

Recording of single γ -aminobutyrate- and acetylcholine-activated receptor channels translated by exogenous mRNA in *Xenopus* oocytes

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High resolution ('giga-seal') patch clamp recording in *Xenopus* oocytes was used to measure single channel currents from ACh- and GABA-activated receptors. The proteins that make up these receptors had been translated from mRNA derived from, respectively, denervated cat muscle and chick optic lobe.

Exogenous messenger RNA (mRNA) injected into *Xenopus* oocytes is translated and processed to form functional drug receptor channels. For example, nicotinic acetylcholine (ACh) receptor channels are formed after injection of mRNA derived from *Torpedo* electric organ (Barnard *et al.* 1982) or cat muscle (Miledi & Sumikawa 1982; Miledi *et al.* 1982*a*), while receptors to γ -aminobutyric acid (GABA) are formed after injection of mRNA from chick optic lobe (Miledi *et al.* 1982*b*). This constitutes a powerful technique for the study of many drug receptors that are otherwise relatively inaccessible to electrophysiological techniques (such as those in the central nervous system). We describe here a further extension of the technique, with use of patch clamp recording (Neher & Sakmann 1976; Hamill *et al.* 1981) of single channel currents induced by GABA and ACh in oocytes that had been injected with mRNA from chick optic lobe and denervated cat muscle.

Poly(A)-mRNA was extracted from chick optic lobe and cat muscle, and injected into *Xenopus* oocytes. Experiments were made on oocytes that had formed both ACh- and GABA-activated channels (for further details see Miledi & Sumikawa 1982; Miledi *et al.* 1982*a, b*). Oocytes were treated with collagenase to remove follicular cells and to clean the membrane surface (cf. Kusano *et al.* 1982). Oocytes were bathed in normal frog Ringer solution (18–20 °C), and impaled with two microelectrodes for measuring the membrane potential and passing steady polarizing currents (Kusano *et al.* 1982). Single channel currents were measured by the cell-attached patch technique (Hamill *et al.* 1981). Patch pipettes were filled with drug solutions in normal Ringer solution. The ACh solution contained additionally 5×10^{-7} M atropine, to block any 'native' muscarinic ACh receptors present in the oocyte (Kusano *et al.* 1982).

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Figure 1 illustrates single channel currents recorded from 'giga-seal' patches on two oocytes, with pipettes containing 10^{-4} M GABA (figure 1a) and 2×10^{-7} M ACh plus 5×10^{-7} M atropine (figure 1b). The drug concentrations were chosen to give a convenient rate of channel openings, without causing appreciable desensitization. It is not clear how many channels were active in the membrane patches, but for the GABA-activated record at least two must have been present, as indicated by the occurrence of occasional double openings (middle trace, figure 1a).

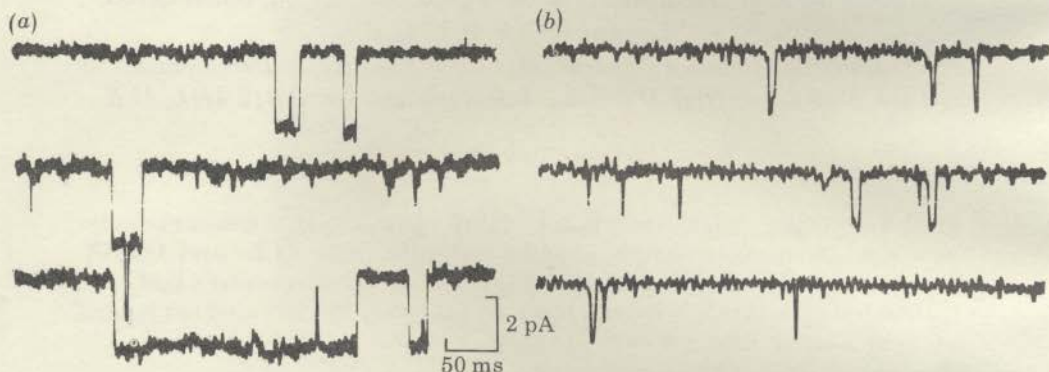


FIGURE 1. Single channel currents induced by GABA (a) and ACh (b) acting on receptors translated in *Xenopus* oocytes from mRNA derived from chick optic lobe and denervated cat muscle. Both records were obtained from membrane patches where seals of > 10 G Ω were achieved with the patch electrode. Patch pipettes contained 10^{-4} M GABA in (a), and 2×10^{-7} M ACh plus 5×10^{-7} M atropine in (b). Downward deflexions of the traces correspond to inward currents, and indicate channel openings. Calibration bars apply to all records. The membrane potential across the patch was about -110 mV in (a) and -90 mV in (b). Records were filtered at 1 kHz (a) and 500 Hz (b). Temperature, $18-20$ $^{\circ}$ C.

Almost certainly, the channel currents illustrated resulted from drug-activated channels, rather than, for example, from any voltage-activated (Miledi 1982) or spontaneously active channels (Kusano *et al.* 1982) normally present in the oocyte membrane. This conclusion is supported by the observation that the frequency of opening of channels depended upon the concentration of drug in the pipette, while changes in membrane potential did not elicit channel openings. Also, the mean lifetimes of the channels opened by GABA and ACh were clearly different (figure 1, and see later), which shows that the two agonists were activating two types of channels with different kinetics.

The channels activated by GABA and ACh both appear to have similar conductances of around 30 pS (at 20 $^{\circ}$ C). Recordings of ACh-activated channels at membrane potentials between -70 and -140 mV gave a mean value for the single channel conductance of 29.3 pS (s.e. 1.0 pS; six patches), based on an equilibrium potential of -10 mV (Miledi & Sumikawa 1982; Miledi *et al.* 1982a). The mean single channel conductance obtained for GABA-activated channels was 28.5 pS (s.e. 1.5 pS; four patches). However, there was some indication, with both GABA- and ACh-activated channels, for the existence of at least two populations of channels, showing different conductances. Further investigation is necessary to

clarify this point. The conductance estimates given above were obtained simply by pooling all measurements.

The mean open times of the GABA- and ACh-activated channels differed considerably. A mean lifetime of 3 ms was obtained for the ACh channel illustrated in figure 1*b* (mean of 129 openings), while for the GABA channel in figure 1*a* the mean lifetime was 16 ms (131 openings). The value for the ACh channel lifetime may have been overestimated because some brief openings may have been lost owing to the restricted bandwidth of the recordings (500 Hz).

These estimates of single channel lifetime and conductance of the GABA- and ACh-activated channels in the oocyte membrane are similar to those of channels in their 'native' cells, e.g. ACh receptor channels in cat muscle (Wray 1980) and GABA receptors in cultured mammalian neurons (Barker & McBurney 1979). Receptor channels translated in the oocyte also resemble the membrane channels of the 'native' cells in other ways. For example, high concentrations of ACh in the patch pipette (10 and 100 μM) caused bursts of channel openings, interspersed with silent periods of several seconds. This resembles the desensitization bursts observed with ACh in frog muscle at high agonist concentrations (Sakmann *et al.* 1980). Also, many of our records of GABA-activated channels showed brief intervening closings during an opening (upper and lower traces, figure 1*a*), similar to the brief closings described for ACh channels in frog muscle (Colquhoun & Sakmann 1981) and glutamate channels in locust muscle (Cull-Candy & Parker 1982).

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