For example, the affinity of atropine, methylatropine, 3-quinuclidinylbenzilate and dodecyltrimethylammonium are reduced by 3–6 fold, but that of scopolamine and N-methylscopolamine by 35-fold. On the other hand the affinity of propylbenzilylcholine is increased 3-fold.

At higher concentration, PCMB reduces the binding capacity of the receptors. It appears very likely that the effects of PCMB on affinity and on capacity are due to reaction with independent SH groups as suggested by Aronstam, Abood & Hoss (1978).

E.H.F. Wong is an MRC student.

References

ARONSTAM, R.S., ABOOD, L.G. & HOSS, W. (1978) Influence of sulfhydryl reagents and heavy metals on the functional state of the muscarinic acetylcholine receptor in rat brain. *Molec. Pharmac.*, **14**, 575–586.

HULME, E.C., BIRDSALL, N.J.M., BURGEN, A.S.V. & MEHTA, P. (1978). The binding of antagonists to brain muscarinic receptors. *Molec. Pharmac.*, **14**, 737–750.

Kinetic effects of tubocurarine on skeletal muscle at high agonist concentrations

D. COLQUHOUN & R.E. SHERIDAN

Department of Pharmacology, St. George's Hospital Medical School, London SW17 0RE

Experiments have been performed on voltage-clamped end-plates of *cutaneus pectoris* muscles from *Rana temporaria*, at 7–8°C. The concentrations of agonist that are conventionally employed are low in the sense that they cause a small fraction of ion channels to open at equilibrium, i.e., $\alpha \gg \beta'$ where α is the rate constant for channel shutting and β', which increases with agonist concentration, is the effective rate constant for channel opening. Under these conditions the rate constant, $\tau^{-1} = \alpha + \beta'$, for the exponential relaxation of the agonist-induced membrane current following a step change in membrane potential (a voltage jump), is approximately α, the reciprocal of the mean channel lifetime.

We have examined the effects of high carbachol concentrations (up to 500 μM) after treatment of the muscle with α-bungarotoxin (100 nM) for long enough to reduce the agonist response sufficiently to allow adequate voltage clamp. Desensitization is rapid under these conditions and the peak response appeared to occur before the agonist concentration at the end-plate membrane had reached its maximum value, unless rapid application of the agonist was achieved (confirming observations of B. Sakmann, personal communication). Fast local superfusion of the end-plate area gave responses to low (e.g. 10 μM) carbachol concentrations that reached 90% of maximum in about 6 seconds. Under these conditions the time constant for current relaxation during 64 ms voltage jumps to −150 mV was essentially independent of the degree of desensitization even with high agonist concentrations. All relaxations were fitted with an exponential curve plus a sloping baseline, thus slow relaxations could not be resolved.

The rate constant at −150 mV, τ^{-1}, changed only slightly with carbachol concentration up to 100 μM ($\tau^{-1} \simeq \alpha \simeq 240$ sec^{-1}) but thereafter increased (presumably due to an increase in β') with agonist concentration, as found by Sakmann & Adams (1976), Adams & Sakmann (1978) and, in electroplaque, by Sheridan & Lester (1977). The rate constant was doubled (i.e. 480 s^{-1}) at roughly 380 μM carbachol at which point the rate of increase was about 1.2×10^6 M^{-1}s^{-1}.

The addition of a low concentration, 0.4 μM, of tubocurarine (close to the equilibrium constant for competitive block) might be expected to increase the rate constant by about 11 s^{-1} as a result of 'channel block' (Colquhoun, Dreyer & Sheridan, 1979). In fact, the rate constant was reduced towards α by tubocurarine (e.g.) from 2α to 1.2α with 380 μM carbachol), and could be restored by a further increase in agonist concentration. This observation is similar to that made in electroplaque by Sheridan & Lester (1977). One explanation of it is that tubocurarine equilibrates rather rapidly with the acetylcholine receptor, thus reducing β'. If the dissociation rate constant was much slower than 1 ms^{-1} (implying an astonishingly fast association rate of over 10^9 M^{-1} s^{-1}) two component relaxations would be expected, which we have not been able to detect with any certainty under the difficult conditions of these experiments. The interpretation of the observations is, however, greatly complicated by the rates of diffusion of agonist and antagonist in the synaptic cleft, so the apparently fast rate of competitive block by tubocurarine requires further investigation.

References

ADAMS, P.R. & SAKMANN, B. (1978). Agonist-triggered end-plate channel opening. *Biophysic. J.*, **21**, 53a.

COLQUHOUN, D., DREYER, F. & SHERIDAN, R.E. (1979). The actions of tubocurarine at the frog neuromuscular junction. *J. Physiol. Lond.*, **293**, 247–284.

SAKMANN, B. & ADAMS, P.R. (1976). Fluctuation and relaxation analysis of endplate currents of frog neuromuscular junction induced by different concentrations of bath applied agonists. *Pflügers Archiv.*, **365**, R145.

SHERIDAN, R.E. & LESTER, H.A. (1977). Rates and equilibria at the acetylcholine receptor of *Electrophorus* electroplaques. *J. Gen. Physiol.*, **70**, 187–219.

Effects of glucocorticoids on acetylcholine release at the neuromuscular junction

H. VAN WILGENBURG

Pharmacological Laboratory, University of Amsterdam, Amsterdam, The Netherlands

It has been shown in our laboratory (Veldsema-Currie, Wolters & Leeuwin, 1976) that glucocorticoids have a direct presynaptic effect on neuromuscular transmission in the rat diaphragm, i.e. a stimulation of the choline uptake in the endplate region. It has been suggested that this direct presynaptic action of the glucocorticoids might contribute to the favourable effect of glucocorticoids in myasthenia gravis. The present study has demonstrated that prednisolone exerts several effects on the miniature endplate potentials (MEPPs), endplate potentials (EPPs) and muscular contraction. Concentrations of 4–32 μmol/l cause an increase in the mean MEPP amplitude, with a maximum of 134% of the control value at 16 μmol/l while concentrations of 260 μmol/l and higher cause a decrease below the control value. The frequency of the spontaneous MEPPs is significantly increased at concentrations of 32 μmol/l and higher, the frequency being twice the control value at 620 μmol/l. Comparable results have been obtained for EPP amplitudes, in preparations treated with a high concentration of Mg^{2+} or with (+)-tubocurarine. The quantum content and resting potential remain unchanged. An effect on muscle contraction becomes apparent after treatment with (+)-tubocurarine. The gradual decrease in twitch amplitude is retarded in presence of low concentrations of prednisolone.

After pretreatment of rats with prednisolone (1 mg/kg, i.m.) the MEPP amplitude and EPP amplitude in the isolated diaphragm are increased with a maximum at 4 h after the injection. The decrease in twitch amplitude after addition of (+)-tubocurarine is also slower than in the diaphragms of untreated rats. Iontophoretic application of acetylcholine has shown that these effects are not due to a postsynaptic effect of prednisolone. These experiments confirm the notion that glucocorticoids have a direct presynaptic effect on neuromuscular transmission and that this effect may contribute to the therapeutic value of glucocorticoids in myasthenia gravis.

Reference

VELDSEMA-CURRIE, R.D., WOLTERS, E.CH.M.J. & LEEUWIN, R.S. (1976). The effect of corticosteroids and hemicholinium-3 on choline uptake and its incorporation into acetylcholine in rat diaphragm. *Eur. J. Pharmac.*, **35**, 399–402.

Direct effects of glucocorticoids at the neuromuscular junction

R.S. LEEUWIN*& R.D. VELDSEMA-CURRIE

Pharmacological Laboratory, University of Amsterdam, Amsterdam, The Netherlands

There is little doubt at the moment that myasthenia gravis shows a number of features of an auto-immune disease. Circulating antibodies against the acetylcholine receptor protein have been isolated from blood of myasthenic patients and accordingly neuromuscular defects in these patients are considered to be localized postsynaptically (Ito *et al.*, 1978). Glucocorticoids improve muscle function in myasthenic patients, and this effect is attributed to their immunosuppressive action. Work from our laboratory has shown that in addition glucocorticoids have a direct presynaptic effect viz. a stimulation of the choline uptake in nerve endings. The endplates of the rat diaphragm possess a choline carrier system with sigmoidal kinetics, and glucocorticoids are positive cooperative effectors of this system. On the other hand there have been reports of glucocorticoids counteracting the neuromuscular blocking action of curare-like drugs, thus supporting the concept of a post-synaptic effect of glucocorticoids (Arts & Oosterhuis, 1975). We have found that the LD_{50} of (+)-tubocurarine in rats is increased significantly, although not substantially, by dexamethasone. Choline gives some protection against (+)-tubocurarine, and cholinesterase inhibitors give full protection. The blocking effect of (+)-tubocurarine on the phrenic nerve diaphragm