# CHANNEL CURRENTS DURING SPONTANEOUS ACTION POTENTIALS IN EMBRYONIC CHICK HEART CELLS

## The Action Potential Patch Clamp

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ABSTRACT Single-channel currents were recorded with the cell-attached patch-clamp technique from small clusters (2–20 cells) of spontaneously beating 7-d embryo ventricle cells. Because the preparation was rhythmically active, the *trans*-patch potential varied with the action potential (AP). The total current through the patch membrane was the patch action current (AC). ACs and APs could be recorded simultaneously, with two electrodes, or sequentially with one electrode. Channel activity, which varied depending on the number and type of channels in the patch, was present during normal cell firing. This method can reveal the kinetics and magnitudes of the specific currents that contributed to the AP, under conditions that reflect not only the time and voltage dependence of the channels, but also environmental factors that may influence channel behavior during the AP.

### INTRODUCTION

The study of excitability is based on the concept that an action potential results from a conductance change in the cell membrane. The main source of information regarding the kinetics of the individual conductances underlying the action potential has been the voltage-step/current-relaxation technique. Fluctuation analysis of currents in the steady state (Conti et al., 1975), and in the nonstationary state following a voltage step (Sigworth, 1977), has aided in the analysis of the channels responsible for these conductance changes. But it was not until the introduction of the single-channel technique (Neher and Sakmann, 1976) that it became possible to view the activity of channels directly, first in the steady state (Conti and Neher, 1980) and soon afterward in the nonstationary state following a voltage step (Sigworth and Neher, 1980). Here we report a method that allows the measurement of single-channel activity during the action potential itself, as the channels normally function. This method provides a direct, model-independent measurement of the time course of individual ionic currents that contribute to the action potential. Our purpose here is not to identify individual currents but rather to report a new application of the single-channel technique and to indicate its usefulness in understanding excitability.

Analysis of single-channel currents with the cell-attached patch-clamp technique (Hamill et al., 1981) requires a knowledge of the potential across the cell membrane ($V_m$). In most studies, $V_m$ is stable. However, if the preparation is electrically active, $V_m$ is the whole-cell action potential (AP), and the current through the patch membrane is the patch action current (AC) (Lux et al., 1981). Previous workers have used the AC as an indicator of the condition of the patch (Fenwick et al., 1982) or of the whole-cell preparation (Jackson et al., 1982). Here we show that the AC can result primarily from unitary currents through ion channels in the patch. In this case, analysis of the ACs reveals the time course and magnitude of specific ionic currents that underlie the AP. A preliminary report has appeared (Fischmeister et al., 1984).

### METHODS

Chick embryonic ventricular cells were obtained by multiple-cycle trypsinization of 7-d ventricular fragments (DeHaan, 1967). The cells were resuspended in tissue culture medium (Nathan and DeHaan, 1979) and were cultured for 20–24 h on plastic Petri dishes (type 1008; Falcon, Division of Becton, Dickinson and Co., Oxnard, CA) where they attached weakly and formed small rounded clusters (20–40 μm diam) of 2–20 electrically coupled cells. Cells were washed in balanced salt solution; 10–100-GΩ seals were obtained routinely on spontaneously beating clusters with 2–6-MΩ electrodes. Electrodes were filled with an intracel-

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lular- or extracellular-like solution (see legends). Experiments were done at room temperature (22–24°C) at pH 7.35. ACs and APs were recorded simultaneously (Fig. 1) with two electrodes, or sequentially, using a single electrode (Fig. 2). In the latter case we recorded APs immediately after the patch was ruptured.

## RESULTS

Fig. 1 *a* shows the setup for simultaneous recording of spontaneous APs and ACs with two electrodes. One electrode (lower) is in the cell-attached configuration for recording patch current ($I$), while the potential at the external side of the patch is clamped at the bath (or other) potential. The other electrode is in the whole-cell recording configuration and monitors the spontaneous electrical activity of the whole preparation ($V$). In Fig. 1 *b* recordings from such an experiment are shown, when both electrodes were filled with an intracellularlike medium. It demonstrates that when the cluster is bathed in a normal solution, spontaneous APs occur while ACs are recorded through a small fraction of the cluster's external membrane, voltage clamped and bathed in an abnormal solution.

Recording ACs and APs simultaneously is not essential. In Fig. 2 *a* an AC (recorded with a pipette containing high K, low Ca solution) is superimposed on an AP recorded immediately after the patch was ruptured by suction. Fig. 2 *b* shows the instantaneous $i(V)$ relationship of the open channel (dashed line) obtained by plotting the AC against the AP. The $i(V)$ curve shows a strong inward rectification at potentials positive to $V_m = -10$ mV (near $E_K$ under these conditions). The background leak estimated from the slope of the line connecting the closed states is <0.2 pS. While we cannot exclude the possibility of damage to the patch membrane or the existence of small channels, this background leakage is not a serious limitation (Ayer et al., 1983). The open-channel slope conductance in Fig. 2 *b* varies between 30 pS at large negative potentials to <1 pS at large positive potentials. Maximum conductance occurs near the reversal potential. We usually observed this inwardly rectifying channel when the patch electrode contained a high K, low Ca solution.

Fig. 3 illustrates the case of several channels of the same type in a single patch. In *a–e*, five ACs are shown. These were recorded from a patch that contained at least three of the same inward rectifying channels seen in Fig. 2. In the bottom trace ($f$) three of these records are superimposed. Level *1* corresponds to the current through at least one open channel, levels *2* and *3* to the current through one and two additional open channels.

Fig. 4 shows the effect of varying the patch holding potential ($V_p$). The pipette K concentration was 3.5 mM. The channel carried outward current and opened mainly during the plateau phase of the AP, when the patch membrane was depolarized. The current through this channel decreased in amplitude and in frequency as the AP repolarized. By an analysis similar to that shown in Fig. 2 *b*, we estimate that the single-channel conductance of this outward current is 55 pS, and that this conductance is nearly voltage independent. The conductance indicates a reversal potential of −88 mV (near $E_K$ under these conditions). When the patch was depolarized by 20 mV (lower trace) the single-channel current and the frequency of

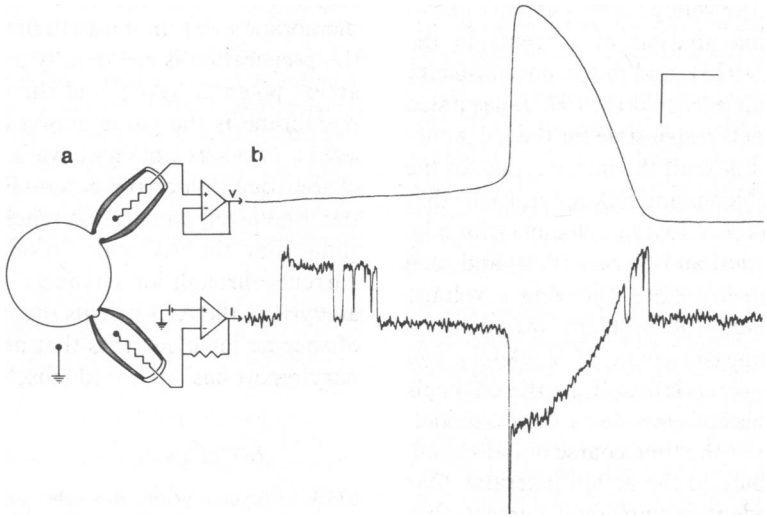


FIGURE 1 (*a*) Schematic diagram of the circuit used to record action potentials and action currents. The *upper* electrode has access to the cell interior through a broken membrane patch. Its amplified signal ($V$) is the whole-cell AP. The *bottom* electrode, sealed to a patch of membrane, may be clamped to bath (or other) potential and records the AC ($I$) through the patch resistance. (*b*) Typical AP and AC recorded simultaneously from a spontaneously beating 35-μm cluster of ~16 cells isolated from 7-d ventricle, after 48 h in culture, in a solution containing (in millimoles per liter): 3.5 K, 13.0 Na, and 1.5 Ca. Both electrodes were filled with intracellular medium containing 100 K, 33 Na, 10 HEPES, 133 Cl, and 0 Ca. The current electrode was clamped at bath potential. Horizontal scale defines 0 mV, 200 ms; vertical scale, 25 mV, 2 pA. An upward deflection indicates an increase in inward current through the patch.

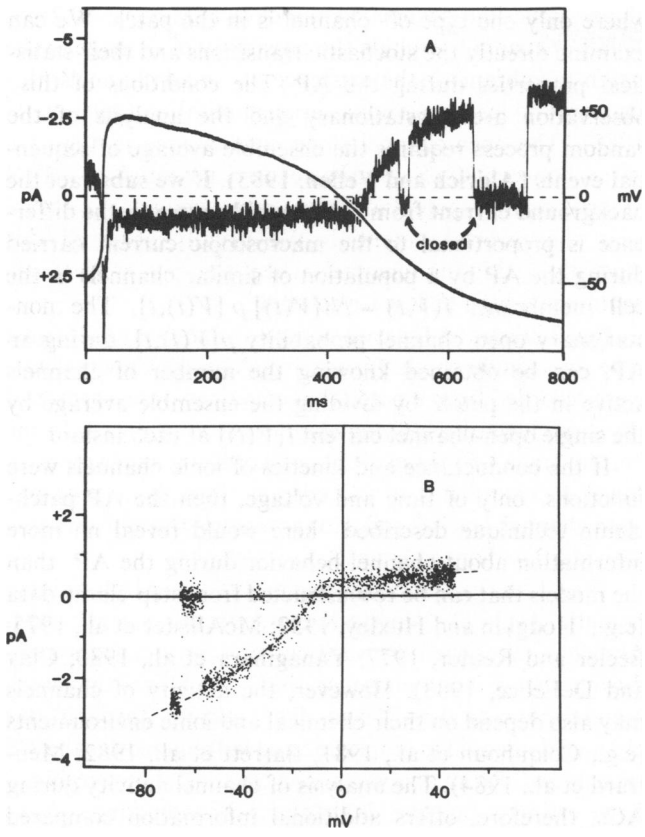


FIGURE 2 (*A*) Spontaneous patch AC, superimposed on a subsequent whole-cell spontaneous action potential after the patch was broken. These records are from a small cluster of repetitively beating 7-d chick ventricle cells in a solution containing (in millimoles per liter): 1.3 K, 143 Na, and 1.8 Ca. The electrode was filled with intracellular medium containing (in millimoles per liter): 100 K, 46 Na, 5 Na EGTA, 38 Cl, 75 $CH_3$ $SO_4$, and 0 Ca (pCa 8). $I$ = 0 was established by adding an offset voltage to compensate for electrode potentials with the electrode tip in the bath, but not again after making the seal. This patch contained one channel that was open during most of the AP except for two momentary transitions to the closed state during the repolarization phase. While recording ACs, the pipette potential was clamped to the bath potential, thus the AC flowed through the membrane patch (including the channel) but not through the seal. When the channel was closed the patch membrane resistance was >100 GΩ. Inward (negative) current through the patch is plotted up from 0. (*B*) The patch $i(V)$ relation, constructed by plotting the AC against the AP. The dashed line (---) shows the open-channel current, an inward rectifier with a reversal potential near $V_m = -15$ mV, in parallel with the patch membrane. The solid dot (●) shows the K equilibrium potential (−7 mV) across the patch assuming 130 mM K inside the cell. Using the closed state as the reference for zero channel current, there appears to be a slight outward current through the open channel at positive potentials. Although we assume it is the open channel that is rectifying, rectification could result either from a nonlinear channel conductance or from a sharp voltage dependency of channel closure near 0 mV. Here, a positive (outward) current is plotted up from 0.

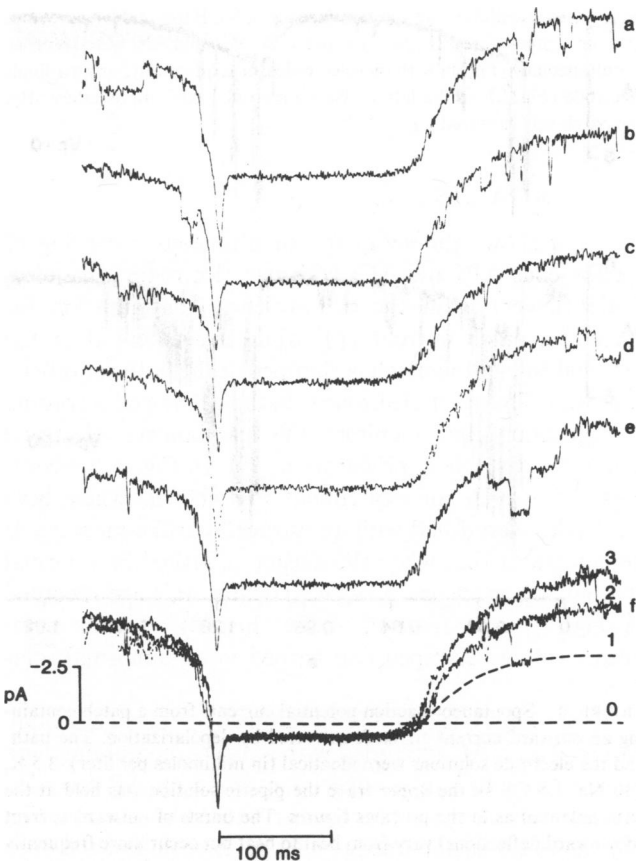


FIGURE 3 Spontaneous action potential currents from a patch exposed to an intracellularlike solution that contains three channels of the type shown in Fig. 2. The pipette potential was clamped to bath potential. The bath and the electrode solutions contain (in millimoles per liter) 1.3 K, 150 Na, 1.8 Ca, and 122 K, 50 Na, 0 Ca (+EGTA), respectively. *a–e* were selected from a sequence of 30 ACs. In *f*, parts of *c–e* are superimposed to emphasize the different current level when at least 1, 2, or 3 channels (marked with the corresponding numbers) are open. Fig. 2 *A*, although from a different experiment, would superimpose on *f* between levels 0 and 1. Because of the sharp rectification of these channels at voltages positive to −10 mV (shown in Fig. 2 *B*), the action currents are the same during the plateau phase, regardless of how many channels open in the patch. For example, *c*, with at least 2 channels open, as evidenced by the current magnitude during repolarization, and *d* with one additional channel open are identical during the plateau phase.

opening both increased, as expected from the behavior during the AP at $V_p = 0$. In a similar experiment, when the patch electrode was filled with 100 mM K, these channels reversed near $V_m = 0$ mV (near $E_K$ under these conditions). A preliminary analysis of this channel has been reported (Levi and DeFelice, 1984) and a complete study is in progress.

## DISCUSSION

There are conditions that make the AP patch-clamp technique unreliable or difficult to use. If the patch is not well broken, the AP appears ragged and the maximum upstroke velocity is low (<10 V/s). When the patch is well broken, the maximum upstroke velocity can be >100 V/s; it is usually impossible to resolve single-channel events during this phase of the AP because of the large capacitive transient lasting 2–3 ms in the AC. Furthermore, the AP may deteriorate with time, although stable recordings

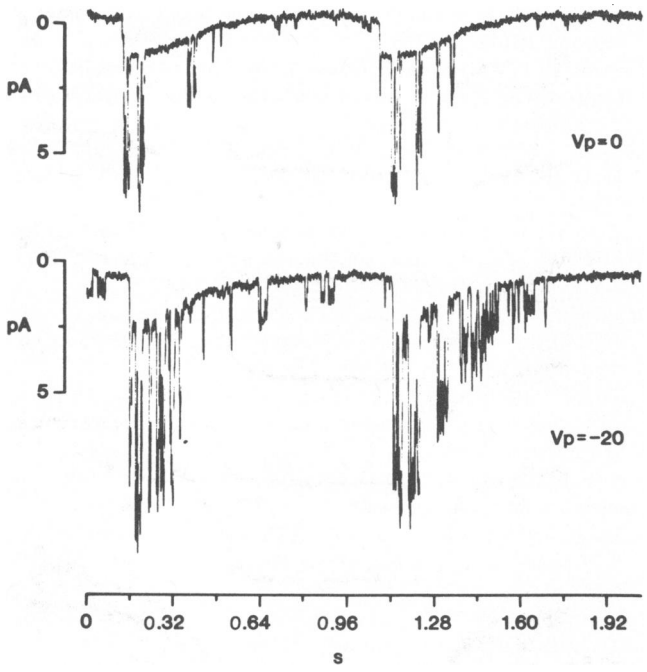


FIGURE 4 Spontaneous action potential currents from a patch containing an outward current channel, activated by depolarization. The bath and the electrode solutions were identical (in millimoles per liter): 3.5 K, 130 Na, 1.5 Ca. In the *upper* trace the pipette solution was held at the bath potential as in the previous figures. The bursts of outward current (downward deflections) vary from beat to beat but occur more frequently during the plateau phase of the AP. In the *lower* trace the pipette solution was held at −20 mV, depolarizing the patch by a constant 20 mV throughout the AP. The probability of channel opening and the amplitude of the current increased.

lasting over 2 h have been obtained. The deterioration is more likely to occur in single cells or in smaller cell clusters. One reason for using single cells is voltage homogeneity, however, the smaller the preparation, the greater is the voltage noise and the tendency to beat irregularly (Clay et al., 1979; Clay and DeHaan, 1979; Jongsma, 1984). Some patches contain an unexplained leakage (or background) current. This leakage results in an unusually large current (>1 pA) during the plateau phase of the AP when, apparently, no channels are opening or closing. The leakage could be due to damage to the patch membrane, to numerous small, unresolved channels, or to channels that are open for long times compared with the AP. Usually such experiments are rejected although it is possible to subtract even relatively large background currents. Patches that contain more than three or four channels of the same type, or more than one type of channel, usually are rejected because they are too complex to analyze. Although certain pipette solutions do promote the appearance of certain channels, as reported above, their presence in the patch is largely a matter of chance. At present, no reliable information on channel density can be obtained from patch data alone.

A more complete analysis is required to identify the channels shown here. As an example, consider the case where only one type of channel is in the patch. We can examine directly the stochastic transitions and their statistical properties during the AP. The conditions of this observation are nonstationary and the analysis of the random process requires the ensemble average of sequential events (Aldrich and Yellen, 1983). If we substract the background current from the ensemble average, the difference is proportional to the macroscopic current carried during the AP by a population of similar channels in the cell membrane: $I(V,t) = Ni[V(t)]\, p\,[V(t),t]$. The nonstationary open channel probability $p[V(t),t]$, during an AP, can be obtained knowing the number of channels active in the patch, by dividing the ensemble average by the single open-channel current $i[V(t)]$ at each instant.

If the conductance and kinetics of ionic channels were functions only of time and voltage, then the AP patch-clamp technique described here would reveal no more information about channel behavior during the AP than the models that can be reconstructed from step-clamp data (e.g., Hodgkin and Huxley, 1952; McAllister et al., 1975; Beeler and Reuter, 1977; Yanagihara et al., 1980; Clay and DeFelice, 1983). However, the activity of channels may also depend on their chemical and ionic environments (e.g., Colquhoun et al., 1981; Barrett et al., 1982; Mentrard et al., 1984). The analysis of channel activity during ACs, therefore, offers additional information compared with previous methods. It can reveal graphically the kinetics and magnitudes of specific currents that contribute to the AP, under conditions that reflect not only the time and voltage dependence of the channels, but also environmental factors that may influence channel behavior during the action potential.

We wish to thank Ms. B. J. Duke for technical help in providing our tissue cultures and Mr. W. N. Goolsby for his help with electronics and computer analysis.

This work was supported by the National Institutes of Health Grant PO1 HL27385.

*Received for publication 8 November 1983 and in final form 13 April 1984.*

## REFERENCES

Aldrich, R. W., and G. Yellen. 1983. Analysis of nonstationary channel kinetics. *In* Single Channel Recording. B. Sakmann and E. Neher, editors, Plenum Publishing Corp., New York. 287–299.

Ayer, R. K., Jr., R. L. DeHaan, and R. Fischmeister. 1983. Measurement of membrane patch and seal resistance with two patch electrodes in chick embryo cardiac cells. *J. Physiol. (Lond.).* 345:29P.

Barrett, J. N., K. L. Magelby, and B. S. Pallotta. 1982. Properties of single calcium-activated potassium channels in cultured rat muscle. *J. Physiol. (Lond.).* 331:211–230.

Beeler, G. W., and H. Reuter. 1977. Reconstruction of the action potential of ventricle myocardial fibers. *J. Physiol. (Lond.).* 268:177–210.

Clay, J. R., and L. J. DeFelice. 1983. Relationship between membrane excitability and single channel open-close kinetics. *Biophys. J.* 42:151–157.

Clay, J. R., and R. L. DeHaan. 1979. Fluctuations in interbeat interval in rhythmic heart-cell clusters. Role of membrane voltage noise. *Biophys. J.* 28:377–390.

Clay, J. R., L. J. DeFelice, and R. L. DeHaan. 1979. Current noise parameters derived from voltage noise and impedance in embryonic heart cell aggregates. *Biophys. J.* 28:169–184.

Colquhoun, D., E. Neher, H. Reuter, and C. F. Stevens. 1981. Inward current channels activated by intracellular Ca in cultured cardiac cells. *Nature (Lond.).* 294:752–754.

Conti, F., L. J. DeFelice, and E. Wanke. 1975. K and Na ion current noise in membrane of the squid giant axon. *J. Physiol. (Lond.).* 248:45–82.

Conti, F., and E. Neher. 1980. Single channel recordings of K currents in squid axons. *Nature (Lond.).* 285:140–143.

DeHaan, R. L. 1967. Regulation of spontaneous activity and growth of embryonic chick heart cells in tissue culture. *Dev. Biol.* 16:216–249.

Fenwick, E., A. Marty, and E. Neher. 1982. A patch-clamp study of bovine chromaffin cells and of their sensitivity to acetylcholine. *J. Physiol. (Lond.).* 331:577–597.

Fischmeister, R., L. J. DeFelice, R. K. Ayer, Jr., R. Levi, and R. L. DeHaan. 1984. Channel currents during spontaneous action potentials in embryonic chick heart cells. *Biophys. J.* 45(2, Pt. 2):134*a*. (Abstr.)

Hamill, O. P., A. Marty, E. Neher, and F. Sigworth. 1981. Improved patch-clamp techniques for high-resolution current recording from cells and cell-free membrane patches. *Pfluegers Arch. Eur. J. Physiol.* 391:85–100.

Hodgkin, A. L., and A. F. Huxley. 1952. A quantitative description of membrane current and its application to conduction and excitation in nerve. *J. Physiol. (Lond.).* 117:500–544.

Jackson, M. B., H. Lecar, D. E. Brenneman, S. Fitzgerald, and P. G. Nelson. 1982. Electrical development in spinal cord cell culture. *J. Neurosci.* 2:1052–1061.

Jongsma, H. J., A.C.G. van Ginneken, and W.P.M. van Meerwijk. 1984. The membrane potential of single isolated heart cells. *Biophys. J.* 45(2, Pt. 2):53*a*. (Abstr.)

Levi, R., and L. J. DeFelice. 1984. A potassium channel that opens during the cardiac action potential. *Biophys. J.* 45(2, Pt. 2):308*a*. (Abstr.)

Lux, H. D., E. Neher, and A. Marty. 1981. Single channel activity associated with the calcium-dependent outward current in *Helix pomatice*. *Pfluegers Arch. Eur. J. Physiol.* 389:293–295.

McAllister, R. E., D. Noble, and R. W. Tsien. 1975. Reconstruction of the electrical activity of cardiac Purkinje fibers. *J. Physiol. (Lond.).* 275:1–59.

Mentrard, D., G. Vassort, and R. Fischmeister. 1984. Calcium-mediated inactivation of the conductance in cesium loaded frog heart cells. *J. Gen. Physiol.* 83:105–131.

Nathan, R. D., and R. L. DeHaan. 1979. Voltage-clamp analysis of embryonic heart cell aggregates. *J. Gen. Physiol.* 73:175–198.

Neher, E., and B. Sakmann. 1976. Single-channel currents recorded from membrane of denervated frog muscle fibers. *Nature (Lond.).* 260:799–802.

Sigworth, F. J., 1977. Sodium channels in nerve apparently have two conductance states. *Nature (Lond.).* 270:265–267.

Sigworth, F. J., and E. Neher. 1980. Single Na channel currents observed in cultured rat muscle cells. *Nature (Lond.).* 287:447–449.

Yanagihara, K., A. Noma, and H. Irisawa. 1980. Reconstruction of sino-atrial node pacemaker potential based on the voltage-clamp experiments. *Jpn. J. Physiol.* 30:841–857.