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OBSERVATIONS ON THE PHARMACOLOGY OF CHOLINOCEPTIVE NEURONES IN THE RAT BRAIN STEM

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1 The pharmacology of spontaneously active cholinoceptive neurones in the brain stem of rats anaesthetized with urethane has been investigated using microiontophoresis to administer muscarinic and nicotinic agonists and antagonists.

2 Acetylcholine (ACh) excited most cells but occasionally depressed their activity. Muscarine, and the muscarinic agonists methacholine and bethanechol produced prolonged excitation or inhibition of cells whereas nicotine produced prolonged excitations but no inhibitions.

3 Atropine selectively antagonized ACh excitations and both excitation and inhibition of neuronal activity produced by muscarine and muscarinic agonists, but not the excitations produced by nicotine, glutamate or DL-homocysteic acid.

4 Dihydro-β-erythroidine (DHBE) and tubocurarine antagonized both ACh and nicotine excitations but not those induced by glutamate or DL-homocysteic acid. Inhibitions by ACh or muscarine were not affected.

5 It is concluded that excitations of cholinoceptive neurones in the rat brain stem may be mediated by activation of both muscarinic and nicotinic receptors whereas inhibitions are mediated by activation of a muscarinic receptor.

Introduction

The firing rates of single neurones in the brain stem of the rat can be increased or decreased by microiontophoretically applied acetylcholine (ACh) (Bradley & Dray, 1972; 1973; Duggan, Headley & Lodge, 1974). However, although the pharmacology of these responses is known for the cat, little is known about their pharmacology in the rat, although it has been reported that ACh excitation of medullary neurones shows a different susceptibility to nicotinic receptor antagonists in different anaesthetic states (Duggan *et al.*, 1974).

In the present study, the responses of rat medullary neurones to iontophoretically applied ACh and other cholinomimetics have been compared and their interactions with cholinoceptor antagonists evaluated.

Methods

Adult albino rats were anaesthetized with urethane (1.3–1.8 g kg^{-1}, i.p.) and prepared for brain stem recording by the method previously described (Bradley & Dray, 1973). One barrel of a multibarrelled glass micropipette was used to record the extracellular activity of single spontaneously active neurones in the medulla. Other barrels contained aqueous solutions of the drug for electrophoresis onto the neurone being studied. The ejection of Na^+ was used as a control for current effects. Recordings were made by conventional methods. The effects of muscarine, methacholine, bethanechol or nicotine were compared on neurones which responded reproducibly to ACh. Studies with the antagonists atropine, dihydro-β-erythroidine (DHBE) or tubocurarine were made on cells which responded submaximally but consistently to applications of ACh and other agonists.

The following drugs were used in this study: acetylcholine chloride, 0.3–0.6 M, pH 4.0–5.0 (Hopkin and Williams, B.D.H. or Sigma); monosodium glutamate, 0.2 M, pH 8.0–9.0 (L. Light & Co.); DL-homocysteic acid, 0.2 M, pH 8.0–9.0 (Koch-Light); nicotine or nicotine hydrogen tartrate, 0.6 M, pH 3.0–4.0 (BDH Ltd); acetyl-β-methylcholine chloride (methacholine), 0.5 M, pH 4.0–5.0 (Koch-Light); β-methylcholine carbamate chloride

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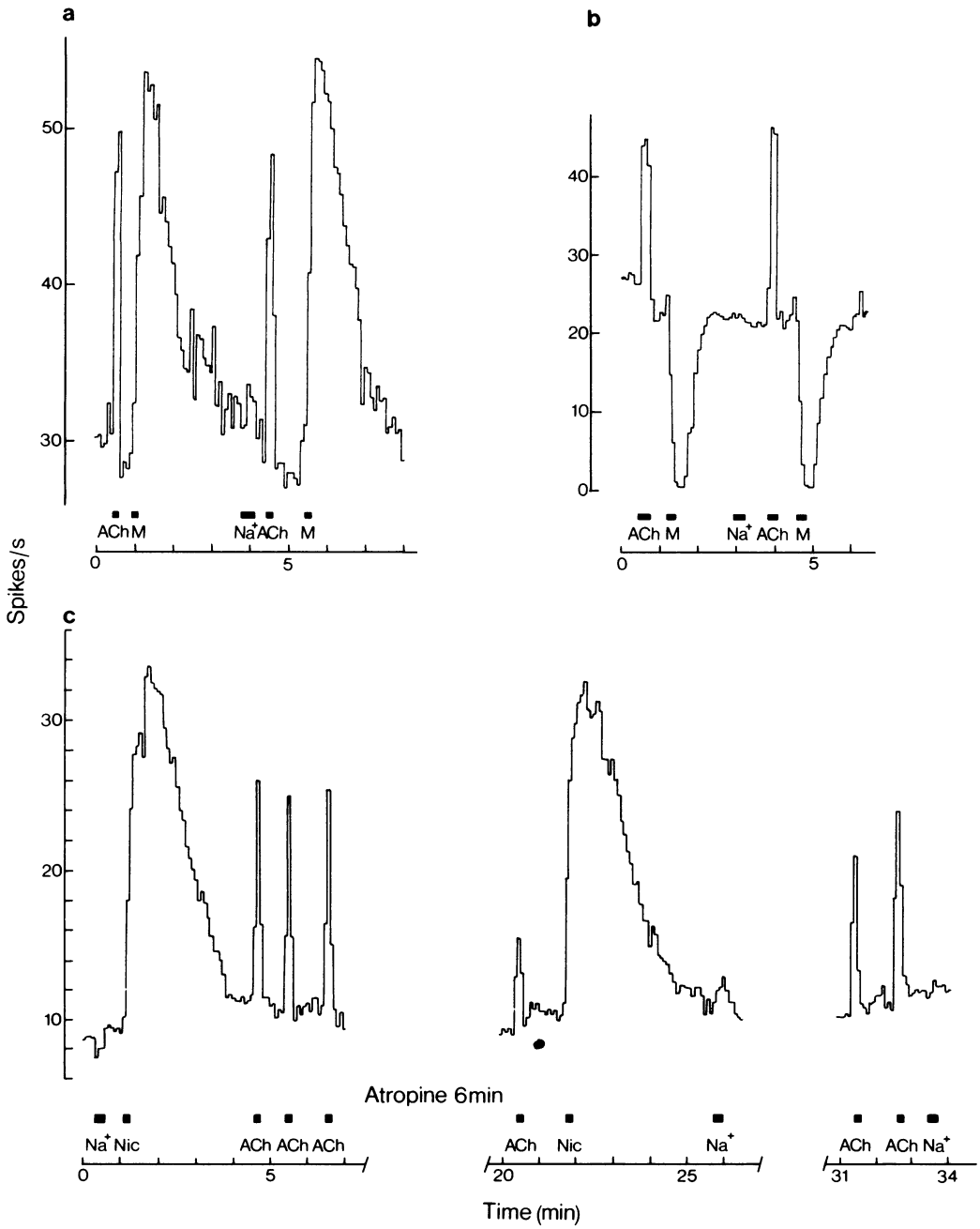

Figure 1 Histograms of neuronal firing rate (spikes/s against min) showing (a) excitatory responses to acetylcholine (ACh) and muscarine (M); (b) excitation by acetylcholine but inhibition by muscarine; (c) selective reduction of acetylcholine but not nicotine (Nic) excitation by atropine (10 nA) and subsequent recovery. All agonist expelling currents were 50 nA.

(bethanechol), 0.5 M pH 4.0–5.0 (Martindale Samoore Ltd); atropine sulphate, 0.03–0.06 M, pH 5.0–6.0 (BDH Ltd); dihydro-β-erythroidine, 0.08 M, pH 4.0–5.0 (Merck, Sharp & Dohme); (+)-tubocurarine chloride, 0.02 M, pH 4.0–5.0 (Burroughs Wellcome & Co); (+)-muscarine iodide, 0.3 M, pH 4.0–5.0 (gift from Prof. A. Herz).

Results

Most cells were excited (241/295) by ACh (5–50 nA for 5–15 s) though some were inhibited (20/295). Two types of excitatory response could be distinguished on the basis of the latency of onset; an excitation of short latency (0.2–0.8 s), and less frequently (49/295) an excitation of longer latency (5–20 seconds). Inhibition by ACh occurred after a relatively short latency (5 s) and rarely outlasted the period of ejection.

Desensitization of ACh excitation was not normally observed, but could be produced during a prolonged administration (1–2 min) of ACh or occasionally by a prior administration of nicotine. No desensitization of ACh inhibition was observed.

Muscarine, methacholine and bethanechol (15–50 nA) excited or inhibited cells but these responses were not always in the same direction as ACh responses (Table 1). It was notable that these muscarinic receptor agonists depressed cell activity more frequently than did ACh (Figure 1b). Responses to these agents were usually more prolonged than the responses to ACh (Figure 1a and b).

Nicotine (15–50 nA) excited most cells (Table 1) and these responses were more prolonged than ACh excitation of the same neurones (Figure 1c). Desensitization to nicotine excitation was sometimes observed but especially when the period between repeated ejections was short. Although nicotine was never observed to depress neurones, there was a notable increase in the number of cells inhibited by ACh when nicotine had been tested.

Prolonged applications of atropine (10–50 nA for 1–10 min) depressed neuronal firing (15/50) but occasionally increased it (2/50). Excitation by ACh was selectively reduced by atropine (48 of 50 cells) (Figure 1c). In addition both excitation and inhibition by muscarine (7/7 excitations; 5/5 inhibitions) or methacholine (10/10 excitations; 1/1 inhibition) were antagonized, but excitations by nicotine (0/12), glutamate (0/15) or DL-homocysteic acid (0/11) were unaffected.

DHBE (50–75 nA for 1–10 min) and tubocurarine (10–50 nA for 1–10 min) produced changes in cell firing. Thus 5 of 18 cells were excited and 3 depressed by DHBE and 4 of 7 cells were excited by tubocurarine. Both ACh (10/17) and nicotine excitation (10/14) were selectively reduced by DHBE. However, neither inhibition by ACh (1 cell) or muscarine (5 cells), nor excitation by glutamate (9 cells) or homocysteic acid (7 cells) were affected. Tubocurarine selectively reduced ACh (2/5) and nicotine (3/8) excitation but not that to homocysteic acid (0/5).

Discussion

In the present experiments, most (88%) spontaneously active medullary neurones were cholinoceptive, 80% being excited and a smaller proportion (8%) inhibited by ACh. Similar responses have been reported and described in detail from other studies in rats (Bradley & Dray, 1972, 1973; Duggan *et al.*, 1974). However, these findings differ from those obtained in the cat, where the number of cholinoceptive neurones appears to be smaller (< 50%) but a higher proportion (22%) are inhibited (Avanzino, Bradley & Wolstencroft, 1966; Bradley, Dhawan & Wolstencroft, 1966).

Muscarine, methacholine and bethanechol generally produced responses in the same direction as ACh. However these substances inhibited neurones more frequently than did ACh. Nicotine, however, only excited neurones. Acetylcholine inhibition was observed more frequently during testing with nicotine, suggesting that possible desensitization of excitatory receptors by nicotine allowed the ACh activation of inhibitory receptors to predominate. Responses to nicotine and to muscarinic agonists were more prolonged than those to ACh and this may be explained by differences in the efficiency of inactivation of these substances.

Desensitization of ACh excitation has been reported previously (McCance, Phillis & Westerman,

Table 1 Summary of the effects of muscarine, methacholine, bethanechol and nicotine on cells on which acetylcholine was also tested

		Muscarine			*Methacholine*			*Bethanechol*			*Nicotine*		
		+	O	−	+	O	−	+	O	−	+	O	−
Acetylcholine	+	37	1	19	41	13	9	44	7	12	37	5	0
	O	1	4	4	0	4	1	2	10	0	4	1	0
	−	0	0	0	0	0	0	0	0	9	10	1	0

Figures refer to the number of cells studied: + = excitation; O = no effect; − = inhibition.

1968; Tebēcis, 1970) and was observed in the present study only after prolonged administration of ACh or after a prior administration of nicotine but not muscarinic receptor agonists. Furthermore, inhibition of cell firing was only observed with ACh and muscarinic agonists but not with nicotine and this response did not desensitize. This suggests that desensitization is more closely related to actions involving nicotinic receptors.

Atropine selectively antagonized the excitatory effects of ACh as well as excitation and inhibition by muscarine and methacholine. Nicotine, glutamate or homocysteic acid excitation were unaffected by atropine. Such interactions provide additional evidence for the presence of excitatory and inhibitory muscarinic receptors.

DHBE selectively anatagonized some ACh excitations as well as those by nicotine. However, inhibition by ACh or muscarine was unaffected, and neither was excitation by excitant amino acids. In addition tubocurarine antagonized nicotine and ACh excitation but not excitation by homocysteic acid. It appears therefore that the receptors on cholinoceptive medullary neurones in the rat are similar to those found in the cat (Bradley *et al.*, 1966) and possess both nicotinic and muscarinic properties (Duggan *et al.*, 1974). Excitation may be mediated by activation of both types of receptor while inhibitions are mediated by activation of muscarinic receptors.

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