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Research Article

Modulation of the Passive Membrane Properties of a Pair of Strongly Electrically Coupled Neurons by Anaesthetics

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Abstract

Differential effects of volatile general anaesthetics and sodium pentobarbital on input resistance [R_m], membrane time constant [τ_m] and capacitance [C_m] of VD1 and RPD2 neurons of Lymnaea are reported in this paper. The volatile anaesthetics halothane and isoflurane caused a significant reduction in the input resistance, membrane time constant and capacitance in a dose dependent manner and halothane was more potent than isoflurane. The clinical concentration [100 μM] of pentobarbitone caused no change in R_m, τ_m and C_m of VD1 and RPD2, whereas 2.0 and 0.5 mM pentobarbitone increased them significantly during phase I but caused no change in these parameters during phase II.

Keywords: *General Anaesthetics; Electrical Coupling; Identified Neurons; Input Resistance; Time Constant; Capacitance*

Abbreviations

C_m: Membrane Capacitance; R_m: Membrane Input Resistance; τ_M: Membrane Time Constant; PDS: Paroxysmal Depolarizing Shift; RPD2: Right Parietal Dorsal Neuron 2; VD1: Visceral Dorsal Neuron 1

Introduction

Electrical synapses [gap junctions] are now known to be important in promoting synchronicity in many regions of the mammalian brain [1], in the brains of lower vertebrates [2] and also in invertebrate preparations [3] as first described in the crayfish by Furshpan and Potter [4,5]. In the pond snail *Lymnaea stagnalis* there are numerous examples of electrically coupled neurons and neural clusters [6-9]. Recent evidence has shown that gap junctions may exhibit synaptic plasticity, being dynamically regulated by neuromodulators such as dopamine [10]. It is therefore of some importance that the actions of volatile and systemic anaesthetics on electrical synapses should be understood, although a previous study suggested that electrical synapses are less sensitive to most anaesthetics than chemical synapses [11].

In a previous paper [12] we demonstrated that barbiturates and volatile anaesthetics have markedly different effects on electrically coupled neurons. For example, volatile anaesthetics cause a significant reduction in the coupling coefficient between the giant VD1 and RPD2 neurons of *Lymnaea stagnalis* [12] and also between the smaller neurons of the left and right pedal A clusters [9]. However, sodium pentobarbitone, which is widely used as a veterinary anaesthetic [13,14] had very different actions. Pentobarbitone, within the clinical concentration range, caused no change in the coupling coefficient though the action potential was abolished and the cells became quiescent. In contrast, 0.5 and 2.0 mM pentobarbitone first generated paroxysmal depolarising shifts [PDS] while increasing the coupling coefficient but it then returned to control values as the neurons became quiescent.

The effects described above are may be partly caused by alterations in the passive membrane properties of the nerve cells for instance by altering the membrane input resistance [R_m] and capacitance [C_m] which will also have direct effects on the membrane time constant [τ_m]. The details of these passive properties of nerve cell membranes are well described elsewhere [15,16], but although subthreshold changes in passive membrane properties may reflect changes in the lipid membrane, other components of the membrane will also be affected. Here we describe the actions of the same concentrations of these anaesthetics as we used in our previous experiments [12] on the passive membrane properties of VD1 and RPD2: i.e. R_m, τ_m and C_m. Preliminary data have appeared elsewhere [17].

Methods

Intact *Lymnaea* brain preparations were prepared and intracellular recordings of neuronal electrical activity were made from target neurons according to the methods of Benjamin and Winlow [6] and Qazzaz and Winlow [12]. Sub-threshold hyperpolarizing current pulses [0.1 nA for 1 sec] were injected into VD1 or RPD2 neurons via the recording microelectrode using Neurolog NL102 bridge balance preamplifiers. Data was captured using a CED 1401 data acquisition interface and a personal computer with Spike2 data capture and analysis software installed. All data were statistically analyzed using the paired Student's t-test and expressed as mean ± standard error of the mean [SEM]. Values were considered statistically significant at a confidence level of 95%. All recordings were made in the summer months (June to August) since date collected at other times of year were variable and inconsistent.

When a square current pulse is injected into a neuron two individual and separate stages are seen before it reaches the final potential value. First, there is a very fast stage followed by a slower exponential stage (Figure 1). This is due to the membrane acting as a capacitor, where the membrane represents a good insulator between two good electrolytic conductors. Being a capacitor means that it stores charge across its surface opposing any changes in its potential. Membrane capacitance can be defined as the ratio between the charge separated by the membrane and the change in its potential. Furthermore, membrane capacitance is directly proportional to its surface area is and inversely proportional to its thickness.

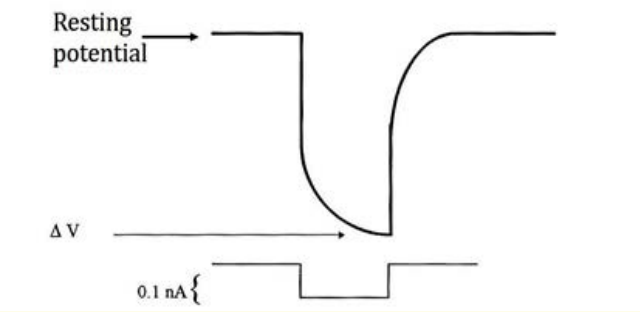

Figure 1: *Calculating input resistance - Injection of a hyperpolarizing current pulse into a neuron cell body causes a drop in membrane potential for the duration of the pulse where ΔV [change in membrane voltage] can be calculated from Ohm's law [ΔV = I.Rm] where I is the applied current and R_m is the calculated input resistance of the cell membrane.*

To calculate membrane capacitance, two passive properties are required; the input resistance and membrane time constant. The former can be determined by passing constant current pulse through the neuronal membrane to earth as shown in Figure 1. Knowing both the applied current and the induced voltage change the input resistance of the membrane can be calculated according to Ohm's Law [voltage [V] = current [I] x input resistance [Rm]] The membrane time constant is defined as the time needed for a current pulse to charge the membrane capacitance to 63% of its final value [15]. Figure 2 illustrates how to measure the time constant [τ_m] and the equation below demonstrates the relationship between the membrane time constant [τ_m], input resistance [R_m] and capacitance [C_m]:

$$\tau_m = R_m \times C_m$$

Membrane capacitance [C_m] was calculated using input resistance and the time constant data, according to this equation.

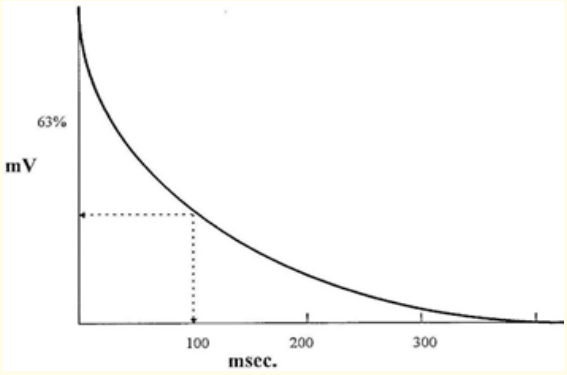

Figure 2: *Measuring membrane time constant - A diagram showing the change in membrane potential during the exponential stage as a function of time after the injection of a hyperpolarising current pulse. Membrane time constant [τ_m] is calculated by measuring the time at 63% of the potential change. Adapted from Kandel [15].*

Results

Input Resistance

The input resistance of VD1 and RPD2 were measured separately in the presence and absence of the anaesthetics. Because VD1 and RPD2 are strongly electrically connected, in 10 preparations we either crushed the commissure between the visceral and right parietal ganglia to isolate the neurons from one another or destroyed one of the pair of neurons. There was no change in the input resistance of either neuron under these conditions.

Effects of halothane: Both neurons became quiescent in 1% and 2% halothane as previously reported [12]. The control input resistance of VD1 [285 ± 19 MΩ; n = 10] and RPD2 [344 ± 11 MΩ; n = 8] significantly decreased [193 ± 6 MΩ and 243 ± 7 MΩ respectively] after application of 1% halothane. 2% halothane further decreased the input resistance of both neurons [143 ± 6 MΩ and 163 ± 7 MΩ respectively] and these effects were completely reversed by washing in anaesthetic free saline as illustrated in Figure 3 for RPD2.

Effects of isoflurane: Isoflurane was applied as for halothane and at the same percentage concentrations, again resulting in quiescence of both neurons. The control input resistances of VD1 and RPD2 were 277 ± 10 MΩ [n = 10] and 344 ± 11 MΩ [n = 8] respectively. As with halothane, isoflurane decreased the input resistance reversibly in a dose dependent manner, as illustrated in Figure 3. The input resistances of VD1 and RPD2 in 1% halothane were 245 ± 7 MΩ and 290 ± 12 MΩ respectively and in 2% isoflurane the respective values were 219 ± 7 MΩ and 205 ± 7 MΩ.

Effects of sodium pentobarbitone: Three concentrations of pentobarbitone were applied, 100μM [within the clinical range], 0.5mM and 2mM, but the order of application was varied and in some cases, they were applied after halothane and/or isoflurane with a 30 min delay between each concentration and/or drug. No differences in the responses to pentobarbitone were noted as a consequence of these different application regimes.

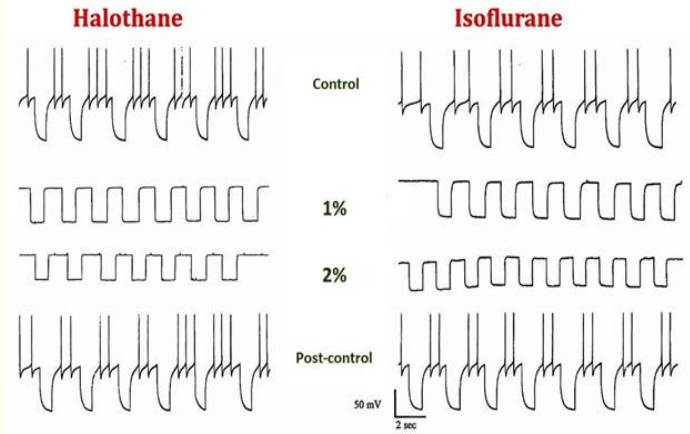

Figure 3: *Effects of halothane and isoflurane on RPD2 – The effects on VD1 [not shown] were similar. The actions of the anaesthetics on both neurons were dose dependent and both neurons became quiescent in the presence of the anaesthetics. Halothane was more potent in reducing the input resistance than isoflurane as demonstrated in Figures 5 and 6.*

Application of 2mM pentobarbitone: 2mM pentobarbitone produced two distinct phases of activity in VD1/RPD2 (Figure 4a) as previously reported [12]. Phase I started within a few seconds of the application of the drug and action potential activity was modified into paroxysmal depolarizing shifts [PDS] within a few minutes. This was followed by quiescence of the neurons in phase II. There was a clear and distinctive difference in the input resistances of VD1 and RPD2 in these two phases. In phase I, from control values of 298 ± 7 MΩ [n = 12] and 355 ± 9 MΩ [n = 14] for VD1 and RPD2 respectively, the input resistances significantly increased to 373 ± 8 MΩ and 410 ± 14 MΩ respectively. However, the input resistances were not significantly different from control values in phase II [288 ± 10 MΩ and 356 ± 9 MΩ respectively].

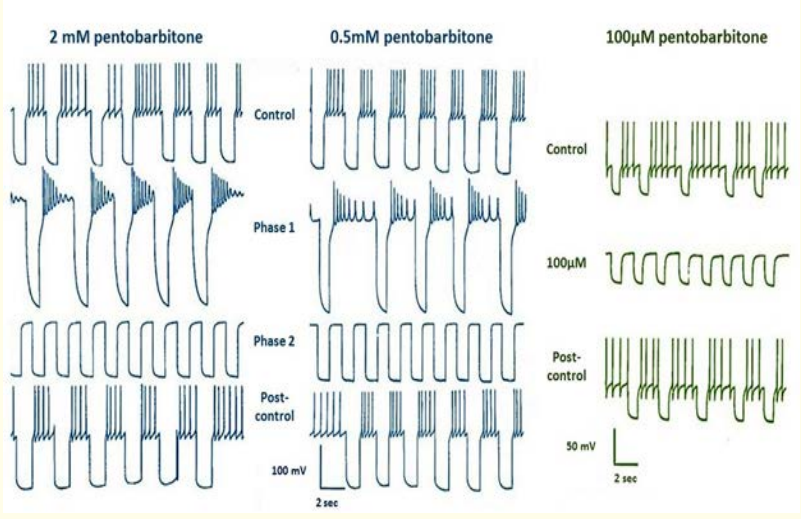

Figure 4: *Effects of sodium pentobarbitone on VD1 on input resistance – The effects on RPD2 [not shown] were similar. High concentrations of sodium pentobarbitone caused paroxysmal depolarizing shifts [PDS] [phase 1] followed a few minutes later by quiescence [phase 2]. During phase 1, Rm increased significantly, but this was not dose-dependent and then declined to control values in phase 2, again not dose dependent at these higher concentrations. At clinical concentrations of 100 µM, the neurons simply became quiescent without any intervening PDS, but Rm did not differ from control values.*

Application of 0.5mM pentobarbitone: Very similar results were obtained as compared with 2mM pentobarbitone, with apparently no dose-dependency at these concentrations outside the clinical range, but their effects were completely reversible (Figure 4b). Control values for input resistance of 286 ± 7 MΩ [n = 12] and 369 ± 11 MΩ [n = 14] were obtained for VD1 and RPD2 respectively. During phase I these values increased to 362 ± 13 MΩ and 427 ± 13 MΩ respectively, while during the quiescence of phase II there was no significant difference from control values [287 ± 12 ± 9 MΩ MΩ and 376 ± 12 MΩ respectively].

Application of 100μM pentobarbitone: 100μM pentobarbitone, which is in the clinical range, had little effect on input resistance of VD1/RPD2, although both cells became quiescent (Figure 4c). Control values were 295 ± 13 MΩ [n = 8] and 322 ± 8 MΩ [n = 6] respectively, which were little different from experimental values of 280 ± 15 MΩ and 329 ± 10 MΩ respectively.

The data for pentobarbitone, isoflurane and halothane are summarized in Figure 5.

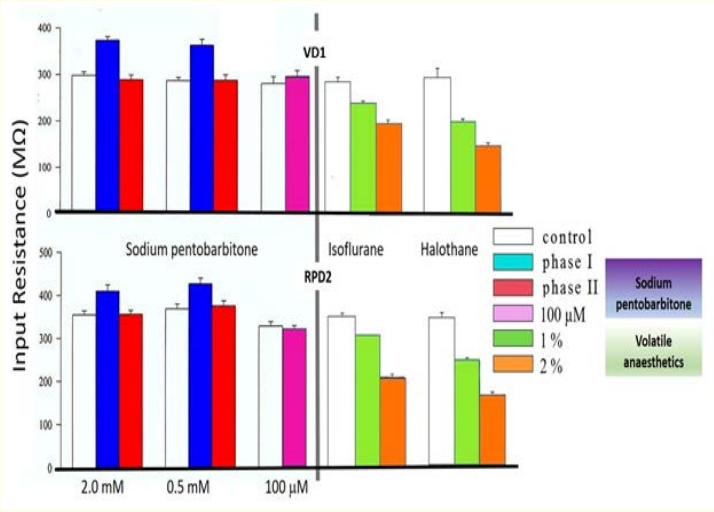

Figure 5: *Summary of the actions of sodium pentobarbitone, halothane and isoflurane on input resistance of VD1 [upper panel] and RPD2 [lower panel]. The Rm of VD1 was significantly increased during the PDS phase [I] induced by both 0.5 mM and 2.0 mM pentobarbitone [n = 10, P ≤ 0.01] but there was no significant change in phase II in comparison with control when the cells became quiescent. Rm was significantly reduced by both 1% and 2% isoflurane in a dose dependent manner [n = 10, P ≤ 0.01]. 1% and 2% halothane induced a greater reduction in Rm in comparison with isoflurane [≤ 0.01] again in a dose dependent manner. The input resistance of RPD2 was affected by the two volatile anaesthetics [n = 12, P ≤ 0.01] and barbiturates [n = 12, P ≤ 0.01] in a similar way to VD1. Error bars represent mean ± standard error.*

Time Constant and Membrane Capacitance

The time constant can be affected differently depending upon the drug or anaesthetic agent as shown in figure 6. Clearly the changes in time constant and input resistance also determine changes in membrane capacitance and changes in both τ_m and C_m are summarized as percentages of control values for VD1 and RPD2 in Figures 7 and 8.

The calculated C_m of VD1 and RPD2, showed that control values of 0.43 ± 0.036 nF [n = 10] and 0.57 ± 0.042 nF [n = 8] decreased to 0.27 ± 0.017 nF and 0.32 ± 0.021 nF with 1% halothane and 0.17 ± 0.015 nF and 0.22 ± 0.019 nF with 2% halothane respectively (Figure 8).

Halothane: Perfusion of the CNS with HBS-containing halothane [1% and 2%] produced a significant reduction of τm in both neurons VD1 and RPD2. Control values of 121 ± 11 msec and 202 ± 14 msec respectively were decreased to 54 ± 7 msec and 77 ± 6 msec after the application of 1% halothane and 29 ± 05 msec and 36 ± 09 msec with 2% halothane. In all experiments, the control values were regained when the CNS was washed with HBS (Figure 7).

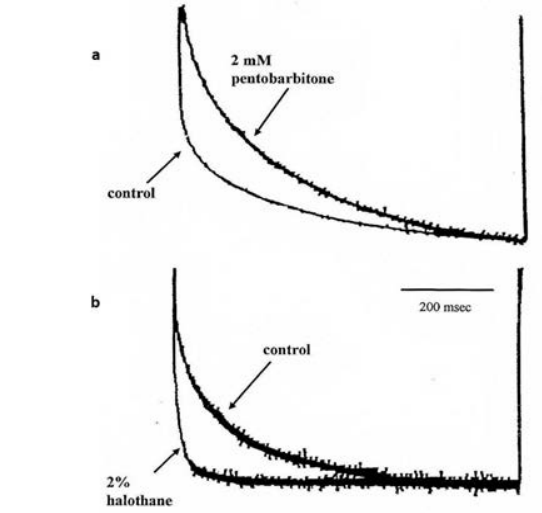

Figure 6: *An example to demonstrate the differential effects of general anaesthesia on membrane time constant. The exponential curves in the diagrams a and b represent the second stage of an injected hyperpolarizing current pulse into a single VD1 neuron. [a] Shows the effect of 2 mM pentobarbitone on the control [τ = 121 ± 11 msec]. After application, the time constant increased significantly [τ = 201 ± 08 msec; n = 12]. [b] In sharp contrast 2% halothane significantly reduced the time constant [τ = 29 ± 05 msec; n = 10].*

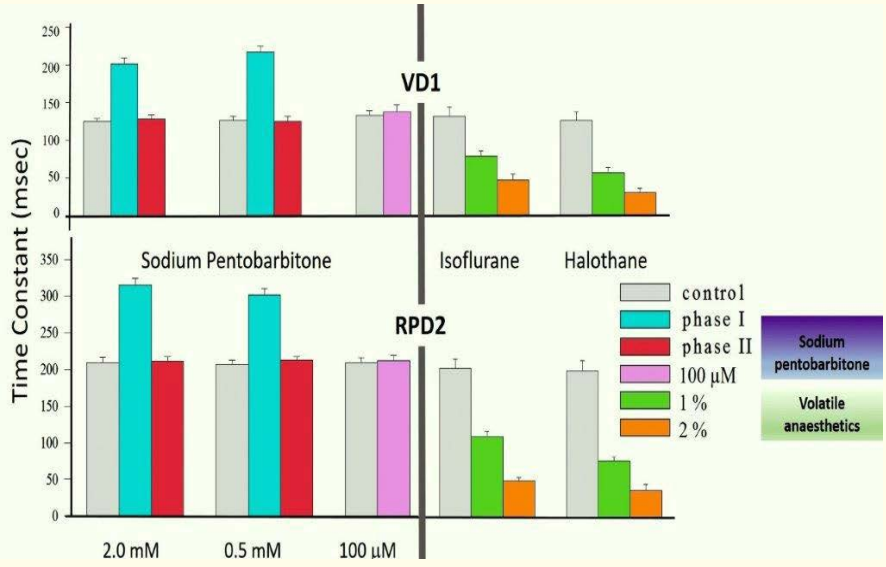

Figure 7: *Effects of volatile anaesthetics and barbiturates on time constant of VD1 and RPD2 neurons. The different anaesthetics [halothane, isoflurane and pentobarbitone; at different concentrations] were applied to the CNS in different order. These anaesthetics did not alter the effect of each other. Volatile anaesthetics decreased the time constant in a dose dependent manner and the effect of halothane was greater than that of isoflurane. However, there was no significant change in their values from the control value when 100 μM pentobarbitone was applied. Furthermore, 2.0 mM and 0.5 mM pentobarbitone significantly increased their time constant during phase I, but they showed no significant difference from their control value in phase II. Note that the bars were calculated as a percentage of the halothane control. Bars are mean ± SEM.*

Isoflurane: Isoflurane produced similar results to those produced by halothane but its effects were slightly weaker. 1% isoflurane significantly reduced the τ_m of VD1 and RPD2 from the control value [0.126 ± 12 msec, n = 10 and 205 ± 13 msec, n = 8] to 75 ± 07 msec and 111 ± 08 msec respectively. A greater reduction was demonstrated with 2% isoflurane [45 ± 07 msec and 50 ± 04 msec] (Figure 7).

When C_m of VD1 and RPD2 was calculated, it was found to be reduced in a dose dependent manner after perfusing the CNS with HBS-containing 1% and 2% isoflurane. Control values of 0.45 ± 0.42 nF [n = 10] and 0.55 ± 0.034 nF [n = 8] were decreased to 0.31 ± 0.023 nF and 0.39 ± 0.033 nF by 1% and 0.20 ± 0.019 nF and 0.24 ± 0.014 nF with 2% (Figure 8).

Sodium pentobarbitone: Pentobarbitone affected the measured parameters of the VD1 and RPD2 neurons differently from that produced by volatile anaesthetics.

Effects of 2 mM pentobarbitone: During phase I of the effect of 2 mM pentobarbitone, τ_m of VD1 and RPD2 was found to increase significantly from the control [125 ± 04 msec [n = 12] and 209 ± 08 msec [n = 14]] to 201 ± 08 msec and 315 ± 09 msec respectively. No significant difference from the control values in phase II was noticed [128 ± 06 msec and 211 ± 07 msec respectively] (Figure 7).

2 mM pentobarbitone produced an increase in the Cm of VD1 and RPD2 during phase I [0.58 ± 0.022 nF [n = 12] and 0.71 ± 0.028 nF [n = 14] compared to controls of 0.44 ± 0.018 nF and 0.57 ± 0.035 nF respectively. However, no significant change was noticed during phase II [0.43 ± 0.015 nF and 0.57 ± 0.019 nF respectively (Figure 8).

Effects of 0.5 mM pentobarbitone - After the application of 0.5 mM pentobarbitone on the neurons VD1 and RPD2, their τ_m was found to increase significantly from their controls [126 ± 06 msec, n = 12 and 207 ± 06 msec, n = 14] to 217 ± 08 msec and 302 ± 08 msec respectively (Figure 7). These values returned almost to control values during phase II [125 ± 07 msec and 213 ± 05 msec respectively.

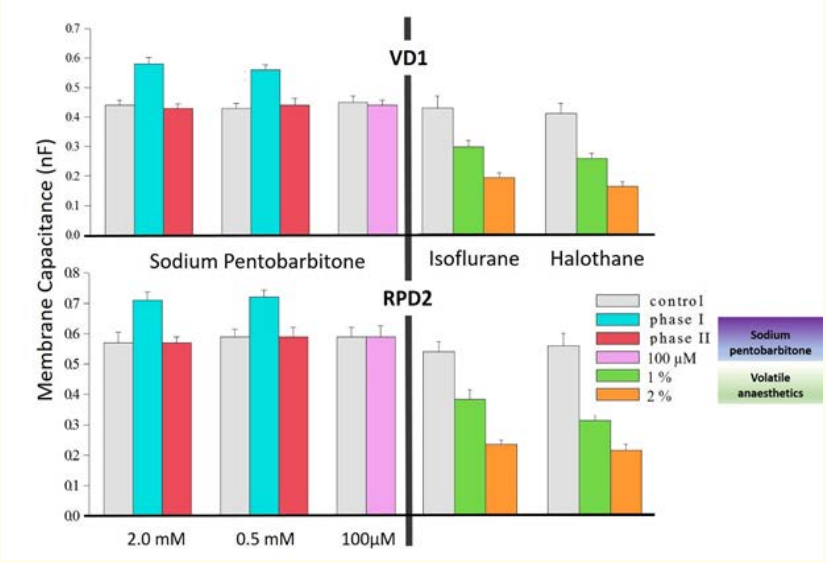

Figure 8: *Effects of volatile anaesthetics and barbiturates on membrane capacitance of VD1 and RPD2 neurons. The different general anaesthetic agents [halothane, isoflurane and pentobarbitone] was tested at different concentrations and their application was in different order. They did not alter the effect of each other on membrane capacitance. Volatile anaesthetics decreased the membrane capacitance in a dose dependent manner with the effect of halothane being greater than that of isoflurane. However, there was no significant change in its value, from its control value, when 100 µM pentobarbitone was applied. Furthermore, 2.0 mM and 0.5 mM pentobarbitone significantly increased the membrane capacitance of VD1 [a] and RPD2 [b] neurons during phase I, but they showed no significant change in their capacitance value in phase II. Note that the bars were calculated as a percentage of the halothane control. Bars are mean ± SEM.*

Similar results to those with 2 mM pentobarbitone were gained after the application of 0.5 mM pentobarbitone. Control values of C_m for VD1 and RPD2 were 0.43 ± 0.016 nF [n = 12] and 0.59 ± 0.025 nF [n = 14] respectively. There was a significant increase in their C_m during phase I [0.56 ± 0.017 nF and 0.72 ± 0.032 nF] but no significant difference from the control values during phase II [0.44 ± 0.023 nF and 0.59 ± 0.013 nF] respectively (Figure 8).

Effects of 100 μM pentobarbitone: After the application of 100 μM pentobarbitone, the effects on τ_m and C_m of VD1 and RPD2 were completely different from the effect of the other concentrations. There was no significant difference on either of these parameters from the control [τ_m = 133 ± 06 msec [n = 8] and 209 ± 07 msec [n = 6] which became 138 ± 09 msec and 212 ± 07 msec (Figure 7) and [C_m] were equal to 0.45 ± 0.021 nF [n = 8] and 0.59 ± 0.031 nF [n = 6] and became equal to 0.44 ± 0.017 nF and 0.59 ± 0.036 nF respectively (Figure 8).

Discussion

It is already known that the effects of general anaesthetics are very complex and, furthermore, the effects on one neuron cannot be generalized to other neurons [18-20]. Over the years, a variety of effects on ionic currents, intracellular components and on chemical and electrical synapses have been reported [12,21-29]. Here we have shown that there are distinct differences between the effects of volatile anaesthetics and barbiturates on the same neuron. The data we report clearly demonstrate that that the application of the volatile anaesthetics isoflurane and halothane reduce Rm, Cm and τ_m in a dose dependent manner, whereas the responses to sodium pentobarbitone were quite different with a significant rise in all parameters in phase I, followed by a fall to control values in quiescent phase II.

Variability between nerve cells: The effects of anaesthetics on neuronal function are very diverse, for example halothane caused different effects on different neurons in the same and in different species. It hyper-activates some *Lymnaea* neurons, such as in the CGCs, J cells, right parietal A group and visceral M group causing them to exhibit PDS [19]. This response was seen in the intact brain [20,30] as well as in isolated cultured neurons [31]. In sharp contrast, other *Lymnaea* neurons became hyperpolarized and consequently quiescent [20,26 32,33]. Indeed, the spontaneous firing activity of RPeD1, RPD1 and VV1/2 [20] and VD1 and RPD2 [26] decreased, eventually leading to quiescence after the application of halothane or isoflurane. In a study on the small pedal A cluster neurons of *Lymnaea* [9] we discovered that they became quiescent after the application of volatile anaesthetics, with no observed change in the resting membrane potential. This indicates that general anaesthetics shut off the voltage-gated channels involved in action potential generation confirming the results of Winlow and Yar [34] in pedal I cluster neurons, who recently demonstrated that a high-voltage activated Ca current was reversibly depressed in a dose dependent manner by the exposure of cultured *Lymnaea* neurons to clinical concentrations of halothane, again in pedal I-cluster neurons [35]. Similarly, Winlow., et al. [36] found that general anaesthetics blocked voltage-gated calcium and potassium currents [25,36]. The likely molecular targets of general anaesthetics are discussed in detail elsewhere [28,29].

What is happening in the neuronal membrane?

Time constant and Membrane capacitance

It could be argued that the effects of anaesthetics on the measured τm and the calculated C_m are entirely due to interactions with membrane lipids, since under normal circumstances C_m is not expected to alter very much, but small changes could occur due to changes in the physical properties of the cell membrane [specifically in the lipid bilayer], i.e. surface area or thickness [15]. If the neuronal membrane was a true capacitor any increase in τ_m and C_m would suggest that membrane had become thinner while a decrease in τm and C_m would suggest membrane thickening. Thus, the volatile anaesthetics which decrease C_m would thicken the membrane while the barbiturate would be expected to thin it during phase I. However, cell membranes consist of both lipids and proteins and surround complex intracellular constituents which may have effects on the membrane and may themselves be modified by applied anaesthetics. Since the now outdated lipid hypothesis of general anaesthesia has been replaced by the membrane protein hypothesis of general anaesthetic action [29,37] it is more probable that the changes in the passive membrane properties induced by anaesthetics are due largely to effects on membrane proteins. In particular, these same results could also be produced by an increases or decreases in membrane conductance [≡ increases or decreases in input resistance respectively] i.e. the membrane becomes more or less leaky, perhaps due to the direct actions of anaesthetics on membrane proteins.

Input resistance

Actions of volatile anaesthetics: Our data clearly demonstrate that volatile anaesthetics and sodium pentobarbitone have differential effects on input resistance and we suspect that they are due to modifications of membrane proteins by the anaesthetics. Volatile anaesthetics are known to inhibit or potentiate ligand-gated and voltage-gated ion channels at clinically relevant concentrations [29]. Franks and Lieb [33] reported that, in the CNS of *Lymnaea* there are two different types of neurons; sensitive and insensitive neurons to anaesthetics. They suggested the presence of a novel potassium current in the sensitive neurons which they called the anaesthetic-activated potassium current [$I_{K[an}$]]. They proposed that this current was activated by the application of halothane, but the activation of $I_{K[an}$] caused the membrane potential to hyperpolarize far below the threshold that is necessary to generate an action potential. We previously demonstrated that the membrane potentials of VD1 and RPD2 were significantly hyperpolarised after the application of halothane or isoflurane and during phase II of 0.5 or 2.0 mM pentobarbitone [9,27] in agreement with of Franks and Lieb [27] and Nicoll and Madison [38]. Thus, a reduced input resistance indicates an increased neuronal conductance implying the presence of either $I_{K[an}$] or other anaesthetic potentiated currents in VD1/RPD2.

Actions of pentobarbitone: The application of pentobarbitone [100 μM] abolished the action potentials and slightly hyperpolarised the membrane of VD1 and RPD2 neurons with no change in their input resistance. Calcium-activated potassium current is one possible pathway through which general anaesthetics hyperpolarise neuronal membranes. Alternatively, activation of a chloride current is another possibility. γ-amintobutyric acid [GABA] is the principle inhibitory amino acid neurotransmitter known to induce a chloride current [33,38-40]. Nicoll and Madison [38] demonstrated that a reduction in the extracellular concentration of chloride in frog motoneurons had no effect on the hyperpolarisation caused by anaesthetics. They concluded that general anaesthetics do not produce their effects by increasing chloride conductance. However, sodium pentobarbitone also causes hyperpolarization of VD1/RPD2 and this is mimicked by 100μM GABA which causes hyperpolarization (from -45.5Mv to -53.4 mv, n = 7; P = 0.002 – Moccia., *et al*. [40]) of the giant dopamine containing, multiaction neuron RPeD1 [42] in *Lymnaea* via $GABA_A$ receptors. We therefore suggest that a GABAA chloride current is responsible for the pentobarbitone induced hyperpolarization in VD1/RPD2.

Moody., *et al.* [43] found that inhalational anaesthetics increase the chloride conductance in rat cortical synaptoneurosomes and this was supported by findings from Nakahiro., *et al*. [44] on cultured rat dorsal root ganglion neurons. They concluded that inhalational anaesthetics augmented the sustained current evoked by GABA and this work was supported by several further studies [39,40] and Zimmerman., *et al*. [45] found that the responses to the application of GABA to rat hippocampal neurons were prolonged by many general anaesthetics. Thus, the actions on GABA receptors are not limited to barbiturate anaesthetics.

Paroxysmal depolarising shifts [PDS]: VD1 and RPD2 neurons generated PDS after the application of 0.5 and 2.0 mM pentobarbitone before becoming quiescent. Furthermore, during which the input resistance was significantly increased. PDS is a kind of neuronal epileptiform activity that can be produced under pathological conditions in different species, which "typically consists of an abrupt depolarisation of the cell membrane to a level at which the cell is unable to generate a full-sized action potential, usually followed by repolarisation for a brief period before the membrane depolarises again, producing square-wave membrane potential oscillation" [46]. A full explanation of PDS is given elsewhere [47]. However it is believed to be connected to rising intracellular calcium concentration [Ca^{2+}]I [46-49] and Moghadam and Winlow [50] have demonstrated that pentobarbitone raises [Ca^{2+}]I while blocking both calcium and potassium channels. An increase in Na+ conductance may also explain the induction of PDS [47,51,52] by high concentrations of pentobarbitone but it does not explain the accompanying increase in the membrane input resistance of VD1 and RPD2 and this needs further attention, unless the decreased conductance of sodium and calcium channels is much greater than the presumed increase in sodium conductance.

Does electrical coupling vary on a seasonal basis?

Assessment of R_m in VD1/RPD2 appears to vary significantly between different laboratories. Here we have shown the control R_m to be in the order of 300 MΩ for VD1 and 350 MΩ for RPD2 [see Figure 5]. This is in the normal range for molluscan neurons [10^5 to 10^8 Ω – [15]], but was found to be rather variable, in the region of 14 - 80 MΩ by Benjamin and Pilkington [53]. Further studies [54] suggested that R_m

for VD1 was 100 MΩ and for RPD2 R_m was 50 MΩ. The explanation of these differences may be due to the well-known seasonal plasticity in the nervous system of Lymnaea [55-59]. Furthermore, we were only able to obtain consistent data on the passive membrane properties of VD1/RPD2 during the summer months. The chemical synaptic connections between the giant dopamine cell and its follower cells fail in winter snails [56], which disables the respiratory central pattern generator [60,61] and Wood and Winlow [62] reported a difference in the frequency-half width correlation in specific neurons between summer and winter. Furthermore, Qazzaz [58] demonstrated seasonal variation in the weak electrical coupling between small pedal A cluster neurons [9]. During the summer months (June to August), the electrical connections between these cells were present and very clear, but disappeared completely during the winter season (October to January). Further studies on the electrical connections of VD1-RPD2 to determine whether any changes in R_m are related to seasonal factors are therefore required. Another possible cause of variation in R_m may be due to the ageing process which is known to reduce Rm VD1/RPD2 [63] and in some *Aplysia* neurons [64]. This is largely due to the increasing size of the neurons and may disturb the synchrony of firing of neuron discharges in the VD1-RPD2 pairing and may also reduce their sensitivity to synaptic inputs [63]. According to Benjamin and Pilkington [53] there is a low resistance/high conductance pathway between VD1 and RPD2 of about 20MΩ when recorded in the cell bodies. The cell membranes have much higher resistance which ensures access of currents between the cells which is sufficient to keep them synchronized. Although we now know the effects of the anaesthetics on the soma membranes, their effects on the junctional resistance remain to be clarified.

Conclusions

It is now appreciated that electrical synapses are highly plastic, modifiable by a variety of agents, including neurotransmitters [27,29], and subject to block by a wide variety of agents, many of which are non-specific [64].

Here we have demonstrated that the volatile anaesthetics isoflurane and halothane have similar actions on electrically coupled synapses between the identified neurons VD1 and RPD2 in *Lymnaea*, where they both cause a dose dependent decrease in electrical coupling between the cells. These actions are in contrast to the effects of sodium pentobarbitone which at low doses [100 μm] causes quiescence with no alteration in coupling coefficient, but at higher doses [0.5 and 2.00 mM] causes an increase in coupling coefficient and PDS [phase I], followed by quiescence and a reduction of coupling coefficient to control values [phase II].

Given that both the volatile anaesthetics and sodium pentobarbitone raise $[Ca^{2+}]_i$ further studies to elucidate the modulation of membrane potential and input resistance by anaesthetics, need to be performed while monitoring $[Ca^{2+}]_i$ during application of each class of anaesthetic. Furthermore, the effects of anaesthetics on the junctional resistance and the junctional proteins need to be understood.

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