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INHIBITION OF THE ACETYLCHOLINE RECEPTOR BY HISTRIONICOTOXIN

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1 The action of C_5-decahydrohistrionicotoxin (C_5-HTX) has been investigated on the extrajunctional acetylcholine (ACh) receptors of denervated rat muscle.

2 C_5-HTX causes both a rapid and slow reduction in amplitude of iontophoretic ACh potentials evoked at all frequencies from the extrajunctional receptors.

3 C_5-HTX also causes a time-dependent inhibition of the iontophoretic potentials evoked at frequencies greater than 0.02 Hz. This inhibition was observed either alone or superimposed upon desensitization, and may be caused by a similar mechanism to desensitization.

Introduction

The alkaloid toxin histrionicotoxin (HTX), and its analogues have been found to block reversibly the action of the transmitter acetylcholine (ACh) at the vertebrate neuromuscular junction and on the *Torpedo* electroplax (Albuquerque, Barnard, Chiu, Lapa, Dolly, Janssen, Daly & Witkop, 1973; Kato & Changeux, 1976; Eldefrawi, Eldefrawi, Albuquerque, Oliveria, Mansour, Adler, Daly, Brown, Burgermeister & Witkop, 1977). Binding and fluorescent studies have shown that HTX and its analogues interact with high affinity with a protein fraction from *Torpedo* electric organ, which is distinct from the ACh binding protein (Eldefrawi *et al.*, 1977; Sobel, Heidmann, Hofler & Changeux, 1978). On the basis of these studies, it was suggested that HTX acts either by binding to the ionic channel associated with the ACh receptor (Albuquerque *et al.*, 1973; Eldefrawi *et al.*, 1977) or by binding to a local anaesthetic site which is allosterically coupled to the ACh receptor (Kato, Glavinović, Henry, Krnjević, Puie & Tattrie, 1975; Kato & Changeux, 1976; Sobel *et al.*, 1978). Sodium flux and α-bungarotoxin (α-BuTX) binding studies have also shown that HTX enhances the agonist-induced desensitization of the ACh receptor by causing an increase in the receptor affinity for agonists (Burgermeister, Catterall & Witkop, 1977).

In the present study, electrophysiological techniques have been employed to determine in more detail the potency and time course of the action of HTX on the ACh receptor, and in particular, to determine whether HTX causes an increase in desensitization.

Methods

Experiments were carried out on the soleus muscles of 100 to 200 g female rats. The muscles were denervated by removal of 0.5 to 1 cm of the sciatic nerve in the mid-thigh region. Five to ten days after denervation, the muscles were removed and placed in a bath perfused with saline solution (Na^+ 135 mM, K^+ 5 mM, Ca^{2+} 3 mM, HEPES 2 mM, pH 7.2). The saline solution was oxygenated with 95% O_2 and 5% CO_2.

Intracellular recordings were made with 5 to 15 MΩ microelectrodes filled with 2 M potassium citrate, and ACh was applied iontophoretically with high resistance (100 to 200 MΩ) electrodes filled with 1 M ACh chloride. A bias current of a few nA was applied to the iontophoretic electrode to prevent diffusion of ACh from the tip of the electrode. The iontophoretic current was monitored with an operational amplifier placed between the bath and ground. The sensitivity to ACh was expressed in mV depolarization per nC of charge (Miledi, 1960).

The experiments were performed using C_5-decahydrohistrionicotoxin hydrochloride (C_5-H_{10}-HTX), which was supplied by Dr Y. Kishi of the Department of Chemistry, Harvard University.

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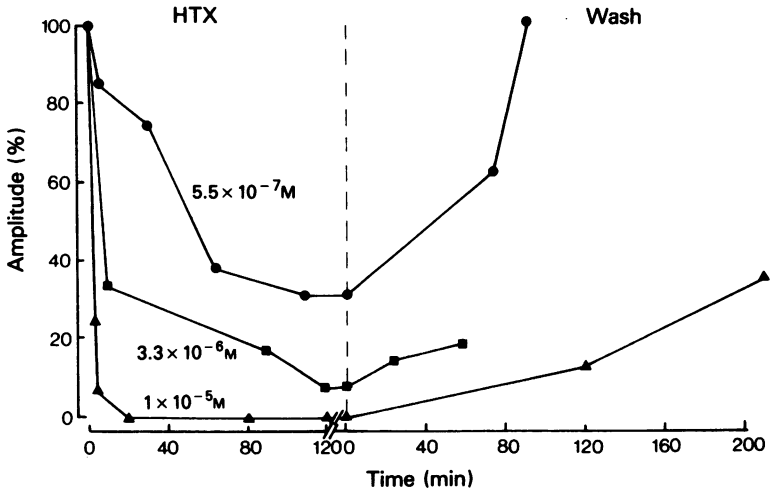

Figure 1 Time course of the onset of, and recovery from, the blocking action of three concentrations of C_5-decahydrohistrionicotoxin (HTX) in reducing the amplitude of the iontophoretic acetylcholine potential evoked in extrajunctional acetylcholine receptors of denervated rat soleus muscle. Concentrations of HTX used were 5.5×10^{-7} M (●), 3.3×10^{-6} M (■) and 1×10^{-5} M (▲).

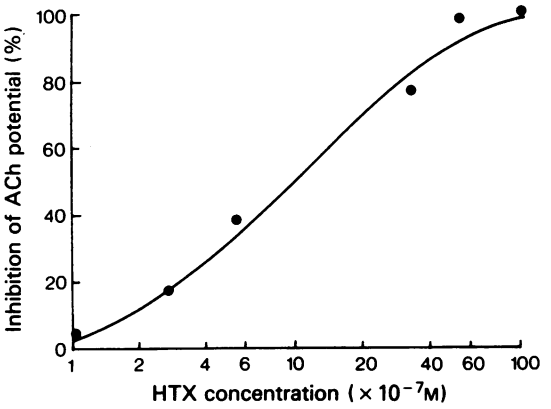

Figure 2 Dose-response relationship for C_5- decahydrohistrionicotoxin (HTX) in reducing the amplitude of the iontophoretically evoked acetylcholine (ACh) potential after 30 min perfusion with the toxin. Half-maximal reduction of the ACh potential occurred at 1×10^{-6} M HTX.

Results

C_5-H_{10}-HTX reduces the amplitude of acetylcholine potentials

Iontophoretic ACh potentials with times to peak of 10 to 20 ms can be evoked over the entire surface of rat soleus muscle fibres 10 to 14 days after denervation. The ACh sensitivity varied from 100 to 300 mV/nC for a 6 mV ACh potential.

The effect of C_5-H_{10}-HTX on ACh sensitivity was measured in two ways. Firstly, the average ACh sensitivity of several muscle fibres was measured in normal saline. The effect of C_5-H_{10}-HTX on the amplitudes of an ACh potential evoked at a very low frequency (0.02 Hz) at one site was then measured up to about 30 min of C_5-H_{10}-HTX perfusion. Slower changes caused by C_5-H_{10}-HTX were investigated by measuring the average ACh sensitivity of several muscle fibres about every 20 min during perfusion of C_5-H_{10}-HTX for about 2 h.

Perfusion of C_5-H_{10}-HTX caused a reduction in the sensitivity to ACh. A large reduction in the amplitude of ACh potentials occurred during the initial 5 to 10 min perfusion of C_5H_{10}-HTX and this was followed by a very slow reduction in amplitude over the subsequent 2 h. Figure 1 shows the time course of the reduction in ACh sensitivity caused by three different concentrations of C_5-H_{10}-HTX. After 10 min perfusion with C_5-H_{10}-HTX, the ACh potential was reduced in amplitude to 85%, 33% and 5% of the control amplitude in 5.5 ± 10^{-7} M, 3.3×10^{-6} M and 1×10^{-5} M C_5-H_{10}-HTX, respectively. The slower blocking action of C_5-H_{10}-HTX then reduced the ACh sensitivity to 31%, 7% and 0% of the control in 5.5×10^{-7} M, 3.3×10^{-6} M and 1×10^{-5} M C_5-H_{10}-HTX, respectively, after 2 h of perfusion. A dose-response curve for the inhibition of the ACh sensitivity by six concentrations of C_5-H_{10}-HTX is shown in Figure 2. The ACh sensitivity was measured

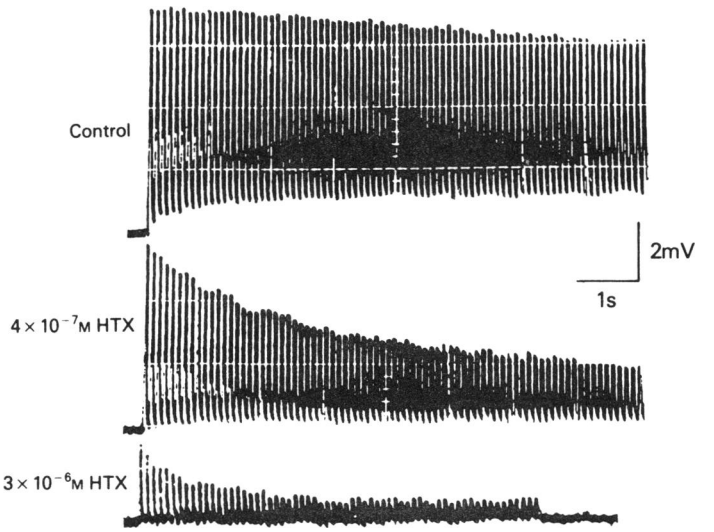

Figure 3 Desensitization in normal saline (control) and the time-dependent inhibition produced by C_5-decahydrohistrionicotoxin (4×10^{-7} M HTX and 3×10^{-6} M HTX) superimposed upon desensitization. Iontophoretic ACh potentials were evoked at a frequency of 10 Hz. The half times of the decline in amplitude of successive ACh potentials was 14.5 s in normal saline, 2.7 s in 4×10^{-7} M HTX and 0.7 s in 3×10^{-6} M HTX. The half-times of the time-dependent inhibition were measured after 30 min in the toxin, at which time the inhibition had reached a maximum.

after 30 min perfusion with C_5-H_{10}-HTX, the same time as time-dependent inhibition reached a maximum. A half-maximal reduction in the amplitude of the ACh potential was caused by 1×10^{-6} M C_5-H_{10}-HTX, while ACh sensitivity was completely abolished in 5×10^{-6} M C_5-H_{10}-HTX. In other experiments, a half-maximal reduction in amplitude of the ACh potential after 10 and 120 min exposure was caused by 2.5×10^{-6} M and 3×10^{-7} M C_5-H_{10}-HTX, respectively. In normal saline, ACh sensitivity remained unchanged for at least 6 h and often over 10 h.

Recovery from the blocking action of C_5-H_{10}-HTX was very slow, especially after treatment with high concentrations of the toxin. For example, complete recovery of ACh sensitivity after perfusion with 5.5×10^{-7} M C_5-H_{10}-HTX was attained only after 40 min of washing with toxin-free saline, whereas after treatment with 1×10^{-5} M C_5-H_{10}-HTX, recovery was only 35% complete after 3.5 h of washing.

Time-dependent blockade by C_5-H_{10}-HTX

ACh potentials always remained constant in amplitude when evoked at frequencies of up to about 1 Hz in normal saline. However, when evoked at higher frequencies, successive ACh potentials increasingly declined in amplitude due to desensitization of the ACh receptors.

Perfusion of C_5-H_{10}-HTX was also found to cause successive ACh potentials to decline increasingly in amplitude. This time-dependent inhibition by HTX was observed either alone when stimulating the ACh potentials at low frequencies (0.02 to 1 Hz) or superimposed upon desensitization when evoking the ACh potentials at 10 Hz. The ACh potentials evoked at 10 Hz were always reduced to zero amplitude after sufficient time, whereas at lower frequencies of stimulation it was often difficult to assess whether the potentials declined to zero amplitude or some steady state amplitude. Quantitative observations on the action of C_5-H_{10}-HTX were therefore always made whilst evoking the potentials at 10 Hz when the HTX inhibition is superimposed on desensitization (Figure 3).

The decrease in amplitude of successive ACh potentials during desensitization and the time-dependent inhibition by C_5-H_{10}-HTX consisted of one slow and at least one fast exponential function with time (see also Anwyl & Narahashi, 1980). The effects of C_5-H_{10}-HTX were therefore expressed simply as half-times of the overall time-dependent inhibition.

A 50% decrease in the half time of the time-dependent inhibition was caused by about 1.6×10^{-7} M C_5-H_{10}-HTX (see Figure 4). At this concentration, only a small (up to 10%) decrease in the amplitude of the initial ACh potential, due to the steady state blockade, occurred.

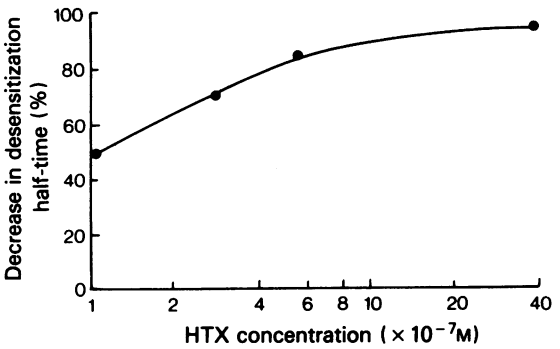

Figure 4 Dose-response curve for the time-dependent inhibition caused by C_5-decahydrohistrionicotoxin (HTX). The half-time of the time dependent inhibition was expressed as a percentage of the desensitization half-time in normal saline. The ACh potentials were all evoked at 10 Hz and the values are those after 30 min in the toxin.

The half-time of recovery from the time-dependent inhibition caused by C_5-H_{10}-HTX was 4.2 s (average 20 expts), and was not altered by the concentration of HTX. This was a very similar value to the recovery half-time from desensitization, which averaged 4.3 s (25 expts.).

Discussion

The results of the present study show that C_5-H_{10}-HTX causes both a constant inhibition of ACh potential evoked at all frequencies, and a time-dependent inhibition of ACh potentials evoked at frequencies greater than about 0.02 Hz. It has previously been proposed that HTX acts by blocking the ionic channel of the ACh receptor (Albuquerque *et al.*, 1973; Albuquerque, Kuba & Daly, 1974). Both types of inhibition found in the present study could be caused by such a channel blockade. However, it is also possible that HTX has two binding sites, one site on the channel, causing the steady state blockade, and a further site responsible for the time-dependent blockade. Biochemical experiments have shown that HTX causes biochemical desensitization i.e. an increase in the affinity of the ACh receptor for ACh. It should therefore be further considered that the HTX time-dependent blockade of the ACh receptor, which is very similar to ACh evoked neurophysiological desensitization, is associated with an increase in the affinity of the ACh receptor for ACh.

The blocking action of C_5-H_{10}-HTX occurs in two stages, a rapid suppression of ACh potentials in the initial 5 to 10 min of toxin perfusion and a slower suppression over the subsequent 2 to 3 h. Several previous studies have shown that HTX is a potent blocking agent of the action of ACh on junctional and extrajunctional ACh receptors (Albuquerque *et al.*, 1973; Eldefrawi *et al.*, 1977). The half-maximal effect of HTX in reducing extrajunctional ACh sensitivity was first reported as 2.4 μM (Dolly, Albuquerque, Sarvey, Mallick & Barnard, 1977), but in a later study the action of HTX was found to be much more potent, with a half-maximal effect occurring at about 10^{-8} M (Albuquerque & Gage, 1978). Depression of ACh responses was even reported with concentrations of HTX as low as 10^{-12} M (Albuquerque & Gage, 1978). However, in the studies in which HTX was found to be very potent, the ACh sensitivity was measured by application of a steady dose of ACh for several seconds (Albuquerque & Gage, 1978). Such prolonged iontophoretic responses are likely to be severely attenuated by time-dependent inhibition in view of the present findings that HTX is extremely potent at causing such inhibition. This may lead to overestimations of the toxin's potency.

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