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SHORT COMMUNICATION

TWO DISTINCT RECEPTORS ARE ACTIVATED BY ARECOLINE ON COCKROACH SIXTH ABDOMINAL GANGLION DUM NEURONES

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During the last decade, extensive biochemical and electrophysiological studies have been performed to characterize the pharmacological properties of cholinergic receptors of insect neurone somata (Sattelle, 1985; Breer and Sattelle, 1987; Benson, 1992). Although most of these studies have been focused on the pharmacological characterization of the cell body nicotinic receptors (Lane *et al.* 1982; Harrow and Sattelle, 1983; David and Sattelle, 1984; Benson, 1992), evidence for a population of acetylcholine (ACh) receptors exhibiting muscarinic and 'mixed' (nicotinic/muscarinic) properties has been reported on insect neurone somata (Benson and Neumann, 1987; Knipper and Breer, 1988; Benson, 1989, 1992, 1993; Trimmer and Weeks, 1989; David and Pitman, 1990, 1992, 1993). One group of insect neurones, called dorsal unpaired median (DUM) neurones, expresses functional cholinergic receptors that differ from the classic vertebrate ACh receptors in that, although DUM neurones were sensitive to ACh, the nicotinic and/or muscarinic nature of these receptors has not been resolved since the cholinergic antagonists α-bungarotoxin and atropine were relatively ineffective in blocking the ACh response (Goodman and Spitzer, 1980; Lane *et al.* 1982; Lapied *et al.* 1990). However, using a range of cholinergic antagonists known to be selective for vertebrate ACh receptors, it has recently been possible to demonstrate the diversity of functional ACh receptors; these include nicotinic, 'mixed' and muscarinic receptors mediating specific responses elicited by different cholinergic agonists, such as nicotine, muscarine, oxotremorine and McN-A-343, on both isolated and *in situ* DUM neurones (Lapied *et al.* 1990, 1992; Bai *et al.* 1992). Extending these studies, we report in this paper that another cholinergic ligand, arecoline, which is known to act preferentially as a muscarinic agonist in vertebrate preparations (Wess *et al.* 1990), induced a complex response mediated by both nicotinic and muscarinic receptors on DUM neurones.

All experiments were performed on *in situ* DUM neurones situated along the dorsal midline of the sixth abdominal (A6) ganglion of the nerve cord of adult male cockroaches *Periplaneta americana* L., obtained from our laboratory stock colony maintained at

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29 °C. Animals were immobilized dorsal side up on a dissection dish. The dorsal cuticle, gut and some dorso-longitudinal muscles were removed to allow access to the ventral nerve cord. The abdominal nerve cord and its A6 ganglion, carefully dissected under a binocular microscope, were stabilized in a Perspex chamber and were continuously superfused at a constant rate (0.12 ml min^{-1}) with cockroach saline of the following composition (in mmol l^{-1}): NaCl, 200; KCl, 3.1; $CaCl_2$, 5; $MgCl_2$, 4; Hepes buffer, 10; pH was adjusted to 7.4 with NaOH. The somata of the DUM neurones were penetrated with conventional intracellular microelectrodes (tip resistances of 40–50 MΩ when filled with 1 mol l^{-1} potassium acetate, 1 mol l^{-1} KCl solution) connected to a VF-180 microelectrode amplifier having current injection capability (Biologic, Claix, France). Spontaneous electrical activity was displayed on a digital oscilloscope (Hameg HM 205-3, Frankfurt am Main, Germany) and continuously monitored on a pen chart recorder for later off-line analysis. The cholinergic agonist arecoline (ARE, 10^{-1} mol l^{-1}) was applied directly by pneumatic pressure ejection (1.05 kg cm^{-2}) onto the cell with a pneumatic pressure system (Miniframe, Medical Systems Corporation, New York, USA) as previously described (Lapied *et al.* 1990). Under these conditions, the pipette could be positioned very close to the cell from which recordings were made. With this method, increasing ejection time at a constant pressure provided a dose-dependent relationship of application of agonist onto the cell membrane. The cholinergic antagonists tested were dissolved in cockroach saline and applied through a gravity perfusion system. All drugs used were purchased from Sigma Chemicals (L'Isle d'Abeau Chesnes, France), except quinuclidinyl benzilate (QNB), 4-diphenylacetoxy-*N*-methylpiperidine methiodide (4-DAMP), methoctramine and mecamylamine (Research Biochemical Incorporation, USA). Pirenzepine was a generous gift from Boehringer Ingelheim. All experiments were performed at room temperature (20 °C).

Pressure application of 10^{-1} mol l^{-1} arecoline (ARE; for 30 ms) onto *in situ* DUM neurones (resting membrane potential −52.8±0.6 mV, *N*=30) produced a slight, slow monophasic depolarization (1.1±0.2 mV, *N*=5; Fig. 1A). Under the same experimental conditions, lower concentrations of ARE (e.g. 10^{-4} mol l^{-1}, 10^{-3} mol l^{-1}) are ineffective on this preparation. For pressure ejection durations greater than 30 ms, ARE induced an unexpected biphasic depolarization composed of a fast initial component followed by a slow second depolarizing phase (Fig. 1A). The maximum peak amplitude of the ARE-induced biphasic response, obtained for a pressure ejection duration of 100 ms, was 6.7±0.5 mV (*N*=15). The duration measured at 33 % of the full amplitude of the response was 2.4±0.3 min (*N*=15). Both the amplitude and the duration of the ARE-induced biphasic response were dose-dependent. These results suggest that the biphasic response is composed of two distinct components with different sensitivities for ARE. This hypothesis is reinforced by the observation that specific nicotinic and muscarinic antagonists can discriminate pharmacologically between the ARE-induced phases (Fig. 1B). Bath application of the muscarinic antagonist scopolamine (5×10^{-6} mol l^{-1}) completely abolished the slow component but failed to block the initial fast depolarization of the ARE-induced biphasic response. The fast depolarization was blocked when the nicotinic antagonist d-tubocurarine (5×10^{-5} mol l^{-1}) was added to the perfusion solution. This indicates that both muscarinic and nicotinic components are

involved in the ARE-induced biphasic response. In several preparations, the amplitude and the duration of the second component of the biphasic response were difficult to measure because an oscillation of the membrane potential and bursting activity were present during application of ARE. This suggests, among other possibilities, a modulation of the electrical activity of *in situ* DUM neurones by presynaptic afferents. To

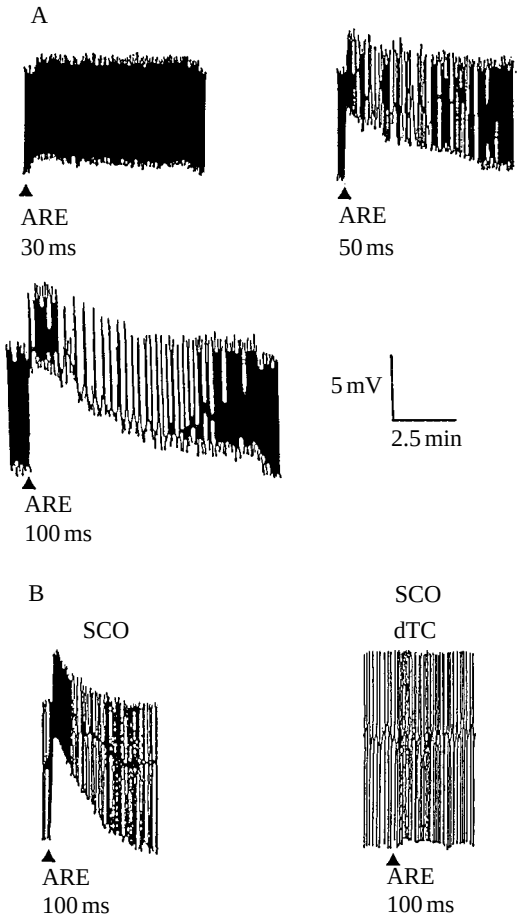

Fig. 1. Effect of arecoline (ARE) on the membrane potential of *in situ* DUM neurones (resting membrane potential −55 mV). (A) Typical examples of the dependence of the effect of ARE (10^{-1} mol l^{-1}) on micropressure ejection duration onto *in situ* DUM neurones. Application of ARE for 30 ms produced a slow membrane depolarization. With pressure applications of ARE lasting longer than 30 ms, a biphasic depolarization composed of a fast initial component followed by a slow second phase was observed. (B) Effect of muscarinic and nicotinic antagonists on the ARE-induced biphasic response (100 ms in duration). A and B were recorded on the same DUM neurone. Bath application of scopolamine (SCO; 5×10^{-6} mol l^{-1}) for 10 min completely abolished the slow depolarization. Addition of d-tubocurarine (dTC; 5×10^{-5} mol l^{-1}) blocked the residual component corresponding to the fast initial depolarization. These antagonist effects were partially reversible after 30 min of saline perfusion.

isolate DUM neurones from presynaptic inputs and to characterize the effects of ARE more precisely, similar experiments were carried out after pretreatment with tetrodotoxin (TTX). After 10 min of TTX (10^{-6} mol l^{-1}) treatment, the rhythmic activity disappeared and the ARE-induced depolarization was potentiated (maximum peak amplitude increased from 6.9±0.7 mV, *N*=10, to 16.4±1.2 mV, *N*=10) with a more pronounced biphasic response (Fig. 2A). The observation that scopolamine (5×10^{-6} mol l^{-1}) and d-tubocurarine (5×10^{-5} mol l^{-1}), used at the same concentration as in control conditions, specifically blocked the slow and fast components, respectively (Fig. 2B), indicates that TTX did not affect the pharmacological properties of the ARE-induced biphasic response. Furthermore, by using TTX, it has been possible to provide a better characterization of the pharmacological profile of this response using a range of classical cholinergic and selective muscarinic antagonists. These different effects are summarized in Table 1. The fast depolarization was sensitive to the nicotinic antagonists d-tubocurarine and mecamylamine, but resistant to α-bungarotoxin (10^{-6} mol l^{-1}) even after 90 min of exposure. It should be noted that, in some further preparations, this fast component was not completely abolished even with higher concentrations of nicotinic antagonists. The slow depolarizing phase was completely blocked by the classical muscarinic antagonists, scopolamine, quinuclidinyl benzilate (QNB) and atropine. To determine more precisely the subtype of muscarinic receptor involved in this slow component, several selective muscarinic antagonists, such as pirenzepine (PZP), methoctramine (MET) and 4-DAMP, which are known to have a high affinity for M1, M2 and M3 vertebrate receptor subtypes, respectively (for a review, see Mei *et al.* 1989),

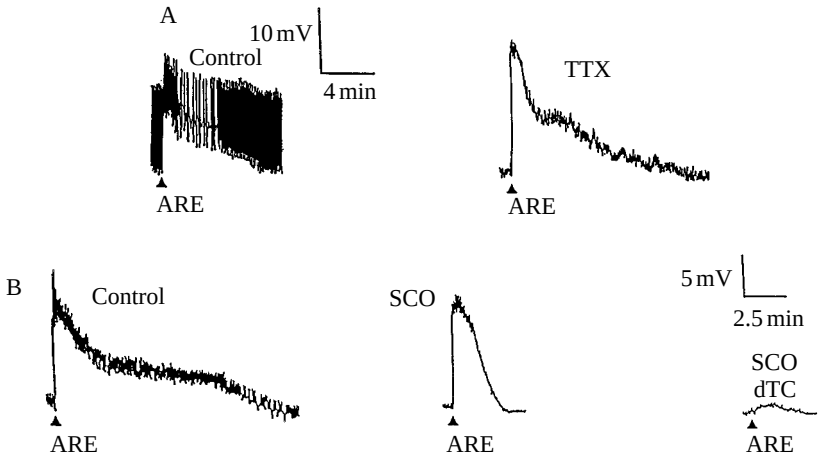

Fig. 2. Effect of pretreatment with tetrodotoxin (TTX) on the arecoline (ARE)-induced biphasic response of an *in situ* DUM neurone. (resting membrane potential −58 mV). (A) Biphasic depolarization elicited by ARE (10^{-1} mol l^{-1}, 100 ms) after perfusion with saline containing TTX (10^{-6} mol l^{-1}). (B) Effects of scopolamine (SCO; 5×10^{-6} mol l^{-1}) and d-tubocurarine (dTC; 5×10^{-5} mol l^{-1}) on the two components of the biphasic depolarization. Pretreatment with TTX produced a potentiation of the ARE-induced biphasic depolarization without any modification of the pharmacological characteristics.

have been tested (Table 1). Only PZP (10^{-5} mol l^{-1}) completely blocked the slow component. In contrast to PZP, MET and 4-DAMP used at the same concentration did not affect the slow depolarizing phase.

Although it seems well established in the literature that ARE is preferentially a muscarinic agonist in vertebrate preparations (e.g. Wess *et al.* 1990), very little is known about the effects of this cholinergic ligand in insect preparations. In contrast to vertebrate studies, our results clearly demonstrate that two distinct functional receptors (i.e. nicotinic and muscarinic receptors) are involved in the biphasic response produced by ARE. Similar effects of ARE have also been observed in the ventral giant interneurone in the cockroach A6 ganglion. In this preparation, ARE-induced depolarization consists of two phases: a fast initial depolarization followed by a slow longer second depolarizing phase, which have been pharmacologically discriminated using nicotinic and muscarinic antagonists (Le Corronc and Hue, 1993). These last results indicate the presence of both postsynaptic nicotinic and muscarinic receptors activated by ARE. Furthermore, it has been reported in isolated adult unidentified locust neurones that ARE was able to evoke both nicotinic (ACh1) and muscarinic (ACh2) responses (Benson, 1992). Interestingly, the ARE-induced depolarization differs qualitatively from all responses already described on DUM neurones using different cholinergic ligands such as nicotine, oxotremorine, muscarine and McN-A-343 (Lapied *et al.* 1990, 1992; Bai *et al.* 1992). Despite this heterogeneity of the cholinergic responses, the use of ARE confirmed that at least two major types of ACh receptors, nicotinic receptors resistant to α-bungarotoxin (Goodman and Spitzer, 1980; Lane *et al.* 1982; Lapied *et al.* 1990; Bai *et al.* 1992) and muscarinic receptors (Bai *et al.* 1992; Lapied *et al.* 1992), are present in DUM neurones. Although this muscarinic component was sensitive to scopolamine, QNB and atropine,

Table 1. *Effects of nicotinic and muscarinic antagonists on the arecoline-induced biphasic response after pretreatment with 10^{-6} mol l^{-1} of TTX*

	Fast depolarization	Slow depolarization
Nicotinic antagonists		
d-tubocurarine	5×10^{-5} mol l^{-1} (N=10)	No effect
Mecamylamine	10^{-4} mol l^{-1} (N=10)	No effect
Muscarinic antagonists		
Scopolamine	No effect	5×10^{-6} mol l^{-1} (N=10)
QNB	No effect	5×10^{-6} mol l^{-1} (N=5)
Atropine	No effect	10^{-5} mol l^{-1} (N=5)
Pirenzepine	No effect	10^{-5} mol l^{-1} (N=5)
Methoctramine	No effect	No effect at 10^{-5} mol l^{-1} (N=5)
4-DAMP	No effect	No effect at 10^{-5} mol l^{-1} (N=5)

The values given in the table represent the concentrations that produced 100 % inhibition.

These antagonist effects were partially reversible after 30 min of saline perfusion.

No effect means that the cholinergic antagonist tested did not affect the response even after 90 min of exposure.

QNB, quinuclidinyl benzilate; 4-DAMP, 4-diphenylacetoxy-*N*-methylpiperidine methiodide.

these muscarinic antagonists do not discriminate between the muscarinic receptor subtypes. Of the vertebrate subtype-selective muscarinic antagonists (i.e. PZP, MET and 4-DAMP) tested in this study, PZP is the most active. These last results, together with previous findings reporting the high sensitivity for PZP (10^{-8} mol l^{-1}) of the slow depolarizing second phase induced by McN-A-343 in isolated DUM neurones (Lapied *et al.* 1992), provide increasing evidence for the presence of an M1-like muscarinic receptor subtype mediating the slow muscarinic depolarizing component in DUM neurones.

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