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SHORT COMMUNICATION

SINGLE-CHANNEL RECORDINGS FROM INSECT NEURONAL GABA-ACTIVATED CHLORIDE CHANNELS

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GABA-receptor-operated chloride ion channels are widely distributed on membranes of insect nervous tissue (for a review, see Sattelle, 1990). For example, in the cockroach *Periplaneta americana* radioligand binding (Lummis and Sattelle, 1986), autoradiography (Lummis and Sattelle, 1985) and GABA-activated ^{36}Cl flux (Wafford *et al.* 1987) studies have demonstrated the presence of GABA-operated chloride channels throughout the nervous system. Electrophysiological studies initiated by R. M. Pitman, J. J. Callec, R. J. Walker and colleagues have demonstrated the presence of many GABA-sensitive neurones in the cockroach central nervous system (Pitman and Kerkut, 1970; Walker *et al.* 1971; Callec, 1974). Recent studies on identified neurones (Wafford and Sattelle, 1986; Sattelle *et al.* 1988) and cultured neurones (Beadle and Lees, 1986; Neumann *et al.* 1987; Shimahara *et al.* 1987) have enhanced our understanding of the pharmacology of cockroach GABA receptors. However, few studies have yielded data on the unitary conductance properties of insect neuronal GABA-operated chloride channels. Shimahara *et al.* (1987) have used noise analysis to estimate that the conductance of chloride channels activated by GABA (5.0×10^{-5} mol l^{-1}) on cultured embryonic cockroach (*Periplaneta americana*) brain neurones is 18.6 pS, with a mean open time of 11.8 ms. Attempts to obtain recordings of insect GABA-activated chloride channels using the patch-clamp technique have proved difficult, and this has been attributed to non-uniformity of the distribution of GABA receptors on embryonic cells (Pichon and Beadle, 1988). No such data are available for adult neurones, and the present study provides an initial description of GABA-operated chloride channels of dissociated neurones from thoracic ganglia of the adult cockroach (*Periplaneta americana*).

Metathoracic (T3) ganglia were isolated from adult male cockroaches (*Periplaneta americana*) and neurones dissociated according to a modified version of the technique of Pinnock and Sattelle (1987). T3 ganglia were desheathed and

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incubated for 1 h in normal saline containing collagenase and hyaluronidase (both type I-S, 0.5 mg ml^{-1}). The ganglia were then rinsed in normal saline and mechanically dissociated by repetitive gentle suctions through a Pasteur pipette, in the same medium supplemented with 10% foetal calf serum and streptomycin sulphate (20 $\mu g\,ml^{-1}$). Cells prepared in this way were allowed to settle on poly-L-lysine- or on concanavalin-A-coated coverslips. All experiments were performed within 10 h of dissociation. Prior to electrophysiological recordings, the medium was replaced by normal cockroach saline of the following composition (in mmol l^{-1}): NaCl, 210; KCl, 3.1; $CaCl_2$, 4; $MgCl_2$, 5; Tes buffer, 5; sucrose, 50; pH 7.2. Patch electrodes were pulled from borosilicate capillary tubes and filled with a solution containing (in mmol l^{-1}): TEA-Cl, 235.1; Tes buffer, 5; pH adjusted to 7.35 with TEA-OH. Patch-clamp recordings were obtained on unidentified freshly dissociated neurones (20–25 μm in diameter) at room temperature (20–23 °C) with the methods described by Hamill *et al.* (1981), using a List L/M EPC 7 or a Biologic RK-300 patch-clamp amplifier. Data were stored on video cassettes (Sony PCM-701ES digital audio processor and Sony Betamax SL-HF100F video cassette recorder) for later off-line analysis on a Compaq Deskpro 286 computer using pClamp 5.5 software (Labmaster TL-1-125 interface, Axon Instruments Inc.). Data were digitized at 20 kHz and filtered at 9.8 kHz. 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 the resting potential of the patched neurones, which is unknown in the cell-attached recording configuration, control experiments were carried out on similar dissociated neurones in the whole-cell recording configuration with a patch electrode filled with intracellular-like solution (in mmol l^{-1}: KCl, 140; NaCl, 15; $MgCl_2$, 2; Tes buffer, 5; pH adjusted to 7.35 with KOH). Under these conditions, the resting potential of isolated cockroach neurones averaged -60.3 ± 3.3 mV (N=6). Similar resting potentials have been measured in the same type of preparation using a conventional intracellular microelectrode recording technique (Sattelle *et al.* 1986).

In the cell-attached configuration, single channel openings were readily detected in about 95% of the patches in the presence of 1.0×10^{-5} or 2.0×10^{-5} mol l^{-1} GABA in the patch electrode. Fig. 1 shows the typical behaviour of single GABA-activated ion channels (2.0×10^{-5} mol l^{-1} GABA) recorded over a 29.5 s period in a patch held at a potential 50 mV more positive than the cell resting potential. This type of channel activity was not observed in the absence of GABA (10 patches), although other channels of much smaller amplitude (conductance of about 3 pS) were detected in some patches. Single-channel current amplitude was voltage-dependent and linearly related to the membrane potential, and the current reversed at -27.3 ± 5.9 mV (N=6) from the resting potential, i.e. at about -87.6 mV. In our experimental conditions E_{Cl}, the equilibrium potential for chloride ions, was -88.7 mV, assuming that $[Cl^-]_i$, the intracellular chloride concentration, was 7 mmol l^{-1} in cockroach neurone, as

estimated by Pinnock *et al.* (1988). Thus, the unitary currents evoked by GABA reversed at a potential very close to E_{Cl}, and were therefore probably carried by chloride ions. Data analysis of single-channel amplitude distributions at different potentials revealed two conducting states. The single-channel conductances, obtained as the slopes of the single-channel current–voltage relationships determined by linear regressions, averaged 17.3±1.5 and 10.6±0.5 pS (N=6). There was no evidence for a voltage-dependence of the channel conductances. As shown in Fig. 1, channel openings were very brief and occurred in bursts, the bursts being grouped in clusters. In general, channel open-and closed-time distributions could be well described by the sum of two and three exponential functions, respectively, suggesting that the channel can exist in at least two open and three closed states. However, it should be noted that in one patch three and four exponentials were needed to describe the open- and closed-time distributions, respectively. Fig. 2 shows that the channel mean open and closed times (observed in six patches containing both conductance levels) were not voltage-dependent (P>0.05) in the potential range studied (−50 to +60 mV from the resting potential). When life-time values obtained at all potentials were considered together, the mean open times were τ_1=0.28±0.03 and τ_2=1.43±0.20 ms, and the mean closed times were τ_1=0.25±0.07, τ_2=2.22±0.21 and τ_3=43.73±5.44 ms. It should be noted that channel activity was often more pronounced at more depolarized potentials (not shown), suggesting that the channel opening probability might be voltage-dependent, as reported for chick cerebral neurones (Weiss, 1988).

The effect of picrotoxin (5.0×10^{-5} mol l^{-1}), the antagonist of the vertebrate $GABA_A$ receptor and of insect (Callec, 1974) GABA-receptor-operated chloride channel, was tested under different conditions (not shown). When both GABA and picrotoxin were present in the patch electrode, no channel activity could be recorded in the cell-attached configuration, whereas channel activity was usually present in 95% of the patches in the presence of GABA alone. In a few outside-out patches, channel activity induced by externally applied GABA rapidly decreased after additional perfusion of picrotoxin. Thus, the GABA-activated single-channel chloride currents were rapidly blocked or prevented by picrotoxin, a result that was expected from the blockade of whole-cell GABA-activated chloride current (not shown).

The channel properties reported here for the first time for dissociated neurones of the adult cockroach reveal similar, but more complex unitary conductance properties than those observed for embryonic neurones using noise analysis (Shimahara *et al.* 1987), in that the channel has several open and closed states and more than one conducting state. Moreover, single-channel analysis has revealed much shorter life-time durations than in embryonic tissue (0.28–1.43 against 11.8 ms). The neuronal GABA-operated chloride channels of adult cockroach neurones resemble those reported for chick cerebral neurones (Weiss, 1988; Weiss *et al.* 1988) both in conductance and in life-time distributions, although four conducting states (6, 12, 20 and 30 pS) instead of two (10.6 and 17.3 pS) have been found in this preparation. GABA-operated chloride channels of slightly larger

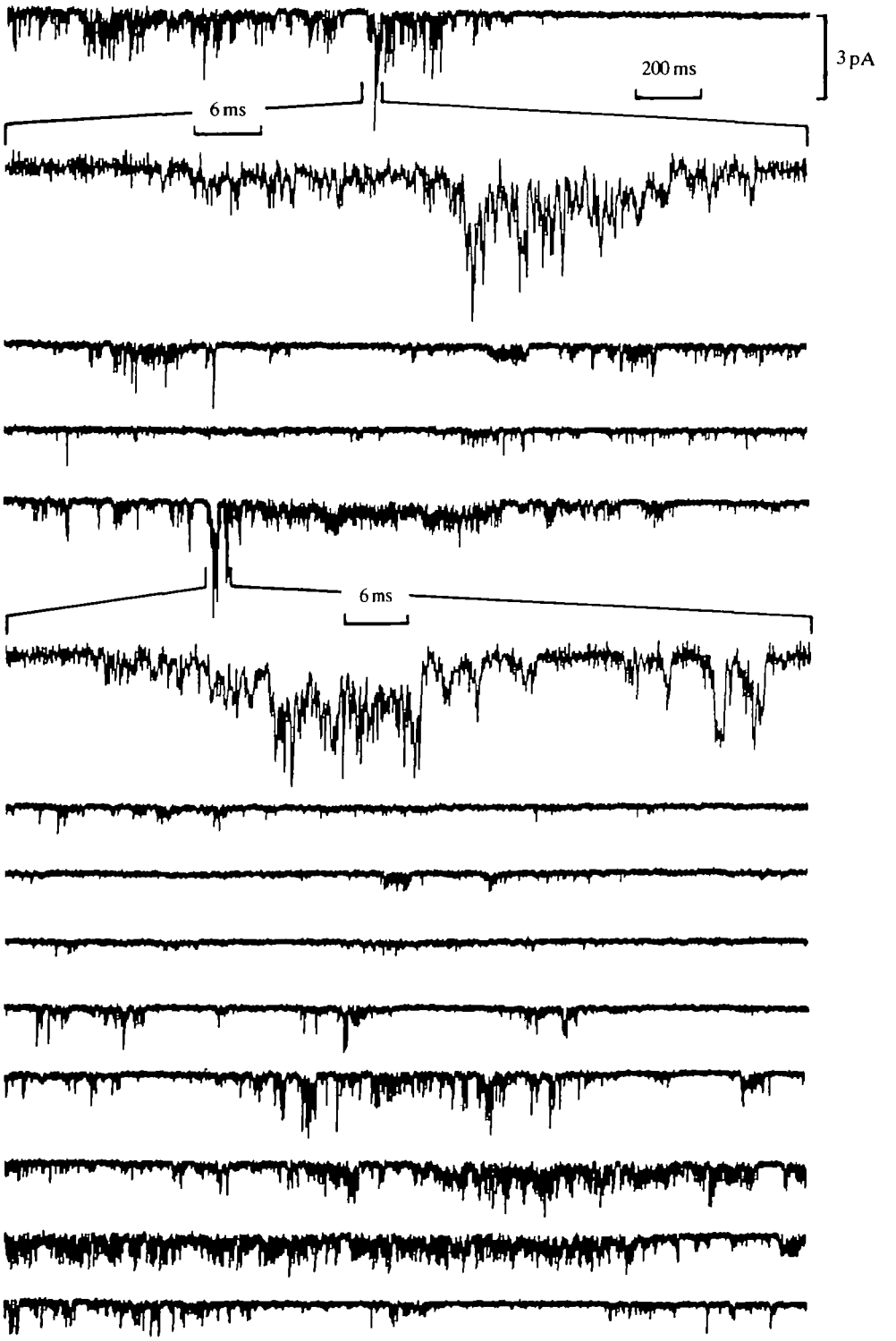

Fig. 1

Fig. 1. GABA-activated chloride channels (2.0×10^{-5} mol l^{-1} GABA) recorded from an unidentified dissociated neurone (diameter 20 μm) isolated from a thoracic (T3) ganglion of the cockroach *Periplaneta americana* in the cell-attached configuration at +50 mV from the resting potential (i.e. −10 mV) over a 29.5 s period. Channel openings are downward deflections and unitary currents are outward at this potential. For illustration purposes, data were acquired at 5 kHz and filtered at 2.2 kHz, except those shown on an expanded time scale (20 kHz sampling and 9.8 kHz filtering).

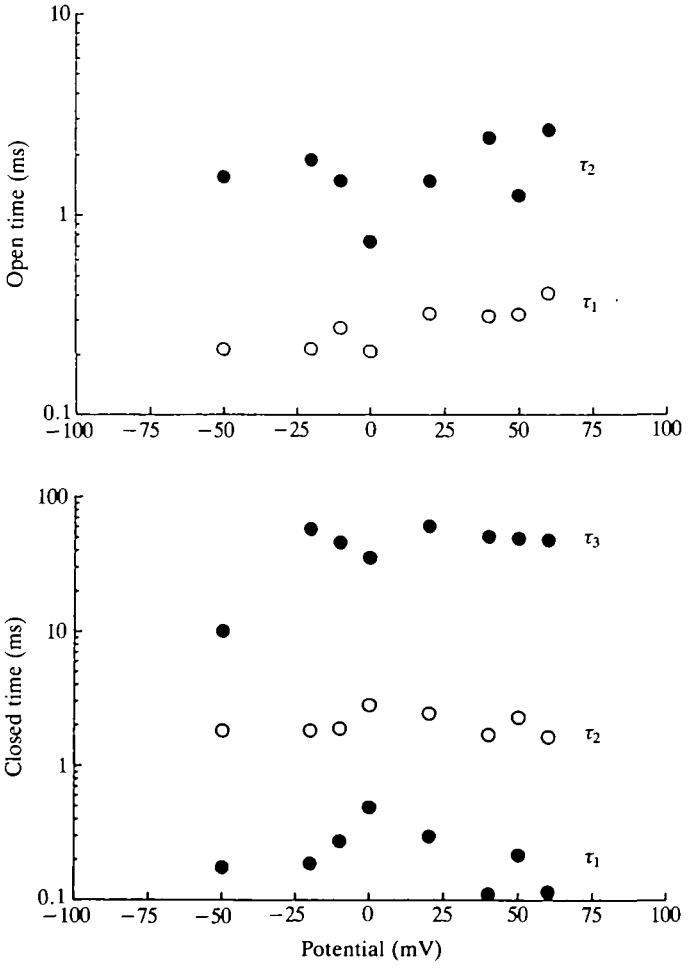

Fig. 2. The mean open (top panel) and closed (bottom panel) times of the GABA-activated chloride channel are voltage-independent. The zero potential corresponds to the neurone resting potential. Mean open and closed times were calculated as the mean time constants of the exponential functions fitted to the life-time distributions observed in six patches. There was no correlation between the potential at which the patch was held and the different mean open and closed times ($P>0.05$).

conductance have been detected on locust muscle using noise analysis (22 pS; Cull-Candy and Miledi, 1981) and on cockroach myosacs using the patch-clamp technique (20 and 30 pS; Bermudez and Beadle, 1988). In locust muscle, the power density spectra of GABA-induced membrane noise could normally be fitted with a single Lorentzian component, from which the mean extrajunctional channel life-time has been estimated as 2.8 ms at −80 mV (Cull-Candy and Miledi, 1981). However, in this latter preparation, the mean life-time of the channel showed a voltage-dependence, being shorter with membrane hyperpolarization (a 180 mV change in membrane potential produced an e-fold change in the mean life-time of the channel). This has not been found in the present study on adult cockroach dissociated neurones, in agreement with results obtained in nematode muscles (Martin, 1985) and in chick cerebral neurones (Weiss, 1988). The observation that the single-channel life-times are not voltage-dependent in our cockroach preparation is not inconsistent with the rectification of the GABA-induced macroscopic membrane current observed in locust muscle (Cull-Candy and Miledi, 1981) and in cockroach (Pinnock *et al.* 1988) and chick cerebral neurones (Weiss *et al.* 1988), if one assumes that the channel opening probability is voltage-dependent (as reported by Weiss, 1988; we have evidence that this might also be the case in cockroach neurones – see above) or that the number of functional receptors decreases at certain membrane potentials (as suggested for acetylcholine receptors by Miledi *et al.* 1980).

The ability to monitor directly GABA-induced unitary conductance and channel open and closed times will permit a detailed understanding of the receptor actions of a wide range of ligands known to modify insect GABA receptor function. Moreover, the patch-clamp technique should yield more information on the nature and pharmacology of the insect neuronal GABA receptor(s).

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