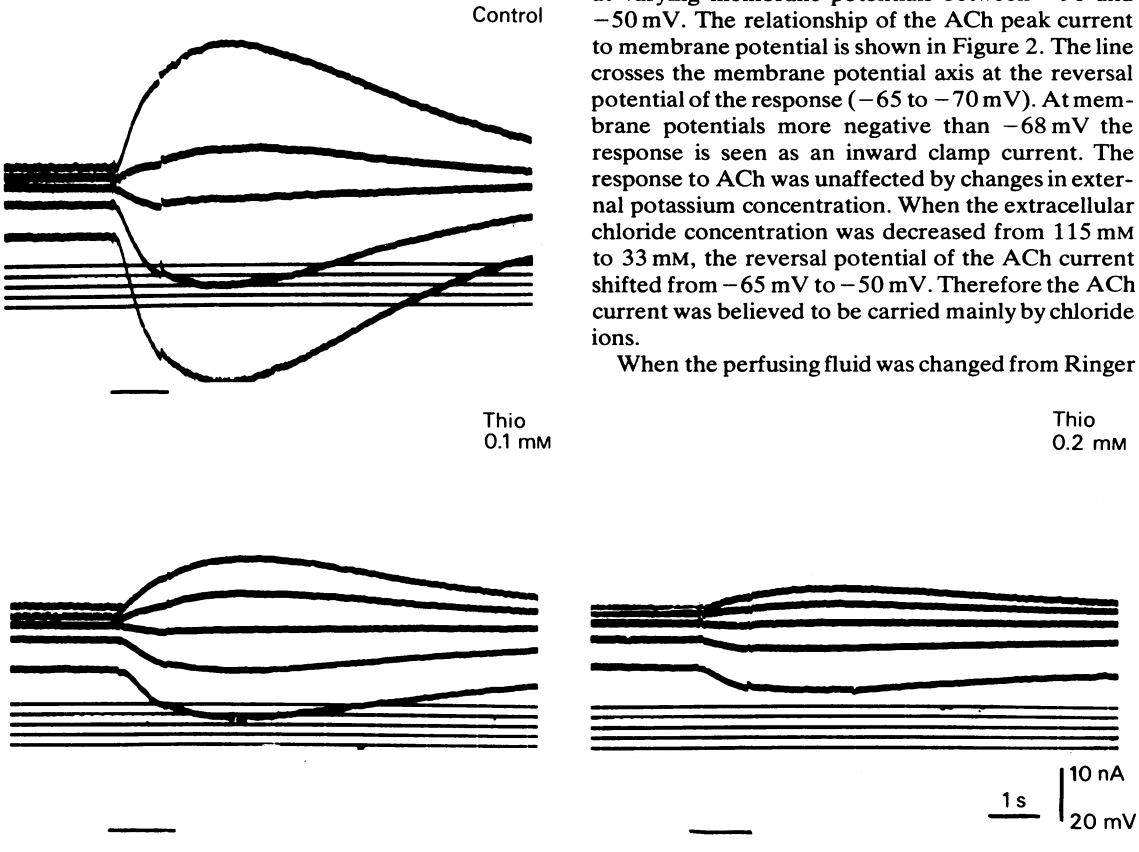


**Figure 1** Effects of thiopentone (Thio) on acetylcholine (ACh)-response in voltage-clamped neurone D2. The cell was clamped at membrane potentials from −90 mV to −50 mV (lower traces) and the ACh-clamp current was recorded at each holding potential (upper set of traces). The response to ACh was a large increase in conductance with a clamp current which reversed at −65 mV. In the presence of 0.1 mM and 0.2 mM thiopentone, the ACh conductance change and ACh clamp currents at all membrane potentials were depressed, in a dose-dependent manner. The responses recovered on washing (not shown). The reversal potential of the ACh response was unaffected by thiopentone.

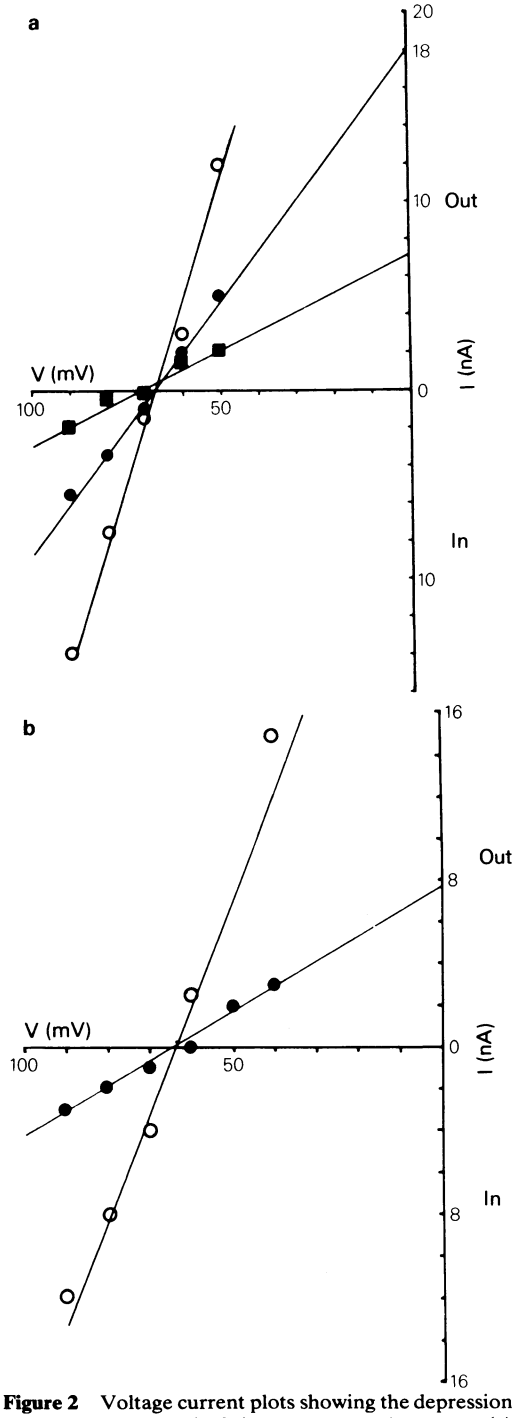


**Figure 2** Voltage current plots showing the depression of the acetylcholine (ACh) response by thiopentone (a) and etomidate (b). In the controls (○), the ACh-induced current reverses at −65 to −70 mV. (a) Thiopentone (● = 0.1 mM, ■ = 0.3 mM) and (b) etomidate (● = 0.1 mM) caused a depression of ACh-induced current without any change in the reversal potential of the response.

to a 0.1 mM solution of thiopentone in Ringer, the resting conductance of the cells remained unchanged. However, the ACh conductance increase was depressed by thiopentone (Figure 1) at all membrane potentials. This effect was dose-dependent (Figure 1) and fully reversible. The onset of the effect occurred within 2 min of perfusing the anaesthetic and the depression of the response was observed for as long as anaesthetic was present. For short applications of anaesthetic (up to 5 min), recovery was achieved by perfusing the preparation with Ringer for 3–6 min. Longer exposure to anaesthetic (20 min) required several hours for recovery to occur. The effect of 0.1 and 0.3 mM thiopentone on the ACh response is also shown in Figure 2a. Although the response was depressed by thiopentone there was no change in the reversal potential of the conductance change and the I–V relationship of the cell was still linear.

Etomidate at concentrations of 0.1–0.5 mM also depressed the ACh conductance change in these cells whilst having no effect on the resting conductance. The reversal potential of the response was again unchanged (Figure 2b). Recovery of the responses was obtained within 5–10 min of perfusing normal Ringer solution. Minaxolone, a steroid anaesthetic, gave similar results at concentrations of 0.05–0.5 mM.

In contrast, ketamine at concentrations of 0.1–0.5 mM was found to have a direct, excitatory effect on these cells, causing a decrease in net outward current (Figure 3). The effect occurred within

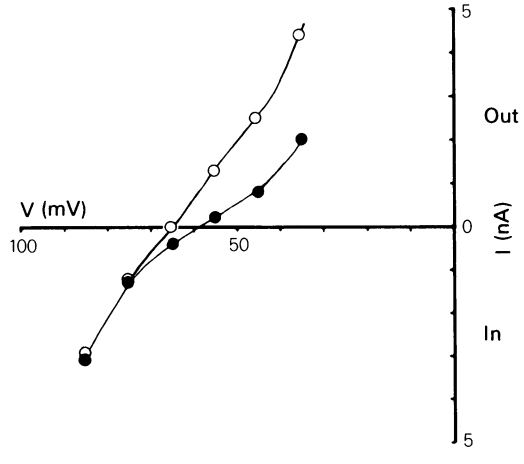


**Figure 3** Voltage-current plot of neurone D2 showing the effect of ketamine (0.3 mM) on the holding current. Unlike the other anaesthetics tested, ketamine caused a decrease in net outward current. The effect occurred within 1 min of application, was dose-dependent and did not persist for more than 1 min. In the unclamped cell this effect was observed as a transient depolarization (<5 mV). (○) = Control; (●) = after 30 s in 0.3 mM ketamine.

1 min of perfusing the anaesthetic, was dose-dependent but did not persist for more than 2 min. Within 2–3 min the ACh response was observed to be depressed as with the other general anaesthetics tested. This effect of ketamine was also dose-dependent and reversible on washing the preparation with normal Ringer solution.

## Discussion

The results show that at concentrations of 0.1–0.5 mM, thiopentone, etomidate and minaxolone have no effect on the resting conductance of identified cells D1 and D2 in *Helix* but that ketamine at the same concentrations has an excitatory effect on these cells. All four general anaesthetics tested depress the ACh-coupled conductance change in these cells which is mainly due to chloride. In *Aplysia*, Cote & Wilson (1980) have shown a depression of chloride-dependent inhibitory responses to both ACh and γ-aminobutyric acid by barbiturates. A chloride-dependent inhibitory response to glutamate in an identified cell in *Helix* has also been shown to be depressed by thiopentone (Judge *et al.*, 1979).

Other reports of similar studies show conflicting results. Barker (1975a,b) concluded from his work that general anaesthetics have a selective action on excitatory postsynaptic responses mediated by sodium ions. These depressant effects on sodium-mediated responses have also been shown in many other preparations (Barker, 1975a; Colton & Colton, 1977; Adams, Gage & Hamill, 1977; Judge *et al.*, 1979; Cote & Wilson, 1980) but the evidence now suggests that these effects are not selective for sodium-mediated events (see Judge, 1980 for review).

Inhibitory responses mediated by potassium ions are also sensitive to barbiturate anaesthetics. Judge *et al.* (1979) have shown a potentiation of dopaminergic potassium-dependent inhibitory events in *Helix* neurones while Cote & Wilson (1980) showed a slight depressant action of barbiturates on cholinergic potassium-dependent inhibitory events.

Due to the wide range of transmitters which evoke these responses in the invertebrate neurones, there is little reason to conclude that the action of general anaesthetics is due to an effect on the receptor-transmitter interaction. It seems probable that these compounds are acting at the level of the receptor-coupled ionophores.

One interesting possibility is that the time course of the receptor-coupled conductance changes determines the sensitivity of the receptor-ionophore to an anaesthetic. Sodium- and chloride-mediated responses are of fast onset and short duration in contrast to potassium-mediated responses. The difference in time course surely reflects the difference in the frequency of activation of receptor-coupled channels.

In invertebrates the potassium-mediated responses are either only slightly depressed (Cote & Wilson, 1980) or potentiated (Judge *et al.*, 1979) by barbiturates, whereas the sodium- and chloride-mediated responses are markedly depressed. This raises the possibility that a high frequency of opening of channels (and hence a potential of long duration) reduces the effectiveness of the anaesthetic. Alternatively, it may simply be the chemical identity of the channel which determines the frequency of activation and sensitivity to anaesthetics. Single channel work will prove invaluable in assessing whether anaesthetic action occurs at the level of the ionophore and in answering these questions.

We wish to acknowledge the gifts of etomidate (Janssen) and minaxolone (Glaxo). This work was supported by an M.R.C. project grant to S.E.J. We are grateful to Dr K.T. Wann for his interest and helpful comments during the preparation of this paper and to Mr G. Eastwood for building the voltage clamp.

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*(Received July 16, 1981.*
*Revised September 23, 1981.)*