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A RE-EVALUATION OF ACETYLCHOLINE RECEPTORS ON FELINE RENSHAW CELLS

K.T. KING[1] & R.W. RYALL

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

1 The effects of atropine, methylatropine and lachesine administered by ionophoresis were examined on responses of Renshaw cells to acetylcholine, acetyl-β-methylcholine, nicotine and DL-muscarine in cats anaesthetized with pentobarbitone or chloralose.

2 The antagonists were as effective in antagonizing excitation by nicotine as they were in antagonizing excitation by acetylcholine and were only slightly more effective in antagonizing excitation by acetyl-β-methylcholine.

3 These results are discussed in relation to the characterization of acetylcholine receptors on Renshaw cells.

4 It is concluded that there are two distinct populations of receptors but that the nicotinic receptors are non-selective in their activation by agonists and antagonists, whereas the muscarinic receptors display greater selectivity.

Introduction

The ionophoretic administration of either nicotinic or muscarinic agonists to Renshaw cells in the cat causes excitation (Curtis & Ryall, 1966a). The excitatory effect of acetylcholine (ACh) and nicotinic agonists is selectively blocked by ionophoresis of dihydro-β-erythroidine (Curtis & Ryall, 1966b). Dihydro-β-erythroidine (DHβE) also selectively blocks an early, but not a late discharge of Renshaw cells evoked by antidromic ventral root stimulation (Curtis & Ryall, 1966c).

These observations, coupled with the fact that intravenous injections of atropine antagonized a component of excitation by acetyl-β-methycholine to a greater extent than excitation by ACh and blocked the late but not the early discharge to ventral root stimulation led to the proposal that Renshaw cells bear both nicotinic and muscarinic receptors for ACh which mediate the early and late, synaptically-evoked discharges respectively. It was suggested on the basis of studies with various agonists and antagonists that acetylcholine receptors on Renshaw cells more closely resembled those in autonomic ganglia than those at the neuromuscular junction. However, in these studies the action of ionophoretic atropine appeared to be non-specific in the sense that excitation by an excitatory amino acid was reduced whenever the response to ACh was more than marginally reduced and no detailed investigation of the action of ionophoretic atropine was undertaken.

It has been found that intravenous and ionophoretic administrations of atropine regularly block the discharges of Renshaw cells evoked by muscle stretch, whereas block by antagonists acting on nicotinic receptors was only occasionally observed (Ryall & Haas, 1975; Ryall, 1976), and it was concluded that predominantly muscarinic receptors are brought into play when Renshaw cells are activated under relatively physiological conditions by muscle stretch, in contrast to the predominantly nicotinic nature of the discharge evoked by synchronous and non-physiological antidromic ventral root volleys.

In another study in rats (Headley, Lodge & Biscoe, 1975) it was concluded that the cholinoceptors on Renshaw cells were unlike those in the cat because it was not usually possible to differentiate between the actions of nicotinic and muscarinic antagonists, administered by ionophoresis. The nature of cholinoceptors on Renshaw cells in cats has therefore been re-evaluated in an attempt to shed some light on the conclusions drawn from the experiments with muscle stretch.

Some of the results have been reported as an abstract (King & Ryall, 1979).

Methods

Experiments were carried out in cats weighing 2–3.5 kg, anaesthetized either with pentobarbitone sodium (35 mg/kg i.p.) or with chloralose (60 mg/kg i.v., after induction with halothane). The

[1] Present address: Neurobiology Dept., Harvard Medical School, Boston, U.S.A.

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spinal cord was transected at the level of the first lumbar segment and Renshaw cells were located in the seventh lumbar or first sacral segments by their characteristic discharges to antidromic ventral root volleys. The discharges were recorded through the centre barrel, containing 4 M NaCl, of a multibarrel assembly. The outer barrels contained either 1 M NaCl as a 'current control' or one of the drug solutions listed below. The recording barrel resistances ranged from 5–15 MΩ and drug barrel resistances ranged from 15–80 MΩ but were usually within the range 20–50 MΩ. Retaining voltages of 0.5 V were routinely applied to all drug-containing pipettes. The overall tip diameters of the multibarrel assemblies ranged from 5–8 μm.

Drugs used in this investigation were acetylcholine (ACh) bromide, 0.5 M, pH 4; sodium-DL-homocysteate (DLH), 0.2 M, pH 8; acetyl-β-methylcholine chloride, 1 M; nicotine, 0.0025–0.5 M; DL-muscarine, 0.24 M; dihydro-β-erythroidine (DHβE), 2.5 or 20 mM; atropine, 0.01–0.1 M, pH 3; atropine methylnitrate, 0.01 or 0.05 M; lachesine (0.01 or 0.05 M). All drug solutions of 0.05 M or less were made up in 0.165 M NaCl.

Extracellularly recorded action potentials were amplified, discriminated from the background noise and the frequency of firing was displayed on a chart recorder. Synaptically evoked potentials were stored in a digital transient recorder and subsequently plotted out on a chart recorder.

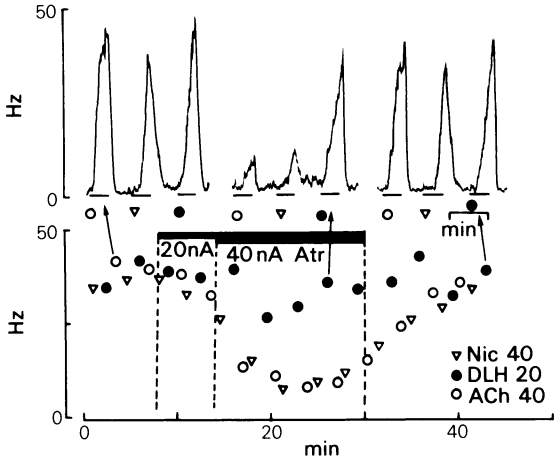

Figure 2 Antagonism of acetylcholine (ACh) and nicotine (Nic) by atropine. Upper traces are sample records of excitation by ACh, Nic sodium-DL-homocysteate (DLH) before, during and after the ionophoretic administration of atropine (Atr) at the times indicated by the arrows on the plots of maximal firing frequencies shown in the lower graph. Electrophoretic currents are in nA.

Results

Agonists

As found in a previous study (Curtis & Ryall, 1966a) the time course of excitation and recovery from excitation of Renshaw cells by ACh was considerably faster than excitation by either DL-muscarine or nicotine but was similar to that of DLH. When the concentration of nicotine in the micropipette exceeded 0.005 M the onset of excitation, when the retaining current was removed or a small ejecting current applied, was faster than observed with lower concentrations but dose-response curves could not be obtained, probably because the rate of passive diffusion from the micropipette was sufficient to produce nearly maximal firing. At lower concentrations of nicotine (0.005 or 0.0025 M) and with DL-muscarine (0.24 M), it was possible to obtain responses which reached a plateau and were graded with the magnitude of the ejecting current. However, the slow time to plateau and for recovery was such that it was rarely possible to hold cells for a sufficient time to compare antagonist potencies. Therefore most of the data on antagonists were obtained with acetylcholine as an agonist.

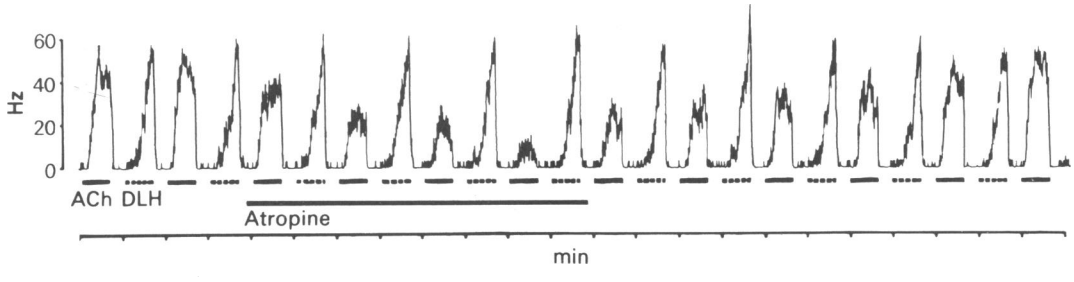

Figure 1 Antagonism of acetylcholine (ACh) excitation of a Renshaw cell by atropine. The recordings are continuous ratemeter plots of firing frequency (ordinates) evoked by ionophoretic administration of ACh (15 nA) or sodium-DL-homocysteate (DLH, 14 nA). Atropine was administered with a current of 25 nA from a 50 mM solution during the period indicated.

Antagonists

DHβE very effectively antagonized the excitatory effect of acetylcholine when the former was ejected with currents of about 10 nA from 2.5 or 10 mM solutions, as found in previous studies (Curtis & Ryall, 1966b). DHβE did not block excitation by DLH. It does not antagonize a muscarinic component of the excitatory action of acetyl-β-methylcholine (Curtis & Ryall, 1966b) and it facilitates the late discharge of Renshaw cells evoked by antidromic ventral root volleys (Curtis & Ryall, 1966c; Ryall, 1970).

It was observed that atropine consistently and selectively blocked the excitatory action of ACh, leaving excitation by DLH unaffected (Figures 1, 2). This observation was rather surprising because it has been concluded (Curtis & Ryall, 1966b) that the major effect of ACh was due to an interaction with nicotinic receptors on Renshaw cells in the cat. The effect was not restricted to atropine, but was also observed with methyl-atropine, one of the other antagonists at muscarinic receptors employed in this study: on 16/20 cells tested with either of these two substances the response to ACh but not to DLH was reduced. On one cell there was no effect on either excitant and on three cells the response to DLH was also reduced.

In ten experiments the time to reach a steady state antagonism of ACh excitation with ionophoretic currents of 5–20 nA ejecting atropine from 50 mM solutions in 0.165 M NaCl was 12 ± 1.9 min (mean ± s.e.). This value is similar to that obtained for DHβE antagonism of ACh and for strychnine antagonism of glycine (King & Ryall, unpublished). Similar time-courses were observed with methyl-atropine or lachesine (Figure 3).

Log-dose response curves to ACh were shifted to the right by atropine and lachesine and remained parallel to control curves (Figure 4) indicating that antagonism was competitive. Similar results were observed with methyl atropine.

If the effect of atropine was indeed due to competitive antagonism of the action of ACh at muscarinic receptors, then it might be expected that it would not block responses to nicotine and would be more potent against muscarinic agonists with less affinity for nicotinic receptors, such as acetyl-β-methylcholine and muscarine.

On all 7 cells tested, atropine, methylatropine or lachesine blocked the excitatory effect of ACh and nicotine to a similar degree (Figure 2) leaving the response to DLH unchanged. In three experiments atropine was only slightly more effective in antagonizing acetyl-β-methylcholine, than it was in antagonizing the action of ACh. In one experiment,

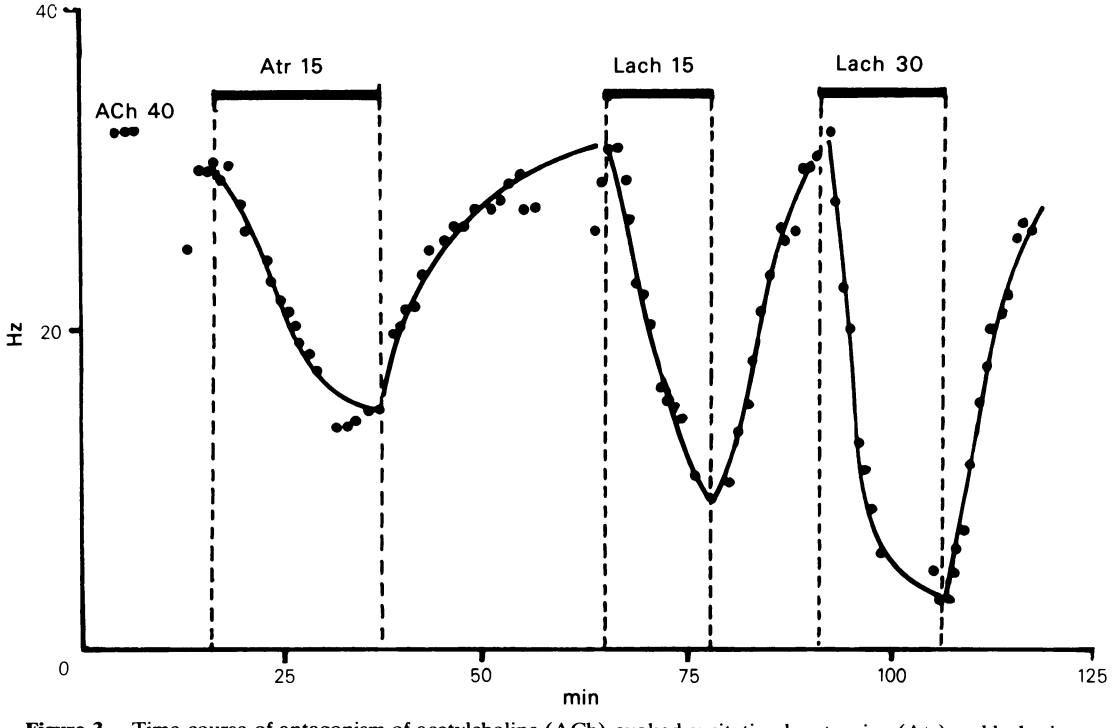

Figure 3 Time course of antagonism of acetylcholine (ACh)-evoked excitation by atropine (Atr) and lachesine (Lach). Ionophoretic currents are shown in nA.

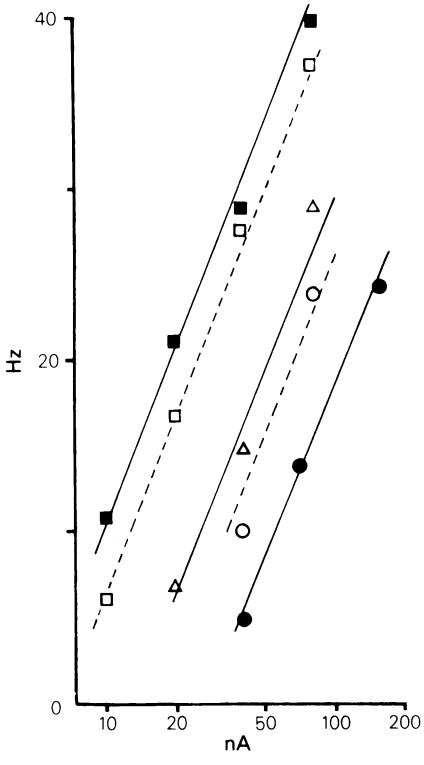

Figure 4 Log dose-response curves for antagonism of acetylcholine excitation by atropine and lachesine: (■) control; (□) recovery; (△) atropine 15nA; (○) lachesine 15nA; (●) lachesine 30nA.

lachesine was no more effective in antagonizing the action of DL-muscarine than it was in antagonizing the action of ACh.

Effects of antagonists on discharges evoked by ventral root stimulation

The effect of atropine or methyl atropine on the early discharge to antidromic ventral root volleys (Curtis & Ryall, 1966c) was observed on 13 Renshaw cells. Figure 5 illustrates the result obtained in one experiment in which the effect of methyl atropine was the largest effect observed with either atropine or methyl atropine. In this experiment, the early discharge was reduced by about 30% with a time course similar to that observed for the reduction of excitation by ionophoretically administered agonists. Similar, but smaller effects were obtained on only four other cells, there being no effect on the remaining eight cells.

The effect of ionophoretic administration of atropine on the late discharge to ventral root stimulation (Curtis & Ryall, 1966c) was examined on three cells and in every one the late discharge was abolished.

Discussion

In the cat it was shown that both DHβE and atropine selectively blocked the ACh-evoked excitation of Renshaw cells, leaving excitation by DLH unaffected. Although atropine was less effective than DHβE, antagonism seemed to be competitive, as judged from the parallel shifts in the log dose-response curves. This action was shared by other antagonists of ACh at peripheral muscarinic receptors and so was not unique to atropine. No differentiation between the action of atropine-like substances could be detected on excitation caused by ACh, acetyl-β-methylcholine, nicotine or DL-muscarine. Although atropine was slightly more potent in antagonizing acetyl-β-methylcholine than in antagonizing ACh, much larger differences would be expected if nicotinic and muscarinic receptors on Renshaw cells were identical to peripheral receptors.

In a previous investigation (Curtis & Ryall, 1966b) it was found that ionophoretically administered atropine was unable to reduce excitation by ACh without altering the sensitivity to DLH. The specific action of atropine observed in the present study is attributed to the use of smaller concentrations of atropine in the micropipettes. This would reduce the passive efflux of atropine which may be sufficient to mask specific actions when high concentrations are used. In the previous study it was observed that the response to acetyl-β-methylcholine was more affected than responses to ACh, as confirmed in the present investigation.

Since DHβE did not affect a component of excitation with acetyl-β-methylcholine which was effectively blocked by atropine (Curtis & Ryall, 1966b), it seems unlikely that the present results are explicable on the basis of only one type of receptor that can be blocked by either DHβE or atropine.

It therefore seems necessary to modify somewhat our previous ideas concerning the characterization of acetylcholine receptors on Renshaw cells, but to retain the concept that there are two types of excitatory receptor for ACh.

If we assume that there is a 'muscarinic' receptor which is similar to the muscarinic receptor in peripheral effector organs and which when activated causes the late discharge from Renshaw cells, but that there is another, quite distinct receptor with characteristics that are intermediate between those of peripheral nicotinic and muscarinic receptors, we can explain the present data and previous observations on cat Renshaw cells. The 'muscarinic' receptor would be activated by agonists such as ACh, acetyl-β-methylcholine, muscarine and carbamylcholine

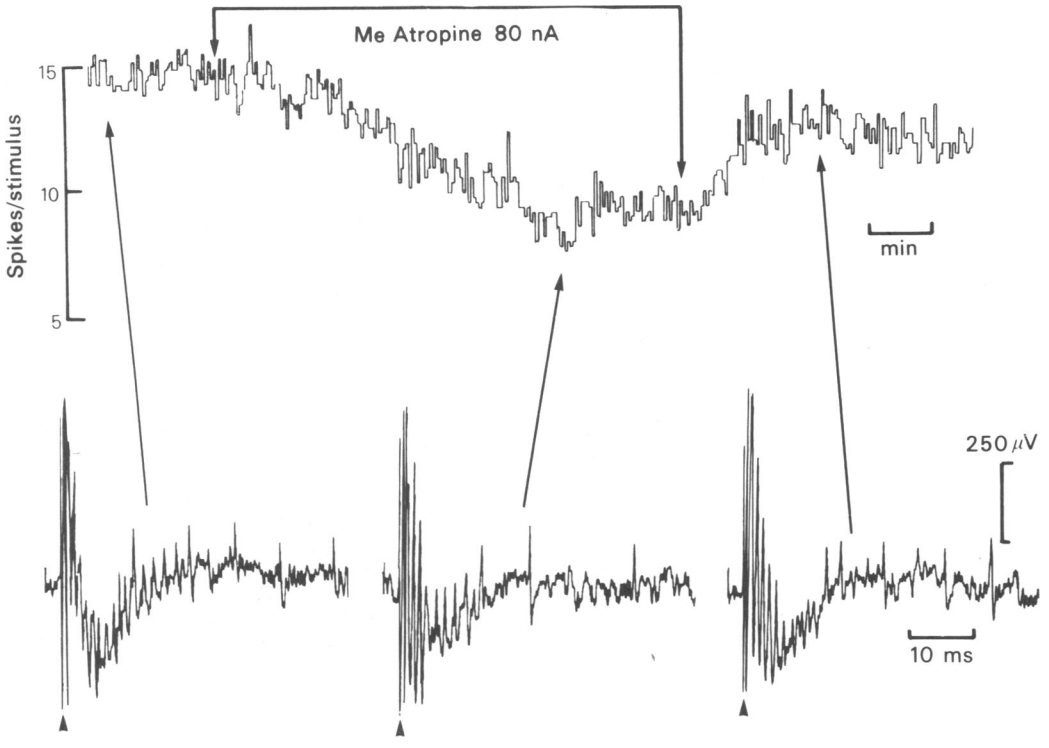

Figure 5 Effect of methyl atropine (Me Atropine) on the early discharge of a Renshaw cell to antidromic volleys in ventral root fibres. The continuous chart recording is a gated count of the number of action potentials evoked by the nerve volley. Sample records are shown before, during and after the administration of methyl atropine, ejected with a current of 80 nA. This effect was the largest seen in any experiment.

but not by nicotine. These receptors would be blocked by atropine-like substances but not by DHβE, a selective antagonist at nicotinic receptors (see Curtis & Ryall, 1966b). This would explain the reduction of the late discharge by atropine but not by DHβE.

The second type of receptor would be activated either by agonists of nicotinic receptors, such as ACh, nicotine or carbamylcholine and, probably to a lesser degree, by agonists of classical muscarinic receptors, including acetyl-β-methylcholine and muscarine. This second type of receptor is blocked either by DHβE or by atropine-like substances to a lesser degree. For this receptor we shall retain the description of nicotinic receptor.

The relative lack of specificity of the nicotinic receptors would explain the partial antagonism of the response to acetyl-β-methylcholine by DHβE (Curtis & Ryall, 1966b). It would also explain the complete block of ACh and nicotine by atropine-like substances and the partial block of the early synaptic discharge observed in this investigation, together with the virtually complete block of all of these responses by DHβE. Atropine is not without effect at the neuromuscular junction (see Headley *et al.*, 1975, for reference). However, the nicotinic receptors on Renshaw cells would appear to be more sensitive to atropine-like substances than are those at the neuromuscular junction.

In experiments on rat Renshaw cells, Headley *et al.* (1975) obtained similar results with atropine to those obtained in the present investigation in the cat. However, they were unable to demonstrate any responses that could not be blocked by DHβE. Since they were also unable to demonstrate a late discharge to ventral root stimulation in that study, although it was regularly found in an earlier study by those authors (Biscoe, Duggan & Lodge, 1973) it is possible that the muscarinic receptors in rats are less readily activated than those in the cat.

α-Bungarotoxin is ineffective in blocking the early discharge or ACh-evoked excitation of cat Renshaw cells (Duggan, Hall & Lee, 1976) and it does not block a cholinergically-mediated dorsal root potential in the frog spinal cord (Miledi & Szczepaniak,

1975), which demonstrates a further difference between nicotinic receptors on Renshaw cells and at the neuromuscular junction.

These conclusions are pertinent to our previous investigations on cholinoceptors involved in physiological activation of Renshaw cells by muscle stretch (Ryall & Haas, 1975; Ryall, 1976).

The present proposal that the muscarinic receptor on Renshaw cells can be differentiated from the nicotinic receptor by the failure of DHβE to interact with the muscarinic site is consistent with our proposal that predominantly muscarinic receptors are activated when Renshaw cells are activated by stretching muscles since these responses were readily blocked by atropine but not by nicotinic antagonists.

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