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SHORT COMMUNICATION

A SULPHYDRYL REDUCING AGENT, DITHIOTHREITOL, MODIFIES AGONIST–NICOTINIC RECEPTOR INTERACTION IN AN IDENTIFIED INSECT NEURONE

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Nicotinic acetycholine receptors are present at high density in the nervous system of insects (Sattelle, 1980). Studies on the structure of nicotinic acetylcholine receptors have shown a degree of homology between insect α-like subunits and α-subunits from other species (Bossy *et al.* 1988; Marshall *et al.* 1990; Sawruk *et al.* 1990*a*). The locust α-subunit, when expressed in *Xenopus* oocytes, is able to form a functional receptor-channel that is apparently homo-oligomeric and mimics several properties of *in vivo* receptor pharmacology (Marshall *et al.* 1988, 1990). It seems likely that the structure of native receptors will include other subunits; non-α-subunits have been described in several species of insect (Hermans-Borgmeyer *et al.* 1986; Sawruk *et al.* 1990*b*). Expression studies show that the α-subunit carries the binding site for acetylcholine and also for a number of other ligands, including nitromethylene insecticides (Leech *et al.* 1991). Sequencing α-subunits from insects has shown the presence of conserved cysteine residues that could form an extracellular disulphide bond (Bossy *et al.* 1988; Marshall *et al.* 1990; Sawruk *et al.* 1990*a*). Compounds that reduce disulphide bonds, such as dithiothreitol (DTT), are known to decrease the sensitivity of nicotinic receptors to acetylcholine (Karlin and Bartels, 1966), suggesting that such a bond is located close to the anionic binding site of the neurotransmitter recognition site on the receptor-channel molecule.

In this report we show that DTT partially blocks the response of the cockroach fast coxal depressor motor neurone (D_f) to nicotine, thereby providing the first direct evidence that these cysteine residues play a role in the agonist binding site of insect nicotinic acetylcholine receptors (nAChRs).

Adult male cockroaches (*Periplaneta americana* L.) were used throughout this investigation. The cell body of the fast coxal depressor motor neurone (D_f) was located visually in an isolated, desheathed metathoracic ganglion and impaled with a microelectrode filled with 2 mol l^{-1} KCl (electrode resistance 15–25 MΩ). The preparation was bathed at a constant rate (2.7 ml min^{-1}) with saline of the

Key words: dithiothreitol, nicotinic acetylcholine receptor, neurone, cockroach, *Periplaneta americana*, disulphide bond.

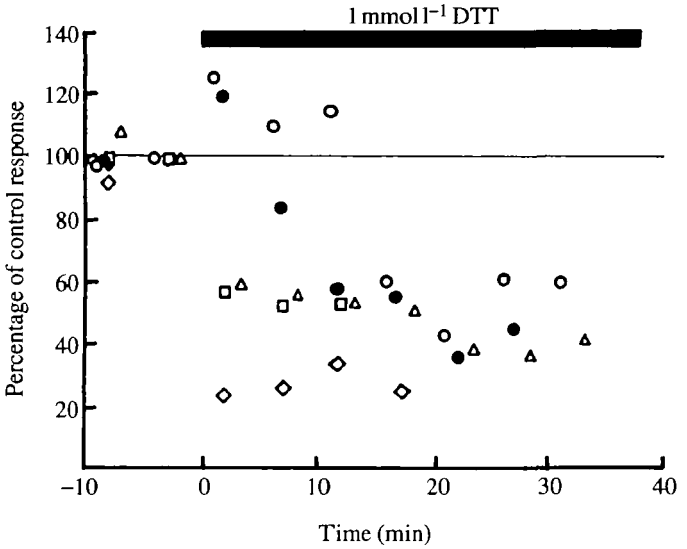

Fig. 1. Application of DTT (1 mmol l^{-1}) reduced the depolarizing response of the cockroach motor neurone D_f to nicotine (10^{-5} mol l^{-1}) by 55±5% (N=5). The maximum effect was reached within a few minutes. Different symbols represent data from different preparations.

following composition (in mmol l^{-1}): NaCl, 214; $CaCl_2$, 9; KCl, 3.1; sucrose, 50; Tes buffer, 10; pH 7.2 adjusted with 2 mol l^{-1} NaOH). Dithiothreitol (DTT) was freshly dissolved in this saline before application. Pulses of a 10^{-4} mol l^{-1} stock solution of nicotine were injected into the perfusion line with a Razel A-99 syringe pump to give a final bath concentration of 10^{-5} mol l^{-1} (Bai *et al.* 1991).

Pulses of bath-applied nicotine induced depolarizations of the membrane of motor neurone D_f. Bath perfusion with 1 mmol l^{-1} DTT reduced the amplitude of these depolarizations within a few minutes and this reduction was not reversed on washout. The inhibition of the nicotine-induced depolarization was not complete, the mean reduction being 55±5% (N=5, Fig. 1). Incomplete inhibition by DTT has been observed for nicotinic receptors in other preparations (Landau and Ben-Haim, 1974) and also for other types of receptor. For example, opioid receptor binding is inhibited by 30–50% following treatment with DTT (Kamikubo *et al.* 1988). Increasing the concentration of DTT to 5 mmol l^{-1} caused the cell to depolarize without application of nicotine, making it difficult to assess the effect on nicotine-induced depolarization, and this was not investigated further. Bregestovski *et al.* (1977) showed that an nAChR of *Limnaea stagnalis* neurones could be protected against the effects of DTT by nicotinic agonists. The cockroach motor neurone D_f could also be protected against DTT by 1 mmol l^{-1} acetylcholine (Fig. 2). Exposure of D_f to ACh before and during application of DTT, followed by washout of both compounds, left nicotine-induced depolarizations

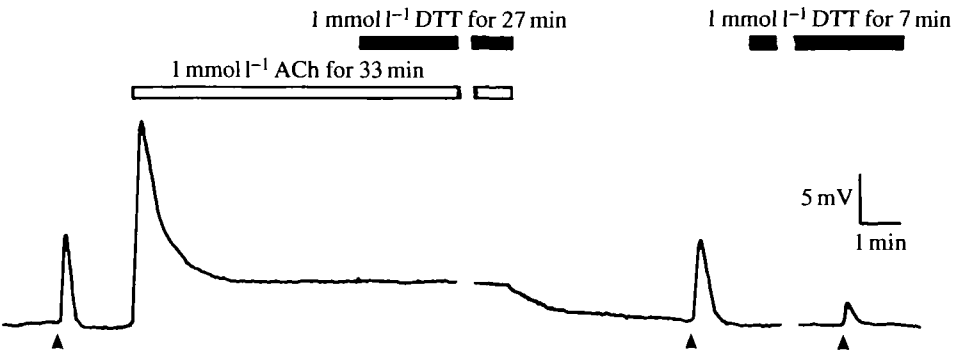

Fig. 2. High concentrations of acetylcholine (ACh) protect the insect nicotinic acetylcholine receptor from reduction by DTT. A control depolarization in response to nicotine was followed by exposure to 1 mmol l^{-1} ACh for 6 min and then 1 mmol l^{-1} ACh plus 1 mmol l^{-1} DTT for 27 min (bars). ACh and DTT were then washed off and nicotine was re-applied, producing a response of the same amplitude as the control response before ACh+DTT treatment. Subsequent application of DTT alone diminished the response to nicotine.

unchanged (N=3). The subsequent application of DTT inhibited nicotine-induced depolarizations as before (Fig. 2). Bregestovski *et al.* (1977) concluded that, for successful protection, the agonist must be bound to the receptor complex. The simplest interpretation of the present finding is that the bound agonist–receptor complex is also required for protection of the insect nAChR, though we have not directly tested this.

These observations confirm the importance of disulphide bonds in the functional nicotinic binding site of nAChRs. Partial inhibition of the response to nicotine, following reduction with DTT, is in agreement with studies on other preparations. It has been suggested that partial inhibition may reflect either differential reactivity of multiple S–S bonds in one receptor or two or more distinct types of receptor that differ in their sensitivity to DTT (Kamikubo *et al.* 1988). We are not able to distinguish between these possibilities in the case of the partial block of nicotine-induced responses by DTT in D_f cells. The subunit structure of insect nAChRs is still uncertain, but there are known to be at least two α-subunits and two non-α-subunits in *Drosophila melanogaster* (Hermans-Borgmeyer *et al.* 1986; Bossy *et al.* 1988; Sawruk *et al.* 1990*a*,*b*). The presence of nAChRs with three different conductances has also been demonstrated in insects (Leech and Sattelle, 1992).

In conclusion, the nicotinic response of cockroach motor neurone D_f is reduced by treatment with a sulphydryl reducing agent (DTT). The effects of DTT can be inhibited by high concentrations of agonist, suggesting that, as in other preparations, occupancy of the ligand binding site masks disulphide bonds against reduction. These data provide evidence that an extracellular disulphide bond plays an important role in the function of insect nicotinic acetylcholine receptors.

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