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SHORT COMMUNICATION

ELECTROPHYSIOLOGICAL EVIDENCE FOR THE MODULATION OF ACETYLCHOLINE RELEASE BY ENDOGENOUS ACETYLCHOLINE IN THE COCKROACH CENTRAL NERVOUS SYSTEM

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Biochemical studies of the central nervous system (CNS) of locusts (Breer and Knipper, 1984; Knipper and Breer, 1988) have provided evidence for a muscarinic negative feedback mechanism in which muscarinic antagonists and agonists, respectively, enhance and decrease the acetylcholine (ACh) output. More recently, this inhibitory action of presynaptic muscarinic acetylcholine receptors (mAChrs) has been demonstrated in cockroach (Hue *et al.* 1989; Le Corronc *et al.* 1991) and in tobacco hornworm (Trimmer and Weeks, 1989) using electrophysiological methods. However, in insects, most experiments have not been performed under physiological conditions but in the presence of acetylcholinesterase inhibitors or exogenous agonists. The aim of this study was to determine whether the release of ACh at a central synapse in the cockroach, *Periplaneta americana*, could be modulated by endogenous ACh acting on presynaptic muscarinic receptors.

Adult male cockroaches were used for all the experiments. Recordings of composite excitatory postsynaptic potentials (cEPSP) and unitary excitatory postsynaptic potentials (uEPSP) were performed using the single-fibre oil-gap method (Callec *et al.* 1971; Callec, 1974) applied to the cercal nerve giant interneurone synapse located within the neuropile of the sixth abdominal (A6) ganglion. A cercus and the corresponding cercal nerve were isolated together with the abdominal part of the nerve cord. The A6 ganglion was desheathed and giant interneurone 2 (GI2) from the ventral group (Harris and Smyth, 1971) was isolated with fine stainless-steel needles between the fifth and sixth abdominal ganglia as close as possible to ganglion A6. The preparation was transferred to an oil-gap recording chamber and continuously superfused ($1 ml min^{-1}$) with the following saline ($mmol l^{-1}$): NaCl, 208; KCl, 3.1; $CaCl_2$, 5.4; $NaHCO_3$, 2; sucrose, 26; pH7.4. Subthreshold cEPSPs were evoked by short electrical stimulation of the ipsilateral cercal nerve and uEPSPs were evoked by mechanical stimulation of a single mechanoreceptor (long bristle hair) on the cercus. Synaptic events recorded in GI2 were displayed on a digital oscilloscope and stored on a video cassette for later off-line analysis. The

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muscarinic agonist arecoline (ARE) was pressure-ejected into the neuropile of ganglion A6 through a glass micropipette connected to a pneumatic pressure-ejection system (Neurophore BM-2H system, Medical Systems Corporation, New York, USA). Atropine (ATR) and scopolamine (SCO) were added to the superfusing saline. When necessary, ionophoretic injections of $1\,\mathrm{mol\,l^{-1}}$ ACh were made into the neuropile near the dendritic tree of the GI2 under test. The synaptic ACh potentials recorded were the reflection of the activation of postsynaptic cholinergic receptors. The measurement of postsynaptic membrane conductance was achieved using a balanced Wheatstone bridge circuit allowing application of hyperpolarizing pulses through the intraganglionic part of GI2. The postsynaptic resting potential was continuously monitored on a pen chart recorder. ARE hydrobromide, ACh chloride, ATR sulphate and SCO hydrochloride were obtained from Sigma Chemicals (USA). Experiments were carried out at room temperature (20°C). For all experiments the measurement of cEPSP or uEPSP inhibition was determined as: [(control−experiment)/control]×100. 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 of less than 0.05 was regarded as significant.

Repeated stimulation of the cercal afferent/giant interneurone synaptic system at various frequencies produced a decrease in the cEPSP amplitude that rapidly stabilized to a plateau level that was correlated to the stimulus frequency (Callec *et al.* 1971; Callec, 1974). To study the possible involvement of negative feedback acting through presynaptic muscarinic receptors activated during this synaptic depression, the effects of the muscarinic antagonist ATR were tested. The effect of a 30s stimulus train at 0.5, 1, 2, 5 or 10Hz was compared with the mean reference cEPSP amplitude obtained at 0.5Hz both in control saline and after 60min of exposure to ATR. The measurements of cEPSP amplitude were made at the end of the train and were the mean amplitude of the last 3–10 cEPSPs depending on the frequency used. Fig. 1A shows that $10^{-6}\,\mathrm{mol\,l^{-1}}$ ATR has little effect on the frequency-dependent depression at 5 and 10Hz, whereas $10^{-5}\,\mathrm{mol\,l^{-1}}$ ATR significantly reduces cEPSP depression at the same frequencies. ATR has no effect between 0.5 and 2Hz. It has been reported (Hue *et al.* 1989) that high concentrations of ATR can exert a blocking action on the postsynaptic receptor channel/complex and can be responsible for a modification of cEPSP depression. The lack of effect of $10^{-5}\,\mathrm{mol\,l^{-1}}$ ATR on ACh potentials (Fig. 1B) suggests that ATR acts at a site other than the postsynaptic receptor/channel complex. Depression of the cEPSP at 10Hz was significantly reduced from 44±3% (N=3) to 32±2% (N=3) in the presence of $10^{-6}\,\mathrm{mol\,l^{-1}}$ SCO (not shown). These effects of ATR and SCO suggest that ACh release evoked by a stimulus train activates muscarinic autoinhibition. However, experiments made at 10Hz in the presence of muscarinic antagonists show that a larger part of the effect can be attributed to independent muscarinic depression (about 28%) than to presynaptic muscarinic inhibition (about 12%) obtained by difference between control and treatments with ATR and SCO.

In order to confirm results obtained in the presence of ATR and SCO, the competition between an exogenously applied agonist (ARE, Le Corronc *et al.* 1991) and endogenously released ACh was tested by recording cEPSPs and uEPSPs in the presence of $10^{-6}\,\mathrm{mol\,l^{-1}}$ ARE at different stimulation frequencies. Experiments were performed

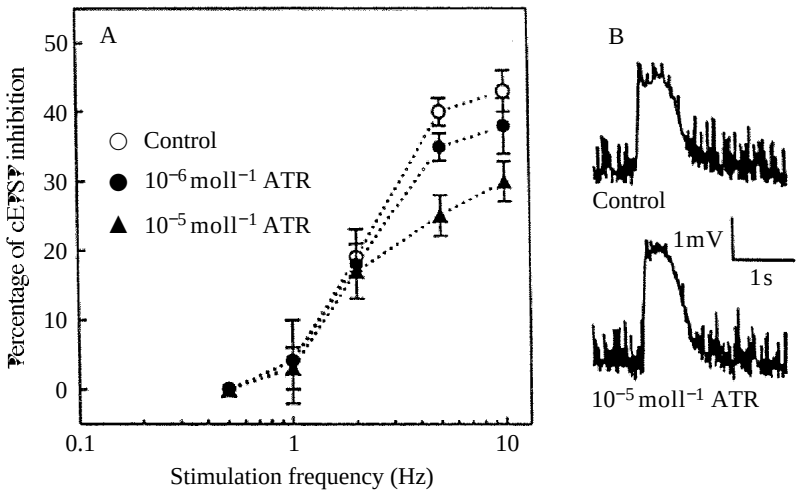

Fig. 1. Effects of atropine (ATR) on cEPSP depression (A) and ACh potential (B). (A) Decrease in frequency-dependent cEPSP depression caused by ATR. Preparations were stimulated for 30s at frequencies of 0.5–10Hz in the absence (open circles) or in the presence of 10^{-6} moll^{-1} ATR (filled circles) or 10^{-5} moll^{-1} ATR (filled triangles). The percentage of cEPSP inhibition was determined from a comparison between the mean cEPSP amplitude at the reference frequency (0.5Hz) obtained immediately before each stimulus train and the mean cEPSP amplitude measured at the end of each stimulus train. Significant differences (P<0.05) between control and ganglia treated with 10^{-5} moll^{-1} ATR were obtained at 5Hz and 10Hz. Data points are means ± S.E. from three preparations. (B) ATR (10^{-5} moll^{-1}) has no apparent effect on the ACh potential, which implies that the nicotinic postsynaptic receptor/channel complex is not the major site of ATR's effects. The thick baseline in the ACh potential recordings represents uEPSPs which are unchanged in the presence of ATR.

as follows. The preparation was electrically stimulated (cEPSP) at a fixed frequency (0.1, 0.5, 1, 2 or 10Hz) for 30s and the mean amplitude of the last 3–10 cEPSPs was determined to be the control amplitude. Pneumatic ejection of ARE was immediately started and was stopped when the cEPSP amplitude stabilized. The total duration of an experiment was never longer than 1min. A similar protocol was performed using mechanical stimulation (uEPSP) at 0.5 and 10Hz. Fig. 2 shows that ARE inhibited cEPSPs for all tested stimulation frequencies, but at 10Hz the cEPSP inhibition was attenuated (28±5%) compared with that at lower frequencies (51±4% at 0.1Hz). Similarly, ARE-induced uEPSP inhibition was significantly reduced from 56±4% (N=3) at 0.5Hz to 34±4% (N=3) at 10Hz.

Because the frequency-dependent cEPSP depression could be partly due to changes in postsynaptic membrane resistance, postsynaptic membrane potential and/or sensitivity of postsynaptic ACh receptors, the postsynaptic membrane resistance and the ACh potential were recorded immediately before and after a 10Hz stimulus train applied to the sensory nerve (duration 30s). No modification of the membrane potential (not shown), the membrane resistance or the ACh potential was observed after the stimulus train (Fig. 3),

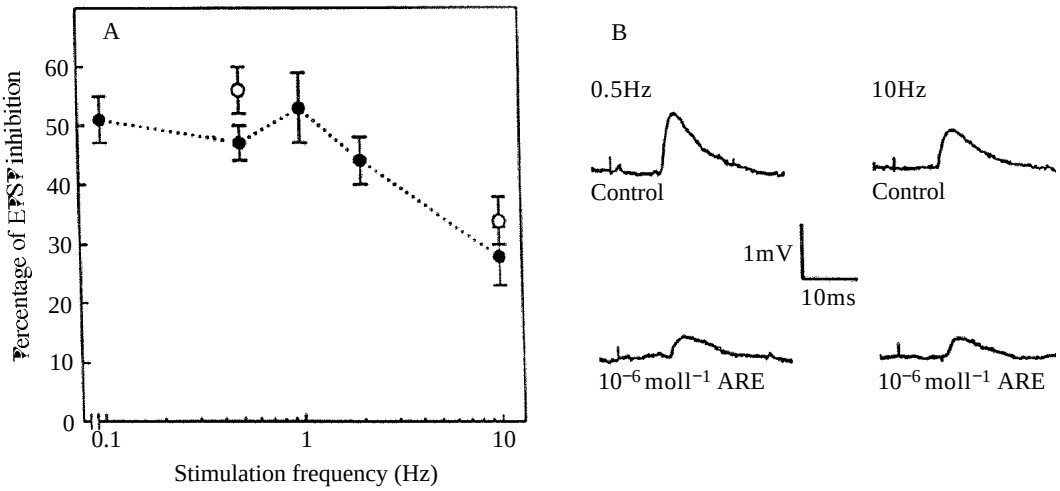

Fig. 2. Effect of the frequency of stimulation of presynaptic afferents on the inhibition of cEPSPs (A) and uEPSPs (A,B) produced by pressure ejection of 10^{-6} moll^{-1} arecoline (ARE). Ejection into ganglion A6 started immediately after a 30s stimulation train at each of the tested frequencies (0.1–10Hz). (A) For cEPSPs (filled circles) and uEPSPs (open circles) the ARE-induced inhibition was significantly reduced (P<0.05) at 10Hz compared to other frequencies (0.1–2Hz for cEPSP and 0.5Hz for uEPSP). Data points are mean values ± S.E. for 3–7 preparations. (B) Typical reduction in inhibition induced by 10^{-6} moll^{-1} ARE of uEPSPs at 10Hz compared with the relatively smaller reduction seen at 0.5Hz. Note that under control conditions a decrease in uEPSP amplitude was observed during repeated mechanical stimulation at 10Hz compared with 0.5Hz.

suggesting that these postsynaptic membrane characteristics remain quite stable during the cEPSP depression.

The synaptic depression in ganglion A6 of the cockroach triggered by repetitive presynaptic stimulation was first described by Callec, who emphasized the possible decrease in the quantity of presynaptic ACh content (Callec *et al.* 1971; Callec, 1974). However, this author also concluded that other factors might be associated with depressive effects; for example, a desensitization of postsynaptic ACh receptors or a shift in the membrane potential. In our experiments the apparent lack of effect of repetitive stimulation on the postsynaptic membrane potential, on the direct postsynaptic response evoked by ACh or on the postsynaptic membrane resistance is in agreement with the above suggestion that depression was due to presynaptic mechanisms. The inhibitory effect of ARE on the cEPSP amplitude was greatest at low frequencies. Conversely, ATR reduced cEPSP depression at high stimulation frequencies. These results are similar to those obtained in the vertebrate nervous system (Kilbinger, 1977; James and Cubeddu, 1984; Wessler *et al.* 1987; Manabe *et al.* 1991) and can be explained by changes in the biophase concentration of endogenous ACh, which is assumed to be increased by high frequencies of stimulation, thus inhibiting subsequent ACh release. The inhibitory effect of an exogenous agonist is thereby attenuated by competition with endogenous ACh on presynaptic mAChrs. Conversely, endogenous ACh did not evoke its inhibitory response

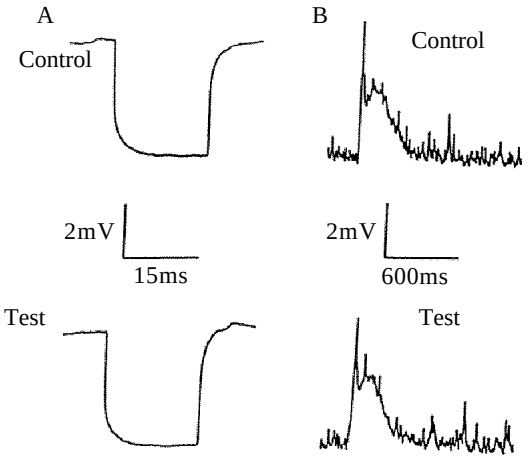

Fig. 3. Lack of effect of a 30s electrical stimulus train (10Hz) applied to the cercal nerve on postsynaptic membrane resistance (A) and acetylcholine potential (B). (A) The response of postsynaptic (GI2) membrane to a hyperpolarizing current pulse. (B) ACh potentials obtained by ionophoretic injection of ACh into the neuropile near the dendritic tree of GI2. In comparison with control conditions, no modification of the membrane resistance or ACh potential was found after the repetitive stimulation (Test).

when muscarinic antagonists were applied to the ganglion. In conclusion, it is suggested that the cEPSP depression partly involves an activation of presynaptic muscarinic autoreceptors by endogenous ACh. Because the stimulation frequencies used in this study correspond to the normal firing rates of sensory cercal fibres it is proposed that muscarinic autoinhibition in the CNS of the cockroach may have physiological significance.

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