Electrophysiological studies of the effects of the general anaesthetic etomidate on frog myelinated nerve fibre

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- 1 The effects of the general anaesthetic etomidate (0.1 to 1 mM) upon the node of Ranvier of frog isolated nerve fibres were investigated under current and voltage clamp conditions.
- 2 When added to the external solution, etomidate reversibly decreased the amplitude of the action potential. The action potential block, induced by the drug, was reversed by increasing the membrane potential.
- 3 Etomidate rapidly and reversibly blocked the Na current with an apparent dissociation constant of 0.6 mM. In the presence of the drug, the steady-state inactivation-voltage curve of the Na current was shifted towards negative voltages.
- 4 The block of Na current by etomidate was partially removed by repetitive depolarization preceded by a 50 ms period of hyperpolarization. In contrast, the block was enhanced when the repetitive depolarization was not preceded by hyperpolarization. This suggests that Na channels were preferentially blocked by the drug in the inactivated state.
- 5 The K current was reversibly blocked by etomidate with an apparent dissociation constant of 0.2 mM. In the presence of the drug, the K current showed an apparent fast inactivation suggesting that K channels were blocked in the open state.
- 6 We conclude that at higher concentrations than those attainable in the mammalian brain following single anaesthetic doses the general anaesthetic etomidate has a 'local anaesthetic-like' action on the peripheral nervous system.

Introduction

Etomidate (Figure 1) is a carboxylated imidazole which when intravenously administered has been used clinically to produce a rapid induction anaesthesia of short duration. Its use is associated with a rapid onset and recovery (Kay, 1976). No significant, or minimal, adverse effects of etomidate have been demonstrated on the cardiovascular system (Criado *et al.*, 1980; Vickers *et al.*, 1984) or respiratory system (Marquardt *et al.*, 1977). Research *in vitro* and microiontophoretic experiments *in vivo* indicated that etomidate has a γ -aminobutyric acid (GABA)-mimetic action (Evans & Hill, 1978). Recently interest has been directed to the suppressive effects on adrenal function first observed in the rat (Preziosi & Vacca, 1982) and found detrimental when etomidate was given as a prolonged continuous infusion (Ledingham *et al.*, 1983), as well as a prolactin-lowering ability of the drug (Preziosi *et al.*, 1983).

The aim of the present work was to study the effects of etomidate on the frog node of Ranvier. This preparation was used to elucidate the mode of action of the drug on excitable membranes, since membrane potential and ionic currents can be measured accurately under current and voltage clamp conditions.

Figure 1 Chemical structure of etomidate.

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Methods

Experiments were carried out on an isolated single myelinated nerve fibre from the frog *Rana esculenta*. The membrane potential and membrane currents were recorded under current and voltage clamp conditions using the method of Nonner (1969). The normal resting potential of fibres (-70 mV) was defined as the potential at which fast Na inactivation occurred by 30%. Na current was monitored with a conventional two pulse protocol. For Na current recordings, a test pulse of various amplitudes was preceded by a 50 ms pulse to -120 mV. For the steady-state inactivation-voltage ($h_{\infty} - V$) analysis of the peak Na current, a test pulse to 0 mV was preceded by a 50 ms pulse of various amplitudes. Membrane currents were calculated assuming an axoplasmic resistance of $10\text{ M}\Omega$. Linear leakage and capacity currents were analogically subtracted from the total current. The series resistance was not compensated for (See Chiu, 1977; Benoit *et al.*, 1985).

The Ringer solution (pH 7.4) contained (in mM): NaCl 111.5, KCl 2.5, CaCl_2 1.8, NaHCO_3 2.4. When recording action potentials or K current, the fibre ends were cut in a solution containing 120 mM KCl, which was used in the end pools throughout the experiments.

When measuring Na current, K current was suppressed by replacing the end pool solution with 110 mM CsCl + 10 mM NaCl and adding 10 mM tetraethylammonium to the external solution. The temperature was $15-16^\circ\text{C}$.

In the present experiments D-etomidate, the active isomer of the etomidate molecule, was tested (Heykants *et al.*, 1975).

Results

Effects of etomidate on action potential

Figure 2 shows the effect of external application of 0.25 and 0.5 mM etomidate on the action potential. In the presence of the drug (0.25 mM), action potential duration increased and amplitude decreased (Figure 2b). The action potential was fully blocked after the addition of 0.5 mM etomidate to the external solution, whatever the amplitude of injected current (Figure 2e(i)). The action potential block, induced by 0.5 mM etomidate, was reversed by hyperpolarizing the membrane by about 20 mV (Figure 2e(ii)). These effects appeared 1 to 2 min after the application of etomidate and were reversed about 5 min after washing out the

Figure 2 Effects of etomidate on action potential recorded from a single myelinated nerve fibre. (a and d) Control action potentials in Ringer solution. (b and e) Action potentials recorded 1 to 2 min after the addition of 0.25 mM (b) and 0.5 mM (e) etomidate to Ringer solution. (e,ii) The membrane was hyperpolarized by about 20 mV. (c and f) Action potentials recorded about 5 min after washout of the drug with Ringer solution. Fibres: 28-03-85 (a, b and c) and 3-07-85 (d, e and f).

drug (Figure 2c and f).

Effects of etomidate on Na current

The action of 0.5 mM etomidate on Na current is presented in Figure 3. About 1 to 2 min after the addition of etomidate to the external solution, Na current was decreased to about one third of its control value without significant changes either in its time course (Figure 3A) or in the shape of peak current-voltage relationships (Figure 3B). However, the maximum peak current was shifted by about 15 mV towards positive values (Figure 3B). This may be due to a series resistance artefact (Neumcke & Stämpfli, 1983). The effects of the drug on Na current were almost totally reversed about 3 to 5 min after washing out the drug with Ringer solution.

Figure 4 shows the effects of 0.5 mM etomidate on the steady-state inactivation-voltage (h_{∞} - V) curve of the peak Na current. In the presence of etomidate (added to the external solution 1 min before), the curve was shifted along the voltage axis towards negative values without significant changes in its shape. The voltage corresponding to half maximum steady-state inactivation was about 15 mV more negative in the

presence of 0.5 mM etomidate than under control conditions. In other experiments with 0.25 mM etomidate, the h_{∞} - V curve was shifted by about 5 mV towards negative values. The shift of the steady-state inactivation-voltage curve was reversed after about 5 min washing with etomidate-free solution (Figure 4).

From the preceding observations, it appears that the effects of etomidate resemble those of local anaesthetics (Hille, 1977). Most local anaesthetics exhibit a frequency-dependent block of Na current (Strichartz, 1973; Hille, 1977). In order to see whether the block of Na current induced by etomidate was also dependent on the frequency of stimulation, we performed the following experiment. In the presence of 0.5 mM etomidate, the fibre was stimulated at frequencies of 1 or 10 Hz, by 19 ms test pulses to either 0 or 30 mV, preceded or not by 50 ms hyperpolarization to -120 mV (Figure 5). In the absence of etomidate, the peak Na current was constant during a prolonged period of stimulation at 1 or 10 Hz. In the presence of etomidate, in order to allow a complete recovery (i.e., return to resting block), the membrane was held between each period of stimulation at a potential of -70 mV for 1 min. The peak Na current measured during each test depolarization, was normalized with respect to the corresponding value measured during the first test pulse of each period of stimulation. When

Figure 3 Effects of etomidate on Na current. (A) Na current recorded under control conditions (a), 1 min after the addition of 0.5 mM etomidate to the external solution (b) and 5 min after washout of the drug (c), during depolarization to 0 mV preceded by 50 ms hyperpolarization to -120 mV. (B) Current-voltage relationship of peak Na current, under control conditions (●), in the presence of 0.5 mM etomidate (◆) and after washout of the drug (○), during periods of depolarization of various amplitudes preceded by 50 ms hyperpolarization to -120 mV. Fibre: 13-03-85.

Figure 4 Effects of etomidate on the steady-state inactivation-voltage curve of the peak Na current. Na current was recorded under control conditions (●), in the presence of 0.5 mM etomidate (◆) and after washout of the drug (○), during depolarization to 0 mV preceded by 50 ms pulses of various amplitudes. Fibre: 13-03-85.

test pulses were preceded by a 50 ms period of hyperpolarization to -120 mV, the etomidate-induced block of Na current was partially removed by pulses of stimulation. Block removal was enhanced by an increased number of pulses and was larger when pulses were applied at 10 Hz than at 1 Hz (Figure 5a). Block removal was identical with test pulses to 0 or 30 mV (Figure 5b). In contrast, the Na current was increasingly blocked by etomidate as the pulse number was increased when test pulses were not preceded by hyperpolarization (Figure 5c).

Effects of etomidate on K current

External application of 0.5 mM etomidate reversibly blocked the K current in about 1 to 2 min (Figures 6 and 7). The K current was reduced by etomidate in a time-dependent manner showing an apparent fast incomplete inactivation (Figure 6b). This apparent inactivation could be described by an exponential function ($\bullet$, in Figure 6b and d), whose extrapolation to time of depolarization gave a value almost equal to that of the steady-state K current recorded in the absence of the drug ($\square$, in Figure 6a and d). The time-dependent block of the K current by etomidate

was consistently observed in three different fibres.

In the presence of the drug, the steady-state K current was reduced to about 25% of its control value and the current-voltage curve appears to change from a sigmoid to a linear relationship (Figure 7; see Discussion). The effects of etomidate on the K current were dependent neither on the frequency of stimulation nor on the membrane potential. They were fully reversed about 5 min after washing out the drug with Ringer solution (Figures 6c and 7).

Dose-response curves for the effects of etomidate on membrane currents

Dose-response curves for the steady-state inhibition of K and Na currents by etomidate (0.1 to 1 mM) are presented in Figure 8a. The steady-state K and peak Na currents were normalized with respect to their corresponding values measured before the application of the drug. Assuming that the inhibition of Na and K currents by the drug is based on a one-to-one reaction between ionic channels and etomidate molecules, the apparent dissociation constants are 0.2 and 0.6 mM for K and Na currents, respectively. However, the theoretical curve deviates considerably from the

Figure 5 Effects of stimulation frequency and membrane potential on the peak Na current block induced by etomidate. The Na current was recorded in the presence of 0.5 mM etomidate. Between each period of stimulation, the membrane was held at a potential of -70 mV for 1 min. The peak Na current was measured during each 19 ms period of test depolarization and was normalized relative to the corresponding value measured during the first test pulse of each period of stimulation. (a) The fibre was stimulated at a frequency of either 1 Hz ($\bullet$) or 10 Hz ($\circ$). The Na current was recorded during depolarization to 0 mV preceded by 50 ms hyperpolarization to -120 mV. (b and c) The fibre was stimulated at a frequency of 10 Hz. (b) The Na current was recorded during depolarization to either 0 mV ($\circ$) or 30 mV ($\bullet$) preceded by 50 ms hyperpolarization to -120 mV. (c) The Na current was recorded during depolarization to 0 mV preceded ($\circ$) or not ($\bullet$) by 50 ms hyperpolarization to -120 mV. Fibre: 12-03-85.

points for Na current. In order to establish whether a one-to-one interaction between ionic channels and etomidate molecules had occurred, we did plots in double logarithmic co-ordinates (Hill plots) of the points of the experiment of Figure 8a and for seven other experiments in which the drug concentration was changed from 0.1 to 1 mM (Figure 8b). Hill coefficients (n_H) of 1.42 and 1.19 and apparent dissociation constants of 0.48 and 0.19 mM were calculated for peak Na and steady-state K currents, respectively. It should be noted, especially for Na currents that n_H values are likely to be more accurate (but see Discussion).

Discussion

The results show that the general anaesthetic

etomidate (0.1 to 1 mM) blocks the action potential and ionic currents at the frog node of Ranvier.

Etomidate has two major effects on Na permeability. It decreases the maximum Na conductance and shifts the steady-state Na current inactivation curve towards negative voltages. Moreover, the inhibition of the Na current is frequency-dependent. The block is decreased by an increased number and frequency of depolarizing pulses when depolarization is preceded by hyperpolarization which removes the resting Na inactivation. In contrast, when depolarization is not preceded by hyperpolarization, i.e., when the Na permeability is partially inactivated before pulses, the inhibition is enhanced by an increased number of depolarizing pulses.

In some respects, the effects of etomidate on the Na current resemble those of local anaesthetics (Strichartz, 1973; Hille, 1977). However, the effects of most

Figure 6 Effects of etomidate on K current. (a, b and c) K current recorded in Ringer solution (a), in the presence of 0.5 mM etomidate (b) and after washout of the drug (c), during an 18 ms period of depolarization to +50 mV preceded by 50 ms hyperpolarization to -120 mV. In (b), (●) represent the exponential extrapolation, to time of depolarization, of the apparent incomplete inactivation, induced by etomidate, whose semilog representation is shown in (d). In (d), (●) represent the K current, in the presence of 0.5 mM etomidate, during its inactivation phase minus the steady-state K current (○) in (b) and (d). In (a) and (d), (□) represent the steady-state K current under control conditions. Extrapolation to time of depolarization of the etomidate-induced inactivation (straight line through (●)) gave 13.59 nA and was determined by a linear regression analysis using the log of the measured values ($r = 0.99$). The steady-state K current, under control conditions and in the presence of etomidate, was 19.08 nA and 4.14 nA, respectively. Fibre: 28-03-85.

local anaesthetics are enhanced by repetitive depolarization and these drugs are assumed to block open channels preferentially (Strichartz, 1973). In contrast, the effects of repetitive depolarization on the inhibition of Na current induced by etomidate can be explained if one assumes that the drug preferentially binds to inactivated channels, either during periods of test depolarization or between periods of test depolarization when the resting Na inactivation is not removed. When depolarizing pulses are preceded by hyperpolarization, etomidate would bind to channels during depolarization and dissociate from channels during hyperpolarization. According to this assumption, the inhibition would be removed by repetitive pulses if the dissociation during hyperpolarization is greater than binding during depolarization. In the absence of a period of prehyperpolarization repetitive depolarization would induce cumulative binding and inhibition.

The removal of the effects of etomidate on the Na current by hyperpolarization also explains the restoration of the action potential by increasing the resting membrane potential, in the presence of 0.5 mM etomidate (see Figure 2e).

From this point of view, the mode of action of etomidate would be similar to that of unprotonated local anaesthetics and of the antiarrhythmic agent ajmaline (Khodorov *et al.*, 1976; Khodorov & Zaborovskaya, 1983).

Etomidate appears to be more efficient in blocking

Figure 7 Current-voltage relationships of the steady-state K current. The K current was measured at the end of an 18 ms period of depolarization of various amplitudes preceded by 50 ms hyperpolarization to -120 mV under control conditions (●), in the presence of 0.5 mM etomidate (◆) and after washout of the drug (○). Fibre: 28-03-85.

potassium than sodium currents (See Figure 8). This is in contrast with local anaesthetics which preferentially block Na currents (Århem & Frankenhaeuser, 1974).

Figure 8 Etomidate dose-response relationship for membrane currents. Peak Na current (○) and steady-state K current (●) were recorded during or at the end of an 18 ms period of depolarization to 0 mV preceded by 50 ms hyperpolarization to -120 mV. (a) Etomidate log dose-response curves for peak Na (continuous line) and steady-state K (broken line) currents. The currents were normalized relative to their respective values in the absence of drug. The curves represent the block of currents assuming a one-to-one reaction between channels and etomidate molecules. Fibre: 28-03-85. (b and c) Hill plots of the effects of etomidate on peak Na and steady-state K currents, according to the equation: $\log(I/(I_c - I)) = \log(K^{-n_H} - n_H \log[\text{etomidate}])$, where K is the apparent dissociation constant and n_H the Hill coefficient. The currents were measured in the presence (I) and in the absence (I_c) of various concentrations of etomidate. Mean values and standard errors of the mean obtained in 2-8 experiments (numbers in parentheses). The straight lines were obtained from linear regression through the points. The correlation coefficients (r) were 0.81 (Na current) and 0.97 (K current).

Similar to derivatives of tetraethylammonium (Armstrong & Hille, 1972), quaternary derivatives of local anaesthetics (Strichartz, 1973), strychnine (Shapiro, 1977), quinidine (Revenko *et al.*, 1982), capsaicin (Dubois, 1982) and the antiarrhythmic agent ajmaline (Khodorov & Zaborovskaya, 1983), etomidate blocks the K current in a time-dependent manner. Taking into account that the etomidate molecule is neutral at physiological pH ($pK_a = 4.10$) and the effects of the drug are rapidly reversed upon washing, the receptor site for etomidate associated with K channels should be located at the external face of the membrane and its affinity (or accessibility) should be larger when K channels are in the open rather than in the resting state.

In the presence of etomidate, the K current-voltage curve appears to change from a sigmoid to a linear relationship (Figure 7) and the etomidate dose-response curves, specially for Na current, are poorly described by a one-to-one interaction between ionic channels and drug molecules (Figure 8), but are better fitted if one assumes that more than one molecule of drug is necessary to block one channel. There are various interpretations of these effects. The reduction of the K current by etomidate should lead to a decrease in K accumulation in the nodal gap and consequently indirectly modify the current (Dubois, 1981a; 1983). Moreover, in the frog node of Ranvier, K and Na currents are made up of several components having different voltage sensitivities and pharmacological properties (Dubois, 1981b; 1983; Benoit *et al.*, 1985; Benoit & Dubois, 1986). Etomidate might be more efficient in blocking one of these components. Furthermore, as shown for tetrodotoxin and niflumic acid (Benoit *et al.*, 1985; Benoit & Dubois, 1986), more than one molecule of drug may be necessary to block

one channel. Further experiments would be needed to clarify these points.

In conclusion, the effects of the general anaesthetic etomidate on the myelinated nerve fibres resemble those of local anaesthetics. However, while most local anaesthetics are thought to interact preferentially with the open Na channels, etomidate may bind preferentially to open K channels and to inactivated Na channels.

Etomidate penetrates into the brain very rapidly, since the peak level occurred less than 1 min after injection: $1.50 \pm 0.35 \mu\text{g}$ of drug per g of brain (0.004 mM) was sufficient to produce general anaesthesia in mammals. With a dose (9.1 mg kg^{-1}), nearly 30 times that employed in humans (0.3 mg kg^{-1}), concentrations of about $15 \mu\text{g}$ per g of brain (0.04 mM) were found (Heykants *et al.*, 1975). In the present research, drug concentrations (0.1–1 mM) 25–250 times higher than are needed in the mammalian brain to obtain anaesthesia with etomidate were employed. On the other hand, on the node of Ranvier several anaesthetic molecules are active at the same dose range (Hille, 1977; Carratù *et al.*, 1983). However, we must point out that extrapolation of our results to mammals may not be justified in view of the importance of species differences.

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