Depolarizations recorded at locust excitatory nerve-muscle junctions in response to DL-ibotenic acid

R.B. CLARK, K.A.F. GRATION & P.N.R. USHERWOOD

Department of Zoology, University of Nottingham, University Park, Nottingham NG7 2RD.

Two pharmacologically distinct populations of extrajunctional L-glutamate receptors, designated D (depolarizing) and H (hyperpolarizing), occur on locust skeletal muscle (Cull-Candy & Usherwood, 1973). It had seemingly been established that DL-ibotenic acid, a rigidly extended analogue of glutamate, activates H-receptors but not D-receptors and junctional glutamate receptors (Lea & Usherwood, 1973; Cull-Candy, 1976). However, we have recently obtained evidence which suggests the existence of ibotenic acid (Ib) receptors at locust glutamatergic neuromuscular junctions.

Iontophoresis of DL-ibotenic acid onto any part of the extra-junctional membrane of a locust muscle fibre evoked hyperpolarizations of up to 3 mV, but at certain locations usually either close to or within the cleft between adjacent fibres depolarizations of up to 15 mV were recorded. These Ib depolarizations were very localized. By using double-barrelled ibotenate/glutamate micropipettes, it was possible to show that regions of muscle membrane giving Ib depolarizations were excitatory junctional sites. By carefully mapping the distribution of ibotenate and glutamate sensitivities at these sites the Ib receptors were found to be restricted to a small area of junctional membrane. In many cases glutamate junctions were apparently devoid of Ib receptors.

Bath-applied glutamate (10^{-5} M) had no effect on the amplitude of currents evoked by iontophoretic application of ibotenic acid to voltage clamped muscle fibres. 10^{-4} M glutamate reversibly depressed the Ib currents by about 80%. Cross-desensitization studies using double-barrelled ibotenate/glutamate micropipettes, showed the desensitization effects of glutamate on the junctional responses and vice-versa to be dose dependent. Whereas it was possible to almost abolish the Ib response with prior application of L-glutamate the reverse was not true.

Reversal potentials of ibotenate and glutamate responses were determined by voltage-clamp. For Ib depolarizations the mean reversal potential ($\pm$s.d.) was -1.56 ± 3.27 mV ($n = 7$). Peak inward Ib current, at membrane potentials of -50 to -60 mV, was 30–40 nA for ibotenate doses of 1–5 nC. For similar membrane potentials peak inward glutamate currents were 80–100 nA. The mean reversal potential of these glutamate currents was -1.7 ± 5.3 mV ($n = 7$).

These results suggest that some of the glutamate receptors which occur at a minority of excitatory junctional sites on locust muscle are activated by DL-ibotenic acid.

This work was supported by the Science Research Council.

References

CULL-CANDY, S.G. (1976). Two populations of extrajunctional L-glutamate receptors in locust muscle fibres. *J. Physiol., Lond.*, **255**, 449–464.

CULL-CANDY, S.G. & USHERWOOD, P.N.R. (1973). Two populations of L-glutamate receptors on locust muscle fibres. *Nature, New Biol.*, **246**, 62–64.

LEA, T.J. & USHERWOOD, P.N.R. (1973). The site of action of ibotenic acid and the identification of two populations of glutamate receptors on insect muscle fibres. *Comp. gen. Pharmac.*, **4**, 333–350.

Interactions of methohexitone sodium and quaternary ammonium compounds at the avian neuromuscular junction

R.C.ELLIOTT

School of Biological Sciences, Brunel University.

Suxamethonium produces neuromuscular block of twitch fibres in the chick biventer cervicis muscle preparation and contracture of the multiply innervated slow fibres (Ginsburg & Warriner, 1960). The contracture is taken as a sign of a 'depolarizing' action. Elliott (1977) showed that a suitable concentration of methohexitone could block the contracture produced by suxamethonium without affecting its neuromuscular blocking action. During attempts to overcome this neuromuscular block produced by suxamethonium in the presence of methohexitone it was noted (unpublished observations) that tetraethylammonium bromide produced a small transient reduction in the block. A study of the interactions between tetraethylammonium and methohexitone is now reported together with observations on tetramethylammonium and tetra *n*-butylammonium.

The drugs used were: methohexitone sodium (METHO) (Eli Lilly), tetraethylammonium bromide

(TEA) (BDH), tetramethylammonium bromide (TMA) (BDH), and tetra *n*-butylammonium iodide (TBA) (BDH). Contractions of the chick biventer cervicis muscle were recorded *in vitro* using an isometric transducer. Indirect stimulation of the muscle was employed using a twice maximal stimulus (usually 50–100 μs pulses of 2.5–5.0 volts, for further details see Elliott (1977). TMA closely resembled suxamethonium in producing a contracture which varied in amplitude with the dose employed, low concentrations of TMA ($3.9–7.8 \times 10^{-5}$ M) produced a small facilitation of transmission (up to 20%), larger doses ($> 1.6 \times 10^{-4}$ M) produced neuromuscular block. The contractures produced by TMA were abolished by $3.5–5.3 \times 10^{-4}$ M METHO, TMA nevertheless still produced neuromuscular block. TEA ($0.2–7.6 \times 10^{-3}$ M) produced no contracture but facilitated transmission in a dose responsive manner, there was no neuromuscular block in the dose range tested. In the presence of low concentrations of METHO ($0.35–1.8 \times 10^{-4}$ M) TEA produced marked contractures, with higher concentrations ($3.5–5.3 \times 10^{-4}$ M) of METHO the contractures were abolished and TEA ($0.6–3.8 \times 10^{-3}$ M) now produced neuromuscular blockade.

TBA ($5.4 \times 10^{-5}–1.7 \times 10^{-3}$ M) produced neither contracture nor facilitation of transmission. Concentrations above 1.1×10^{-4} M produced a dose responsive block of transmission. In the presence of low concentrations of METHO (8.8×10^{-5} M) TBA in concentrations above 8.7×10^{-4} M now produced contractures.

It is concluded that in the presence of suitable doses of METHO all the quaternary drugs tested can produce contracture and neuromuscular block. This may suggest that although these drugs may differ in their effects on transmission at the avian neuromuscular junction their basic modes of action are the same. It is possible that some of the effects observed with METHO may be accounted for if the drug exerts an hyperpolarizing action on multiply innervated fibres and on presynaptic nerve endings.

References

ELLIOTT, R.C. (1977). Interactions of suxamethonium and barbiturate drugs at the avian neuromuscular junction. *Pharm. Res. Comm.*, **9**, 649–655.

GINSBORG, B.L. & WARRINER, J. (1960). The isolated chick biventer cervicis nerve-muscle preparation. *Br. J. Pharmac.*, **15**, 410–411.

Evidence for the presence of histamine H_1-receptors in guinea-pig brain

S.J. HILL & J.M. YOUNG

Department of Pharmacology, University of Cambridge, Hills Road, Cambridge CB2 2QD.

Histamine is present in appreciable amounts in mammalian brain and is stored in both neuronal and non-neuronal compartments, suggesting that it may have multiple roles in brain function. The field of histaminergic mechanisms in brain has been reviewed by Schwartz (1977). Both histamine H_1- and H_2-receptor types have been reported to be involved in histamine function, but in most studies the evidence for H_1-receptors is based on the use of H_1-antagonists at concentrations at which non-specific effects may occur (Schwartz, 1977). To establish whether H_1-receptors are indeed present in guinea-pig brain we have examined the binding of [^{3}H]-mepyramine, which we have recently demonstrated to be a selective ligand for histamine H_1-receptors in homogenates of smooth muscle from guinea-pig intestine (Hill, Young & Marrian, 1977). The results of this study, which we present here, show that there is appreciable binding of [^{3}H]-mepyramine to homogenates of guinea-pig brain, the properties of which are consistent with the presence of histamine H_1-receptors.

Guinea-pig whole brain was homogenized in 5 volumes of 50 mM Na-K phosphate buffer, pH 7.5, treated with a polytron blender at setting 5 for 15 s and centrifuged at 6,000 *g* for 20 minutes. The pellet was resuspended, centrifuged at 8,700 *g* for 1 min in a Beckman microfuge and the resulting pellet suspended in the same buffer. Binding assays were carried out in 50 mM Na-K phosphate buffer, pH 7.5, using a microcentrifugation assay, essentially as described previously (Hill, Young & Marrian, 1977).

The binding of [^{3}H]-mepyramine sensitive to promethazine (2×10^{-6} M), a histamine H_1-selective antagonist, was saturable with an affinity constant near 10^9 M^{-1}, similar to that observed in intestine (Hill, Young & Marrian, 1977) and in the range of values reported from measurements on the antagonism of the contractile response of intestinal smooth muscle to histamine. (+)-Chlorpheniramine was approximately 100-fold more potent than the (−)-isomer in inhibiting the binding of 10^{-9} M [^{3}H]-mepyramine, consistent with the potency ratio of 100 reported for the antagonism of the contractile response of aortic strips to histamine (O'Neill & Patil, 1975). The binding affinities of promethazine, 7×10^8 M^{-1}, and (+)-chlorpheniramine, 8×10^8