Volatile anaesthetics inhibit a cyclic AMP-dependent sodium-potassium current in cultured sensory neurones of bullfrog

*¹T. Tokimasa, **K. Sugiyama, *T. Akasu & **T. Muteki

*Department of Physiology and **Anesthesiology, Kurume University School of Medicine, 67 Asahi-machi, Kurume 830, Japan

1 Cultured dorsal root ganglion cells of the bullfrog were voltage-clamped in the whole-cell configuration.

2 An adenosine 3':5'-cyclic monophosphate (cyclic AMP)-dependent cationic inward rectifier (I_H) was inhibited by bath application of enflurane (0.2–0.8 mM) and halothane (0.2–0.5 mM), which thereby induced an outward current at the resting potential, and a membrane hyperpolarization in unclamped cells.

3 The main effect of enflurane (0.5 mM) was to displace the steady-state I_H activation curve to a hyperpolarizing direction by about 10 mV, as well as to reduce the maximum H-conductance to about 20%.

4 Forskolin (1–10 μ M), which enhances I_H by producing a depolarizing shift in the I_H activation curve and increasing the maximum H-conductance, recruited I_H even when the current had already been eliminated by enflurane (1 mM).

Introduction

It has been proposed that general anaesthetics may affect metabolisms of intracellular cyclic nucleotides (Triner *et al.*, 1980; Vulliamoz *et al.*, 1986) and that adenosine 3':5'-cyclic monophosphate (cyclic AMP) regulates the resting membrane potential of vertebrate nerve cells by changing the voltage-dependence of I_H (Bobker & Williams, 1989; Pape & McCormick, 1989; Tokimasa & Akasu, 1990a). Therefore, the effects of enflurane and halothane were tested on I_H of bullfrog primary afferent neurones which is also regulated by cyclic AMP-dependent protein kinase (Tokimasa & Akasu, 1990b).

Methods

Small (200–250 g) bullfrogs (*Rana catesbeiana*) were used. The whole-cell I_H was recorded from A-cells at 22–24°C as described previously (Tokimasa & Akasu, 1990b): the cells were superfused with a nominally calcium-free Ringer solution containing tetrodotoxin (3 μ M, Sankyo), 4-aminopyridine (1 mM, Sigma) and the chloride salt of sodium (80 mM), potassium (5 mM), magnesium (10 mM), cobalt (2 mM), barium (2 mM) and tetraethylammonium (20 mM) (pH adjusted with 4 mM-HEPES, 1 mM-Tris and HCl to 7.2). A pipette solution contained Na₂ATP (5 mM, Sigma) and EGTA (1 mM, Sigma) (Tokimasa & Akasu, 1990a,b). The concentration of enflurane (Dinabott) and halothane (Takeda) in the recording chamber was determined by gas chromatography (see Fujiwara *et al.*, 1988). Statistics are expressed as mean $\pm$ s.e.mean for the cells tested.

Results

I_H was activated during step hyperpolarizations (10–70 mV, 1–10 s) from a holding potential less negative than –60 mV or the threshold of I_H activation. Cyclic AMP-dependence of the properties of I_H was essentially the same as described previously (Tokimasa & Akasu, 1990a,b). Bath application of enflurane (0.2–0.8 mM) decreased the amplitude of I_H in all twenty cells tested (Figure 1a–c) without significantly changing the reversal potential of I_H ; -27.1 ± 2.1 mV in control and -25.5 ± 2.2 mV in the presence of 0.2 mM enflurane

($n = 3$). The main effects of enflurane (0.5 mM) on I_H were as follows. First, enflurane caused an outward current at the resting potential (e.g. 165 pA at –75 mV in Figure 1a) by reducing a tonic inward flow of I_H , which thereby induced a membrane hyperpolarization (4–6 mV) in unclamped cells ($n = 3$, not shown). Second, the maximum H-conductance (12–21 nS at –120 mV) was reduced to $21 \pm 2\%$ ($n = 6$). Finally, the steady-state I_H activation curve now centred at -102.3 ± 3.1 mV as opposed to -91.6 ± 2.1 mV in control ($n = 5$). Consistent with this, I_H activated more slowly but deactivated more rapidly in the presence of enflurane than in the control ($n = 20$). In seven cells, the voltage-dependence of the time constant for the final exponential phase (see Tokimasa & Akasu, 1990a) of the activation (e-fold per 22.1 ± 2.3 mV) and deactivation (e-fold per 15.5 ± 1.4 mV) was not significantly changed by enflurane. In all three cells, essentially the same results as with enflurane were obtained with halothane (0.2–0.5 mM, not shown).

Enflurane (0.5–0.8 mM) depressed I_H even when the current had already been enhanced by forskolin (10 μ M, Sigma) in all three cells tested. Enflurane now produced an outward (hyperpolarizing) shift of the holding current even at the holding potential of –50 mV ($n = 2$). These observations are compatible with the forskolin-induced displacement in the threshold potential for I_H from –60 to about –45 mV (Tokimasa & Akasu, 1990a,b). Forskolin (10 μ M) recruited I_H even when the current had already been depressed by 0.5 mM enflurane ($n = 3$, Figure 1d) or eliminated by 1 mM enflurane ($n = 1$, not shown). However, comparable I_H recruitment by forskolin was never encountered when I_H was eliminated by 2 mM caesium ($n = 3$).

Discussion

The results presented here have demonstrated that enflurane and halothane at concentrations similar to those used clinically (0.2–1.2 mM, see Fujiwara *et al.*, 1988) inhibit I_H , an action opposite to that of forskolin but quite similar to that of protein kinase inhibitors such as H-8 (Tokimasa & Akasu, 1990a,b). Therefore, it can be speculated that primary target sites for the general anaesthetics could be related to direct actions of the anaesthetics on adenylate cyclase, phosphodiesterase or cyclic AMP-dependent protein kinase. The interaction between forskolin and enflurane on I_H (Figure 1d) may

¹ Author for correspondence.

Figure 1 I_h inhibition by enflurane (0.5 mM). Results in (a)–(c) were obtained from a single cell. The resting potential was -75 mV when examined in the normal Ringer solution. (a) I_h was activated either briefly (1 s) during step hyperpolarizations from the holding potential (HP) of -50 to -100 mV (upper left panel) or continuously by setting the HP at -75 mV (lower left panel) and -100 mV (right panel). I_h was then deactivated for 1 s during step depolarizations to -50 mV either from -75 or -100 mV. Each column within the panels represents I_h before (left), during (middle) and after washout (right) of enflurane. Enflurane caused 170 pA outward shift of the holding current at HP -75 mV (700 pA at -100 mV). (b) The steady-state I_h activation curve in control ($\square$, $\circ$) and in the presence of enflurane ($\blacksquare$, $\bullet$). $I/I_{\max}$ denotes the fractional I_h at given potentials (Tokimasa & Akasu, 1990a). Inset represents a semi-log plot of $I/(I - I_{\max})$ showing that a hyperpolarizing shift in the half-activation voltage of the activation curve from -89 mV to -102 mV. (c) Sample recordings plotted in (b). Commanding levels are indicated beside each current trace. (d) Results were obtained from another cell. Ordinate scale denotes the amplitude of the activating I_h at -95 mV (HP -60 mV) during the course of experiments in which $10 \mu\text{M}$ forskolin was added to the superfusate for a period indicated by a solid bar and then to the superfusate containing enflurane (a hatched bar denotes the bath application of enflurane). Insets show the actual current traces at the time indicated by filled circles and arrows.

strengthen our working hypothesis. Alternatively, enflurane and halothane might change the conformation of some sites within the channel molecule at which the phosphorylation occurs by acting, for example, on the lipid surrounding the channel (Bean *et al.*, 1981; Haydon & Urban, 1986). Further experiments are in progress to answer these questions.

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