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SHORT COMMUNICATION

DO PRESYNAPTIC MUSCARINIC RECEPTORS REGULATE ACETYLCHOLINE RELEASE IN THE CENTRAL NERVOUS SYSTEM OF THE COCKROACH *PERIPLANETA AMERICANA*?

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Although insects have proved to be suitable subjects for neuropharmacological investigations, the role of muscarinic acetylcholine (ACh) receptors remains unresolved. Up to now, this problem has mainly been investigated by studying the characteristics of ACh release from insect synaptosomes (Breer & Knipper, 1984) and by characterizing the binding to central nervous system (CNS) extracts from the cockroach of two muscarinic receptor-blocking drugs, L-[benzilic-4,4′-^{3}H] quinuclidinyl benzilate (Meyer & Edwards, 1980; Lummis & Sattelle, 1985) and [*N-methyl*-^{3}H]scopolamine (Lummis & Sattelle, 1986). These parallel approaches have shown that muscarinic receptors exist in the insect CNS. The functional role of these receptors is investigated in the present study by electrophysiological experiments on a synaptic preparation from the CNS of the cockroach, *Periplaneta americana*.

The synaptic preparation was carefully dissected from the nerve cord of adult males, and consisted of a cercus, the corresponding cercal nerve XI, the sixth abdominal (A6) ganglion (containing the studied synapses) and the ventral nerve cord. The electrophysiological experiments employed the single-fibre oil-gap technique pioneered by Callec (1974), which allows reliable recordings of postsynaptic potentials in an isolated giant interneurone in response to presynaptic stimulation. Unitary excitatory postsynaptic potentials (uEPSPs) were evoked by mechanical stimulation of a single mechanoreceptor (long bristle hair) on the cercus. Composite EPSPs (cEPSPs) were triggered by electrical stimulation of the ipsilateral cercal nerve XI. In the cockroach, EPSPs are known to result from the interaction of ACh with nicotinic ACh receptors of giant interneurones (Sattelle *et al.* 1983). Sensitivity of nicotinic ACh receptors was tested by ionophoretic injection of carbamylcholine (CCh) according to the method developed by Callec *et al.* (1982). Direct measurements of postsynaptic membrane resistance were achieved using a balanced Wheatstone bridge circuit to apply hyperpolarizing square current pulses to the membrane. The saline superfusing the desheathed A6

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ganglion had the following composition (in mmol l^{-1}): NaCl, 208; KCl, 3·1; $CaCl_2$, 10; $NaHCO_3$, 2; sucrose, 26; pH 7·2. Experiments were carried out at room temperature (20°C). The results were expressed as mean ± S.E. when quantified and the statistical significance was assessed by analysis of variance in which a P value less than 0·05 was regarded as significant.

To determine whether muscarinic ACh receptors might modulate ACh release from cercal nerve endings, we recorded the electrical activity of the giant interneurone 2 in the absence and in the presence of muscarinic receptor ligands having either blocking or activating properties. The most striking effect of atropine alone (Fig. 1) was significantly ($P < 0·05$) to enhance both EPSPs without any apparent effect on the direct depolarizing response evoked with CCh: uEPSP and cEPSP amplitudes increased from 1·76 ± 0·076 ($N = 4$) to 3·33 ± 0·047 mV

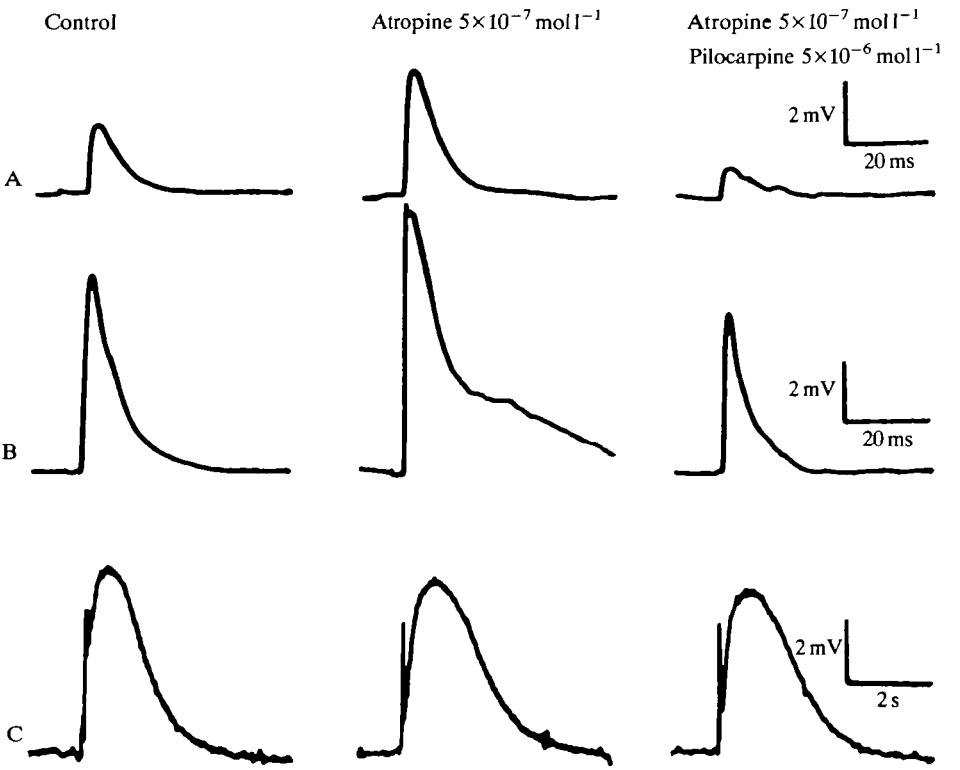

Fig. 1. Effects of 5×10^{-7} mol l^{-1} atropine and 5×10^{-6} mol l^{-1} pilocarpine on the amplitudes of the uEPSP (A), cEPSP (B) and carbamylcholine (CCh) potential (C, 300 nA for 100 ms). Each record represents the steady-state effect reached within 25 min after application of each drug. In A and B, traces represent the average of 10 consecutive sweeps (in B, the small vertical deflection at the top of the cEPSP in the presence of atropine indicates that the spike threshold was reached for one sweep). Note that both EPSPs are enhanced by atropine whereas the CCh potential remains almost unchanged. Pilocarpine added to the superfusing saline abolishes the facilitatory effect of atropine and reduces the amplitudes of EPSPs to even lower values than the control ones.

($N = 5$) and from $5{\cdot}45 \pm 0{\cdot}061$ ($N = 4$) to $7{\cdot}15 \pm 0{\cdot}157$ mV ($N = 3$), respectively. Most often a spike was elicited in the giant interneurone when the cEPSP was increased enough to reach the threshold ($7{\cdot}79 \pm 0{\cdot}233$ mV, $N = 7$). This facilitatory effect was noted over a wide range of atropine concentrations (10^{-8}–10^{-6} mol l^{-1}). Nevertheless, in this preliminary study, no attempt was made to determine the most potent facilitatory dose of atropine. Moreover, an opposite action of atropine was observed at 10^{-5} mol l^{-1}. The facilitatory action of 5×10^{-7} mol l^{-1} atropine could easily be suppressed by application of 5×10^{-6} mol l^{-1} of the muscarinic agonist pilocarpine, reflecting the relative potencies of the ligands (Fig. 1). Under these conditions, uEPSP and cEPSP amplitudes were significantly decreased ($P < 0{\cdot}05$) to even lower values than under control conditions, $0{\cdot}92 \pm 0{\cdot}099$ ($N = 4$) and $4{\cdot}48 \pm 0{\cdot}097$ mV ($N = 4$), respectively. This depressive synaptic action of pilocarpine was also observed without preliminary treatment with atropine. Further investigations were made with the anticholinesterase agent eserine, to increase the amount of endogenous ACh in the synaptic cleft. At the cercal–giant interneurone synapse, 10^{-7} mol l^{-1} eserine transiently increased ($P < 0{\cdot}05$) both the amplitude (from $1{\cdot}41 \pm 0{\cdot}035$ to $1{\cdot}99 \pm 0{\cdot}054$ mV, $N = 6$) and the half-time of decay (from $6{\cdot}57 \pm 0{\cdot}196$, $N = 6$, to $9{\cdot}27 \pm 0{\cdot}405$ ms, $N = 3$) of the uEPSP as well as those of the cEPSP (amplitude: from $5{\cdot}23 \pm 0{\cdot}271$, $N = 5$, to $6{\cdot}87 \pm 0{\cdot}570$ mV, $N = 3$; half-time of decay: from $5{\cdot}80 \pm 0{\cdot}253$, $N = 5$, to $13{\cdot}67 \pm 2{\cdot}293$ ms, $N = 3$) evoked by presynaptic stimulation (Fig. 2 and Lapied *et al.* 1988). The secondary effect of eserine (a decrease of uEPSP and cEPSP amplitudes to values significantly lower than control ones, $0{\cdot}575 \pm 0{\cdot}058$, $N = 4$, and $2{\cdot}31 \pm 0{\cdot}083$ mV, $N = 5$, respectively) might be attributed to the direct effect on presynaptic muscarinic receptors of an elevation in the ACh concentration at the synaptic cleft. If this hypothesis were correct, then atropine should restore the large uEPSP and cEPSP due to eserine. The last panel of Fig. 2 shows that application of 5×10^{-7} mol l^{-1} atropine, following preliminary treatment with 10^{-7} mol l^{-1} eserine, indeed restored the large EPSPs. The levels of membrane resistance and resting potential, which can affect EPSP amplitude, were not changed by the presence of atropine, pilocarpine and eserine.

The present study suggests that the muscarinic ligands atropine and pilocarpine can modulate ACh release from nerve endings in the cockroach CNS, according to the hypothesis developed by Polak (1971) for vertebrate CNS. Although atropine also has some actions on the nicotinic receptor ionophore complex in insect CNS, albeit at higher concentrations (Dudai, 1978; David & Sattelle, 1984), our study gives electrophysiological evidence for a functional role for the presynaptic muscarinic ACh receptors detected biochemically in the insect CNS (Meyer & Edwards, 1980; Breer & Knipper, 1984; Lummis & Sattelle, 1985, 1986). Several arguments support this conclusion. (i) The great enhancement of EPSPs in the presence of low doses of atropine. Because uEPSPs were increased in magnitude, the enhancement of cEPSPs cannot be explained by an increased axonal presynaptic excitability in the presence of atropine. (ii) The antagonistic action of pilocarpine indicates that both muscarinic ligands, atropine and pilocarpine, act at

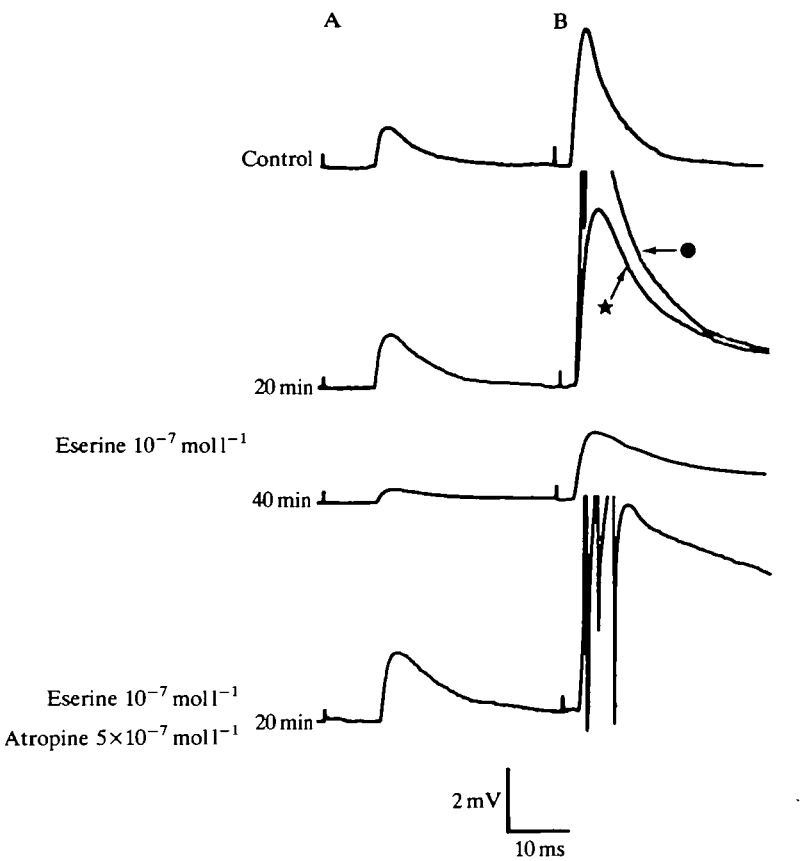

Fig. 2. Effects of 10^{-7} mol l^{-1} eserine on the uEPSP (A) and cEPSP (B), and combined action of eserine and 5×10^{-7} mol l^{-1} atropine. A biphasic action is observed following application of eserine, i.e. an increase in EPSP amplitudes and durations is followed by a progressive decrease of amplitude, but not duration, of the EPSPs. Atropine, added to the saline containing eserine, suppresses the inhibition and enables the cEPSPs once again to reach the spike threshold in B. In B, second row from the top, keeping the same stimulation intensity as that of the control triggered a suprathreshold cEPSP (filled circle), whereas lowering it evoked a subthreshold cEPSP (star) from which the increased duration was measured.

the same site. (iii) The stabilities of the postsynaptic membrane resistance and resting potential, and especially the apparent stability of the CCh-induced potential, argue in favour of a presynaptic localization of the mechanism responsible for the observed effects. (iv) The restoration of large cEPSPs with atropine, after exposure of the synapse to eserine, further supports the hypothesis of a muscarinic presynaptic site regulating ACh release. (v) The observation that pilocarpine not only reverses the action of atropine, but also decreases the EPSP of control synapses, suggests a muscarinic feedback inhibition of ACh release by synaptically released ACh. In conclusion, cercal nerve–giant interneurone syn-

apses of the cockroach seem strongly to regulate ACh release *via* presynaptic muscarinic receptors. Further electrophysiological investigations will be needed to determine the pharmacological properties of such presynaptic muscarinic receptors and the molecular mechanisms underlying this regulation of ACh release.

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