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Effects of H_1-receptor blocking drugs on the frog sartorius neuromuscular junction

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1 The effects of H_1-receptor blocking agents, pyrilamine, promethazine, and diphenhydramine, on the amplitude and time course of endplate potentials (e.p.ps) were studied in the sartorius muscles of frogs.

2 H_1-receptor blockers (10^{-5}–10^{-4} M) reduced the amplitude of e.p.ps recorded intracellularly without affecting the resting membrane potential.

3 The acetylcholine potential was decreased by perfusion of H_1-receptor blockers. However, when the muscle fibre was stimulated directly, threshold and magnitude of action potential were not affected by H_1-receptor blockers.

4 The time constant of decay of the e.p.ps recorded extracellularly was reduced by H_1-receptor blockers and the decay remained exponential with a single time constant.

5 The quantal content was not reduced by perfusion of H_1-receptor blockers at a concentration of 10^{-4} M.

6 It is assumed that the major site of action of these H_1-receptor blocking agents is at the postsynaptic membrane.

Introduction

The side effects of H_1-receptor blocking agents such as sedation, fatigue and lassitude are known (Wyngaarden & Seevers, 1951). These effects are thought to be elicited by the action of these agents on the central nervous system (CNS). However, H_1-receptor blockers also have local anaesthetic and ganglion blocking actions (Dutta, 1949), so that these side effects may not be due solely to the actions on the CNS. It was reported that several H_1-blocking agents depressed the twitch tension of the rat diaphragm (Takiuchi, 1964). However, no further studies concerning the effect of H_1-receptor blockers on the neuromuscular junction have been carried out until now. In the present study, we intended to clarify the influence of H_1-receptor blockers on the neuromuscular junction of frog sartorius muscles by means of intracellular and extracellular recordings.

Methods

The experiments were performed on sciatic nerve-sartorius muscle preparations of *Rana nigromaculata*. The muscles were dissected and mounted at their resting length in a 5 ml Perspex chamber containing Ringer solution adjusted to pH 7.2. Fine dissection was done under a stereomicroscope (× 100). The muscle preparation was perfused continuously with oxygenated Ringer solution at a rate of 3 ml min^{-1} and the perfusate was removed by suction from the surface of the chamber. The Ringer solution was of the following composition (mM): NaCl 115, KH_2PO_4 2, $CaCl_2$ 1.8 (pH 7.2). The experiments were carried out at room temperature (20–25°C).

The muscle contraction evoked by nerve stimulation was abolished by perfusion with (+)-tubocurarine chloride (2×10^{-6}–4×10^{-6} M). Endplate potentials (e.p.ps) and miniature endplate potentials (m.e.p.ps) were recorded intracellularly with glass microelectrodes filled with 3 M KCl, having d.c. electrical resistance of 10–20 MΩ. When e.p.ps were recorded extracellularly, glass microelectrodes filled with Ringer solution and with a tip resistance of 2–5 MΩ were used. An extracellular electrode was used in some experiments to record the external e.p.ps, providing an indication of the endplate current (e.p.c.) through that spot (Steinbach, 1968). In such cases, the tip of the microelectrode was placed very close to the

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endplate, within 20 μm of where the intracellular recording was being carried out; such close proximity allowed recording of the most rapid deflection of the e.p.ps. The sciatic nerve was stimulated through a pair of platinum electrodes insulated except for the tips. Microelectrodes filled with 3 M potassium acetate (with tip resistances of 2–5 MΩ) were used as stimulating electrodes to study the electrical properties of the muscle fibre membrane. This current-passing electrode impaled the muscle intracellularly within 100 μm of the recording electrode. To reduce the artifact, a shield was set between the current passing electrode and the recording electrode. To prevent twitches due to direct stimulation, the preparation was pretreated with glycerol in most cases (Miyamoto, 1975). When the influence of H_1-receptor blockers on depolarization of the endplate provoked by a focal application of acetylcholine (ACh potential) was being investigated, an iontophoretic application of ACh from micropipettes filled with 4 M ACh solution was carried out (del Castillo & Katz, 1955). When the quantal content representing the amount of released ACh was estimated, high $MgCl_2$ (10 mM) and low $CaCl_2$ (1 mM) Ringer solution was used to perfuse the preparation. The average quantal content (m) was calculated by the following equation;

$$m = [1 + (cv)^2]/(CV)^2$$

where CV and cv are the coefficients of variaton of e.p.ps and m.e.p.ps, respectively (Martin, 1966; Miyamoto, 1975). Voltage signals were amplified by a high-input impedance amplifier (Nihon Koden, MEZ-8201) and displayed on a digital storage oscilloscope (Kikusui, DSS-6521). The data are expressed as mean ± s.e.mean and the differences between the means of various groups were tested by Student's *t* test. The criterion for statistical significance was $P < 0.05$.

The drugs used were as follows with sources indicated in parentheses: (+)-tubocurarine chloride (Tokyo Kasei), acetylcholine chloride (Wako Chemical), pyrilamine maleate (ICR Laboratories), promethazine hydrochloride (Yoshitomi Pharmaceutical Industries), and diphenhydramine hydrochloride (Tokyo Kasei).

Results

Effects of H_1-receptor blockers on the amplitude of the e.p.ps

During perfusion of Ringer solution containing (+)-tubocurarine (2×10^{-6} M), the mean amplitude of e.p.ps was 9.6 ± 0.4 mV ($n = 60$). With perfusion of Ringer solution containing pyrilamine (10^{-6}–10^{-4} M), the mean amplitude of e.p.ps in the (+)-tubocurarine-treated muscle was depressed

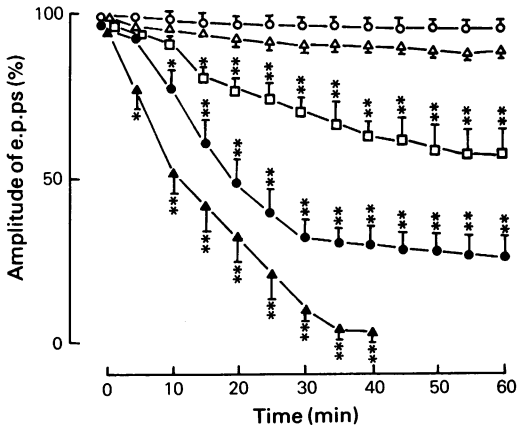

Figure 1 Effect of pyrilamine on the amplitude of e.p.ps recorded intracellularly. When the amplitude of e.p.ps reached a steady level, perfusion of pyrilamine was started and this is depicted as time zero. The magnitude of e.p.ps at time zero was taken as 100%. (○) Control; other symbols show the percentage change determined after perfusion of pyrilamine: (△) 10^{-6} M; (□) 10^{-5} M; (●) 5×10^{-5} M; (▲) 10^{-4} M. Values are mean of $n = 5$–6; vertical lines show s.e.mean. The differences of the mean between control and pyrilamine-treated groups were tested: $^*P < 0.05$; $^{**}P < 0.01$.

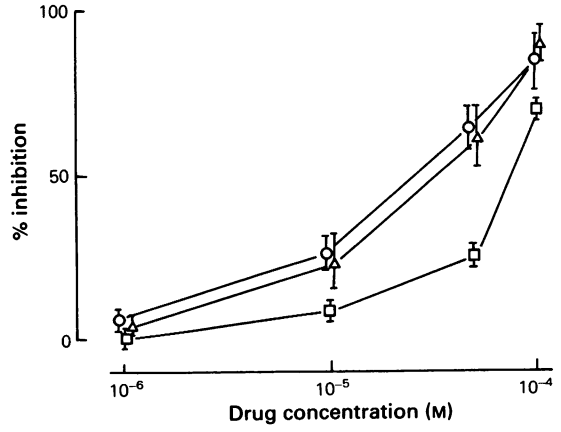

Figure 2 Concentration-response curves for inhibitory effects of H_1-receptor blockers, (○) pyrilamine; (△) promethazine; (□) diphenhydramine, on the amplitude of e.p.ps recorded intracellularly. Ordinate scale represents the percentage inhibition 30 min after drug application. Values are mean of $n = 5$–6 and vertical lines show s.e.mean. In the case of diphenhydramine perfusion, significant inhibition was noted at 10^{-5} M ($P < 0.05$) or higher ($P < 0.01$). In pyrilamine and promethazine containing solutions, significant inhibition ($P < 0.01$) was observed at 10^{-5} M.

Table 1 Effects of H_1-receptor blockers on the time course of the endplate potentials recorded intracellularly

Drug	*Concentration* (M)	*Rise time* (ms)	*Half decline time* (ms)
Control		1.10 ± 0.04	2.89 ± 0.02
Pyrilamine	10^{-5}	1.07 ± 0.09	2.33 ± 0.42
	10^{-4}	0.85 ± 0.08*	1.98 ± 0.15*
Promethazine	10^{-5}	0.96 ± 0.05	2.51 ± 0.30
	10^{-4}	0.88 ± 0.10*	1.95 ± 0.24*
Diphenhydramine	10^{-5}	1.00 ± 0.03	2.22 ± 0.32
	10^{-4}	0.89 ± 0.06*	1.95 ± 0.50

Each measurement was carried out at 20 min after the drug perfusion ($n = 6$). Significantly different from the value obtained in the absence of drug (control): $*P < 0.05$.

gradually, in a concentration-dependent manner (Figure 1). The other H_1-receptor blockers, promethazine and diphenhydramine, also depressed the mean amplitude of the e.p.ps (Figure 2). The average of the resting membrane potentials was -94.8 ± 3.6 mV ($n = 21$), and at the concentrations tested (10^{-6} M to 10^{-4} M), no H_1-receptor blockers significantly affected the resting membrane potential of the sartorius muscle, distributed in the range from -92.0 ± 5.0 to -95.0 ± 4.4 mV.

Effects on the time course of the e.p.ps

The time course of the e.p.ps recorded intracellularly was analysed by measuring the time required to reach the peak (rise time) and the time from the peak to 50% of maximum amplitude (half decline time). After application of H_1-receptor blockers, both the rise time and half decline time were shortened (Table 1): the characteristic features of the e.p.ps after H_1-receptor blockers were a more rapid decay and a reduced peak amplitude. E.p.ps recorded extracellularly are illustrated in Figure 3a. When the muscles were exposed to pyrilamine at concentrations higher than 10^{-5} M, a progressive reduction in both the amplitude and time constant of the decay phase occurred. When the decay phase of the e.p.ps was measured every 0.2 ms and plotted on a logarithmic scale against a linear time scale, the points lie on a straight line indicating that the decay is exponential (Figure 3b). During perfusion of

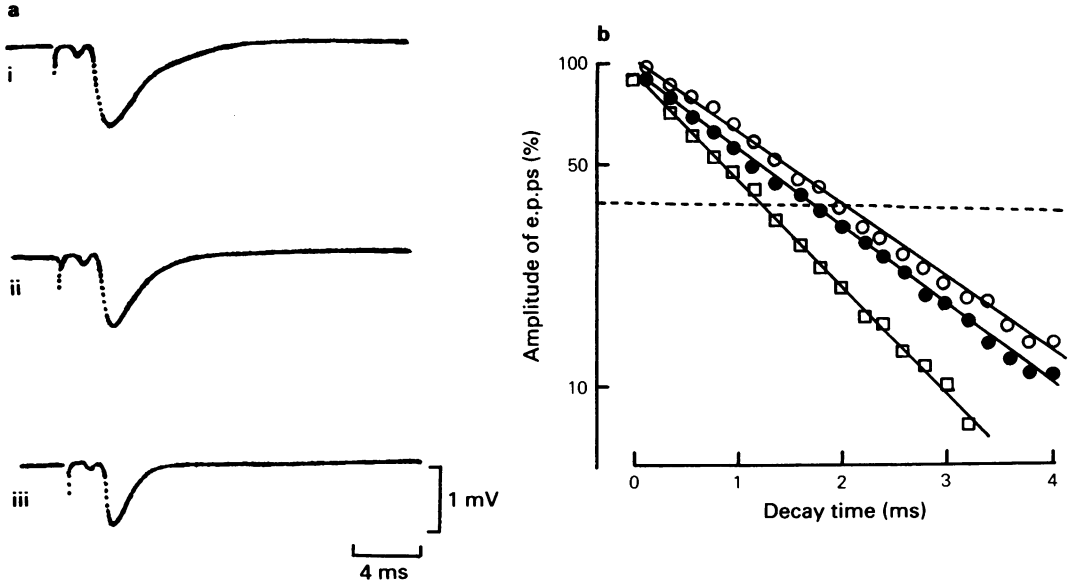

Figure 3 Effect of pyrilamine on e.p.ps recorded extracellularly. The tracings shown in (a) are recorded in the control solution (i), in 10^{-5} M pyrilamine (ii) and in 10^{-4} M pyrilamine (iii). The graph in (b) is a semilogarithmic plot of the e.p.ps amplitude (% of amplitude) against time (ms) elapsed from the peak. Dotted line exhibits the level of time constant in the decay. (○) Control, (●) pyrilamine 10^{-5} M, (□) pyrilamine 10^{-4} M.

Table 2 Effects of H_1-receptor blocking drugs on time constants of decay (τ) of external endplate potentials and quantal contents (m)

Drug	*Concentration* (M)	τ (ms) *before*	τ (ms) *after*	m *before*	m *after*
Pyrilamine	10^{-5}	1.98 ± 0.10	1.88 ± 0.09	17.80 ± 1.46	17.57 ± 0.64
	10^{-4}	2.01 ± 0.06	1.43 ± 0.09**	17.69 ± 0.86	15.65 ± 1.02
Promethazine	10^{-5}	1.93 ± 0.09	1.88 ± 0.07	23.08 ± 0.82	22.68 ± 1.29
	10^{-4}	1.89 ± 0.07	1.38 ± 0.04**	22.88 ± 0.86	20.92 ± 1.50
Diphenhydramine	10^{-5}	1.97 ± 0.06	1.90 ± 0.05	21.72 ± 1.52	21.69 ± 1.09
	10^{-4}	1.93 ± 0.04	1.58 ± 0.04**	21.71 ± 1.07	20.78 ± 1.10

Each value is given before and 20 min after drug application ($n = 6$). Significantly different from the value obtained before the drug application: $^{**}P < 0.01$.

pyrilamine, the decay phase was shortened, and in all cases the decay remained a single exponential. The effect of H_1-receptor blockers on the time constant of the decay phase is summarized in Table 2. All of the drugs tested shortened the time constant significantly at 10^{-4} M.

The electrical properties of muscle fibre membrane in the presence of H_1-receptor blockers

To investigate whether H_1-receptor blockers influence the electrical properties of muscle fibre, square wave pulses were applied directly. When a rectangular depolarizing current (100 nA) of 10 ms duration was applied, similar action potentials were elicited during perfusion of Ringer solution, whether or not it contained pyrilamine, promethazine or diphenhydramine at concentrations of 10^{-5} M and 10^{-4} M. The threshold potential of 27.8 ± 1.4 mV ($n = 21$), measured from the resting potential, and the peak amplitude of action potentials (123.1 ± 3.9 mV, $n = 21$) were not altered by any of the H_1-receptor blockers tested. When hyperpolarizing current (100 nA, 100 ms) was passed, an electrotonic change of the membrane potential (26.2 ± 1.3 mV, $n = 21$) was induced. The input resistance (262.2 ± 5.8 KΩ, $n = 21$) and the time constant (11.4 ± 0.7 ms, $n = 21$) of the membrane of muscle fibres determined from the electrotonic potentials were not changed significantly with the treatment by any of the test drugs. The changes in the actual values before and after the treatment of pyrilamine, a typical H_1-receptor blocking agent, are shown in Table 3. When the measurements were made with the other H_1-receptor blockers, the values are very similar in all parameters at any concentration.

Effects of H_1-receptor blockers on the acetylcholine-induced potential

When ACh-induced potentials were measured before and after application of the H_1-receptor blockers, the inhibitory effect of pyrilamine (10^{-5} M) on the ACh-induced potential was found to be very powerful. Inhibition increased with prolongation of perfusion time, as shown in Figure 4. Similar results were obtained with promethazine and diphenhydramine at the same concentrations.

Effects of H_1-receptor blockers on the quantal contents of the e.p.ps

The estimated value of the quantal content (m) was determined from 40 tracings recorded intracellularly from the one endplate. The quantal contents measured before and 20 min after drug application are summarized in Table 2. At a drug concentration of 10^{-5} M,

Table 3 Effects of pyrilamine on the physical properties of the cell membrane of frog sartorius muscle

Concentration (M)	*Resting membrane potential* (mV)	*Action potential* (mV)	*Threshold potential* (mV)	*Input resistance* (kΩ)	*Time constant** (ms)
Control	− 94.5 ± 3.2	123.1 ± 3.9	27.8 ± 1.4	260 ± 10.6	10.7 ± 1.4
10^{-5}	− 94.8 ± 2.8	121.1 ± 2.1	28.4 ± 0.7	262 ± 12.5	10.1 ± 0.6
10^{-4}	− 94.3 ± 3.6	119.0 ± 4.3	29.7 ± 0.6	262 ± 10.6	11.4 ± 0.7

The measurements were carried out 20 min after the start of pyrilamine perfusion ($n = 6$). *Time constant was calculated from the transient phase of the electrotonic potentials.

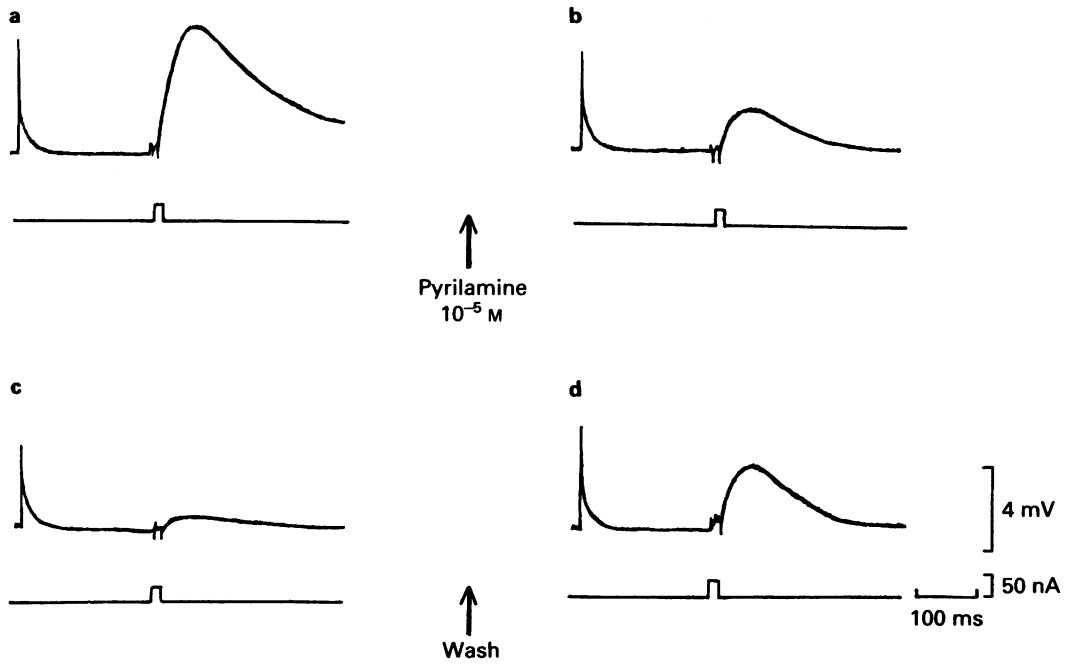

Figure 4 Effect of pyrilamine (10^{-5} M) on the acetylcholine (ACh)-induced potentials provoked by iontophoretic application of ACh (30 nA, 10 ms). In each pair of traces, the upper trace shows the e.p.p (left) and the ACh potential (right), and the lower trace shows the current pulse applied through the iontophoretic pipette. Potentials were recorded before application of the drug (a), 10 min after (b), 20 min after pyrilamine application (c), and 20 min after replacing with normal Ringer solution (d).

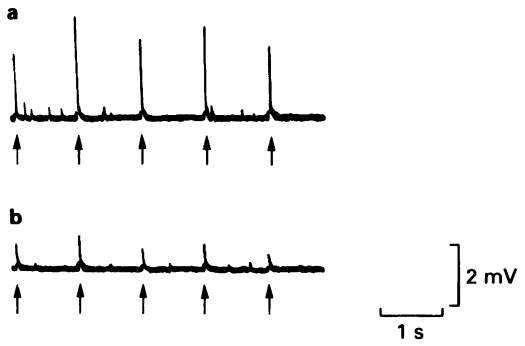

Figure 5 Effect of pyrilamine on the e.p.ps and m.e.p.ps. (a) During perfusion of high $MgCl_2$ (10 mM) and low $CaCl_2$ (1 mM) Ringer solution with no pyrilamine, e.p.ps and m.e.p.ps of frog sartorius muscles were measured. Electrical stimulation (2–5 V, 0.05 ms) was given at a frequency of 1 Hz indicated by arrows in each tracing. (b) After 20 min of pyrilamine (10^{-4} M) perfusion, both the amplitudes of e.p.ps and m.e.p.ps decreased. However, when the m value was calculated by the variance method, the values determined during pyrilamine perfusion decreased only slightly.

there were no changes in the quantal contents. At a concentration of 10^{-4} M, the quantal contents were slightly reduced but not significantly; however, the amplitude of the e.p.ps was reduced by approximately 70% at 20 min after the application of pyrilamine (10^{-4} M) as shown in Figure 5. This indicates that the major site of action of H_1-receptor blockers is located at the postsynaptic membrane rather than at the presynaptic membrane.

Discussion

The present study showed that H_1-receptor blocking agents depress the e.p.ps of (+)-tubocurarine-treated frog sartorius muscle without affecting resting membrane potentials. Takiuchi (1964) reported that promethazine and diphenhydramine elicited a neuromuscular blockade preceded by a transient augmentation of the twitches. Abdel-Aziz & Bakry (1973) also obtained similar results with diphenhydramine. However, neither augmentation of the e.p.ps amplitude nor an increase in the quantal content

was found in the present study. Moreover, H_1-receptor blockers definitely depressed the ACh-induced potential without affecting the excitability of the muscle fibre. This indicates that H_1-receptor blockers inhibit the action of ACh on the postsynaptic membrane. The present study also shows that the presynaptic effect of H_1-receptor blockers is minor compared with the postsynaptic effect, since decrease of the average quantal content is clearly less marked than reduction of the e.p.ps elicited by the same concentration of H_1-receptor blockers (Figure 1 and Table 2).

In the present experiments, a variance method was employed to estimate the quantal content, since the amplitude of m.e.p.ps was severely reduced in the presence of H_1-receptor blockers. This method would cause overestimation of quantal content at high level of release (Miyamoto, 1975) and it is necessary to correct for 'non-linear summation' (Martin, 1955). However, it can be used to compare the values obtained in both the presence and absence of drugs. The values of quantal contents (m) in the control period ranged from 17.69 to 23.08 as shown in Table 2. It has been reported that when the quantal content was estimated at 6°C in normal frog sartorius muscle in frog Ringer solution ($CaCl_2$ 1.8 mM without $MgCl_2$), the average value of m was about 300 (Katz & Miledi, 1979). However, when transmitter release was measured at frog neuromuscular junctions pretreated with glycerol, in perfusate containing various ratios of Ca/Mg, the values of m changed in the wide range from 23.2 in a Ca/Mg ratio of 1.8/4.0 to 158.6 in a Ca/Mg ratio of 2.5/0 (Miyamoto, 1975). In the present experiment, the measurement of quantal content was carried out in a medium containing a Ca/Mg ratio of 1/10, so that the value of m could be smaller than 23.2. Also, it has been shown in rat hemidiaphragm that the value of m was about 3 in a medium containing a Ca/Mg ratio of 2/12.5 (Hubbard *et al.*, 1968). Therefore, the values of quantal content determined in the present experiment may be slightly overestimated but probably remained within a reasonable range.

The time course of the e.p.ps recorded extracellularly reflects the change of the endplate conductance generated by released ACh (Katz & Miledi, 1965; Gage & Hamill, 1976). The time course of the external e.p.ps was shortened by H_1-receptor blockers in the present experiments so that it would seem that these drugs may diminish the mean open time of the endplate channel (Anderson & Stevens, 1973) and consequently, the amplitude of the e.p.ps is reduced (Gage & McBurney, 1973). It has also been shown in the present experiments that the time constant of decay phase of e.p.ps recorded extracellularly during pyrilamine perfusion remains exponential. Drugs interacting with lipid bilayers, such as octanol and several inhalation anaesthetics, decrease the time constant of decay and reduce the peak amplitude of m.e.p.cs. (Gage *et al.*, 1974; Gage & Hamill, 1976). Further, the decay of the e.p.cs remains exponential, even when the time constant of decay was decreased by 80–90% (Gage *et al.*, 1978). The possibility has been raised that octanol may increase the rate constant for the closing reaction of 'ACh-activated channels by increasing the fluidity of lipid in the vicinity of the ACh receptor-channel complex. Since it has been found that H_1-receptor blockers interact with the lipid bilayer (Rooney *et al.*, 1979), it is possible that H_1-receptor blockers may reduce the time constant of decay by shortening the life time of the endplate channel, as in the case of octanol.

H_1-receptor blocking agents are known to have local anaesthetic action and an anti-cholinoceptor effect (Dutta, 1949). Some local anaesthetics, such as procaine and lidocaine, diminish the amplitude and modify the time course of e.p.ps and e.p.cs, producing a biphasic decay (Furukawa, 1957; Steinbach, 1968). On the other hand, atropine reduces both the amplitude and the duration of the e.p.ps (Beránek & Vyskočil, 1968). By comparing sequential changes in the e.p.ps produced by local anaesthetics and atropine, it was noticed that the effect of H_1-receptor blockers is more like that of atropine. Accordingly, this effect is not the same as that seen with agents thought to act as open-channel blockers, i.e. procaine and lidocaine. Although it is difficult to understand clearly the mode of action of H_1-receptor blockers on the neuromuscular junction, it seems that they might affect the action of ACh on its postsynaptic receptor and alter the kinetics of channel closure.

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