SINGLE-CHANNEL OBSERVATIONS ON THE MU-OPIOID RECEPTOR

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The existence of opioid receptors was first reported by Terenius (1972, 1973*a*, *b*) and, soon thereafter, confirmed by several other research groups (Pert and Snyder, 1973; Simon et al., 1973). Since opioids occur neither in the regular diet nor in the known metabolism of vertebrates, the existence of an opioid receptor was an enigma, solved by the subsequent discovery of endogenous opioids (Terenius and Wahlström, 1974, 1975; Terenius 1975; Hughes et al., 1975; Pasternak et al., 1975; Teschemacher et al., 1975; Goldstein et al., 1979). It is now known that there are several types of opioid receptors, distinguishable by their pharmacological response (Takemori et al., 1969; Martin et al., 1976; Lord et al., 1977).

We have shown recently that partially purified mu-opioid receptors can be reconstituted in an artificial bilayer membrane, which they make electrically conducting upon addition of endogenous or exogenous opioids (Smuda and de Levie, 1985*a*, *b*). Here we report on single-channel observations of such reconstituted mu-opioid receptors.

A partially purified receptor preparation was obtained from bovine brain by solubilization with the zwitterionic detergent 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate (CHAPS), followed by ion exchange and affinity chromatography, whereupon the receptor was reconstituted in a Takagi-Montal lipid bilayer, as described earlier (Smuda and de Levie, 1985*b*). The only change in procedure was the use of a somewhat faster operational amplifier (model 3523; Burr-Brown Corp., Tucson, AZ) with a feedback resistance of 1 GΩ as current-to-voltage transducer, followed by a regular 100×postamplifier feeding directly into the analog-to-digital converter of a minicomputer (model PDP 11-20; Digital Equipment Corp., Maynard, MA). Data were obtained at a 1 KHz rate, and stored in 2-s segments. The aqueous solutions on both sides of the membrane contained 0.1 M NaCl + 0.1 M KCl + 5 mM $CaCl_2$, buffered at pH 7.6 with 20 mM HEPES, i.e., *N*-2-hydroxoethyl-piperazine-N′-2-ethanesulfonic acid.

Although it is, in principle, possible to observe single-channel behavior by adding extremely small amounts of agonists, this is a risky procedure because the preparation occasionally contains spurious channels. To safeguard against those, we used the following procedure.

After a lipid bilayer was formed and a potential of 100 mV applied, 10 μl of the partially purified protein fracton was added to the *cis* compartment of the measurement cell. No electrical current resulted from the application of the voltage (the electrode in the *trans* compartment being held at virtual ground by the current-to-voltage converter) or the presence of the protein fraction, until DAGO was added to the *cis* compartment to a concentration of 50 nM; DAGO stands for tyrosine-D-alanine-glycine-*N*-methyl-phenylalanine-glycinol. The resulting electrical current was very noisy, see Fig. 1 *a*, and exhibited poorly resolved current steps of ~7-pA amplitude. We then added a large excess of naloxone, which caused the electrical activity to decay to zero. Fig. 1 *b* shows one of several records obtained during this decay, just before the current remained permanently at baseline level. In these final records of electrical activity, single channels are fully resolved. They have a conductance of about 65 pS, i.e., a resistance of about 15 G Ω, which is of the usual order of magnitude for ion channels (Hille, 1984). The presence of naloxone appears to increase the length of time these channels are closed but not the single-channel conductance. The pharmacological specificity of these channels, i.e., the fact that they open with physiological concentrations of mu-agonist, and close with antagonist, clearly

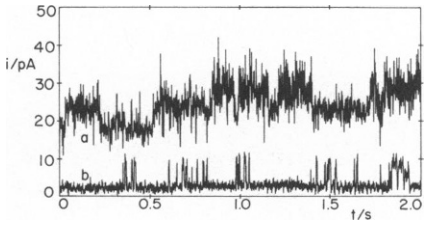

FIGURE 1 Two typical two-second segments of the current flowing in the presence of opioid receptor and 50 nm DAGO, before *a* and after *b* the addition of 1 mM naloxone, which, soon after curve *b* was recorded, completely suppressed all channel activity. Applied voltage 0.1 V, data rate 1 KHz.

identifies them as mu-opioid receptors. Work to characterize these receptor channels in more detail is currently in progress.

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