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EFFECT OF ATROPINE ON THE DECAY OF MINIATURE END-PLATE CURRENTS AT THE FROG NEUROMUSCULAR JUNCTION

ANNE FELTZ & W.A. LARGE
Laboratoire de Neurobiologie, Ecole Normale Supérieure, 46, Rue d'Ulm, 75005, Paris, France

1 The effect of atropine on the time constant of decay (τ) of miniature end-plate currents was investigated.

2 The atropine-induced shortening of τ was accompanied by a reduction in the voltage-sensitivity of τ.

3 This effect of atropine was reversible and dose-dependent, the voltage sensitivity being abolished with high concentrations of atropine (2.5×10^{-5} M).

Introduction

The time constant of decay (τ) of synaptic currents at the neuromuscular junction has been shown to be markedly affected by the post-synaptic membrane potential, being prolonged by a hyperpolarization of the membrane (Gage & Armstrong, 1968; Kordaš, 1969). Making certain assumptions about the fundamental processes underlying the end-plate current (e.p.c.), Magleby & Stevens (1972a, b) demonstrated that τ reflects the life-time of the acetylcholine-induced elementary current pulse; moreover, in 1973, Anderson & Stevens showed that the τ of e.p.cs, the τ of miniature end-plate currents (m.e.p.cs) and the channel lifetime, as deduced from noise analysis, were similarly affected by alteration of the membrane potential.

The action of atropine in reducing the amplitude of miniature and nerve-evoked end-plate potentials at the neuromuscular junction is characterized by a diminution of the decay times of the potentials (Beránek & Vyskočil, 1968). The shortening of the time-course of e.p.cs (Kordaš, 1968; Magazanik & Vyskočil, 1969) can be accounted for by a reduction in the lifetime of the elementary current pulse produced by acetylcholine (ACh), as revealed from noise analysis (Katz & Miledi, 1973). In the present paper, we describe the action of atropine on the voltage-sensitivity of $\tau_{\text{m.e.p.cs}}$

Methods

M.e.p.cs were studied in the cutaneous pectoris muscle of the frog, *Rana esculenta*, by two techniques: (1) focal extracellular recording with glass microelectrodes filled with 2 M NaCl (tip diameter 5–10 μm); (2) intracellularly, by the use of the two electrodes voltage-clamp method. In the latter case the two electrodes (filled with 3 M KCl) were inserted into the end-plate of the muscle fibre within 50–100 μm of each other. A rise time of around 0.5 ms for the m.e.p.cs was used as an index of close vicinity of the releasing sites. The preparation was constantly superfused and all experiments were carried out at room temperature (20–23°C). The composition of the Ringer was (mM): NaCl 161, $CaCl_2$ 1.8, KCl 2.5, Tris maleate 2, acetylglycine 2. The pH was adjusted to 7.2 by addition of NaOH. The extra NaCl was added to increase the osmotic pressure of the bathing fluid and hence the frequency of miniature end-plate potentials. Atropine sulphate (Serlabo) was diluted in the bathing fluid at the given concentrations. The m.e.p.cs were photographed directly on to moving film and subsequently were enlarged and traced on to graph paper. The decays of the currents were plotted according to the equation: $I_t = I_0 e^{-t/\tau}$ on a 9810A Hewlett Packard desk computer. I_t = current at time t; I_0 = current at time zero (i.e. the peak current) and τ is time constant of decay. For each experiment, the decay constants of at least 6 m.e.p.cs were calculated and averaged for each parameter. Unless stated, the mean ±s.e. of decay time constant was calculated from at least 6 cells for each experimental condition.

Results

In normal Ringer solution, at the resting membrane potential (~−90 mV), the time constant of decay

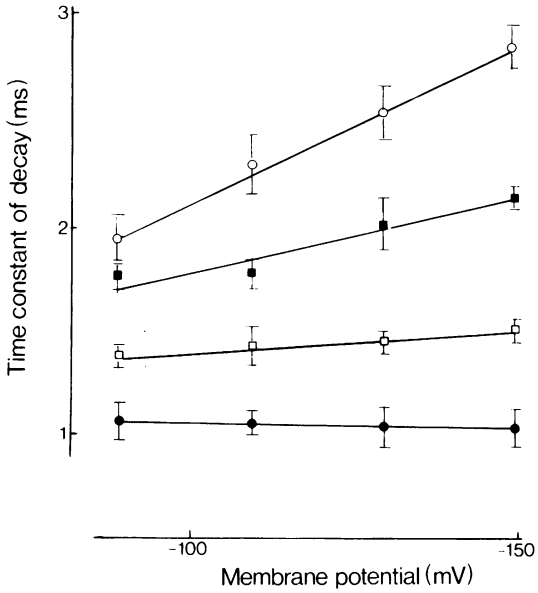

Figure 1 Effect of atropine sulphate on the relation between the time constant of decay of miniature end-plate currents and clamp potential. Control experiments (O); measurements in presence of atropine sulphate: (■) 5×10^{-6} M; (□) 1×10^{-5} M; (●) 2.5×10^{-5} M. Vertical bars represent ±s.e. mean.

of m.e.p.cs in voltage-clamp experiments was 2.20 ± 0.10 ms (mean ± s.e. from 26 cells). This was in good agreement with the value obtained with focal external recording 2.21 ± 0.08 (18 cells). This would suggest that in the voltage-clamp experiments, the electrodes were placed in the end-plate region and also that the membrane potential was adequately controlled (see Magleby & Stevens, 1972a). When atropine was added to the bathing fluid the amplitude of the m.e.p.cs was reduced and there was a concomitant shortening of the time course of decay. These effects were dose-dependent with a threshold concentration of about 5×10^{-6} M. The effects of atropine developed within 2–3 min and appeared to be maximal after 10 min when all measurements were made. In voltage-clamped fibres, the time constants of decay after 1×10^{-5} and 2.5×10^{-5} M were 1.39 ± 0.05 and 1.09 ± 0.08 ms respectively (compared with 2.20 of controls, see above) and in the extracellular experiments the same concentrations of atropine produced τ values of 1.32 ± 0.08 and 1.20 ± 0.05 ms respectively (control = 2.21). However, it is important to note that even at high concentrations of atropine the falling phase of currents conformed to a single exponential and hence atropine differs in its action from the local anaesthetics (see review by Rang, 1975).

It has been shown that in normal conditions $\tau_{m.e.p.cs}$ is sensitive to changes in the membrane potential (Anderson & Stevens, 1973; Gage & McBurney, 1975). Furthermore, over a wide range of membrane potentials, τ can be related to the membrane potential by the equation $\tau_V = \tau_0 e^{V/H}$ where τ_V and τ_0 are the time constants of decay at potentials V and 0 respectively and H is a constant which indicates the voltage-sensitivity of the falling phase of m.e.p.cs (notation of Gage & McBurney, 1975, after Magleby & Stevens, 1972a,b). We have applied the same analysis in the present experiments. Figure 1 shows the change in the time constant of decay, when the membrane potential is hyperpolarized from the holding potential of –90 mV in 20 mV steps before and after various concentrations of atropine. In 17 cells, H had a mean value of -180 ± 13 mV and the mean τ_0 was 1.26 ± 0.09 ms (for each cell, τ_0 was calculated by extrapolation using the least squares method). In the presence of atropine there was a concentration-dependent reduction in the voltage-sensitivity and with 2.5×10^{-5} M atropine sulphate, voltage-sensitivity was abolished. It is interesting to note that assuming the exponential relationship between τ and membrane potential, atropine did not alter the extrapolated τ_0 value (the τ_0 values for the 5×10^{-6}; 1×10^{-5} and 2.5×10^{-5} M atropine were respectively 1.25 ± 0.05; 1.31 ± 0.07 and 1.17 ± 0.09 ms. Both the shortening and the reduction in voltage-sensitivity of τ produced by atropine were reversible and the control values were attained after 10–20 min washing.

Discussion

The results show that the atropine-induced shortening of the time course of miniature end-plate currents is accompanied by a reduction in the voltage-sensitivity of the decay constant. Moreover the action of atropine appears to be different from that of local anaesthetics (Maeno, 1966) inasmuch as the decay remains a simple exponential after the treatment with atropine whereas the latter drugs cause a biphasic decay. In this respect atropine resembles octanol which shortens miniature currents (Gage, McBurney & Van Helden, 1974) and also reduces voltage-sensitivity (Feltz & Large, unpublished results). The loss of voltage-sensitivity after atropine may be explained by a major decrease in the lifetime of the end-plate ionic channels such that another process, for example diffusion of ACh out of the cleft, becomes the rate limiting factor. An alternative explanation may be a direct action of atropine on the ACh-receptor complex and/or the ionic channel. It is interesting to note that at some

other synapses, there is no voltage-sensitivity of the time course of the decay phase (Gardner & Stevens, 1974; Llinás, Joyner & Nicholson, 1974), and from our results with atropine it would appear that ACh can open the channels at the neuromuscular junction also when voltage-sensitivity has been abolished. Therefore voltage-sensitivity does not appear to be a fundamental characteristic of synapses.

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