Single-channel currents trigger action potentials in small cultured hippocampal neurons

(spontaneous impulse/ion channel/patch clamp/random generator)

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ABSTRACT Spontaneous neuronal impulse activity appears to play a key role in some neural processes, such as the normal establishment of interneuronal connections during development. In addition, spontaneous impulses may be essential for the functional operation of neuronal networks. Mechanisms of spontaneous non-pacemaker impulse generation are, however, not well known. In this work, spontaneous electrical activity in small cultured hippocampal neurons from rat was studied with tight-seal recording techniques. The results demonstrate that spontaneous individual openings of single ion channels can trigger impulse generation in these high-resistance cells. First, impulses recorded in the whole-cell mode were apparently induced by spontaneous plateau-potential events showing the characteristics expected from individual openings and closures of ion channels. Second, patch-clamp recordings in the cell-attached configuration showed that openings of single ion channels in the patch membrane could trigger cellular impulses, detected as biphasic current deflections. These findings suggest that the random gating of ion channel molecules can be used as a mechanism for stochastic triggering of spontaneous impulses in mammalian central neurons.

Several neuronal cell types are capable of spontaneously generating electrical impulses in the absence of synaptic input or other external stimuli (1, 2). Some of these neuron types, as well as heart cells, can fire repetitive impulses with remarkably regular intervals. The mechanisms of such regular spontaneous activity have been extensively studied (3, 4). In other cells, impulses can be generated at random time intervals (5). Impulses of such stochastic nature may be critical for neural function. Impulses originating at random are, for instance, thought to play a crucial role for the normal establishment of synaptic connections during development (6). Furthermore, theoretical work shows that random signaling may contribute essentially to the information-processing capabilities of neuronal networks (7–9). In spite of this, the mechanisms underlying random initiation of impulses are not well known.

A possible mechanism for achieving randomly induced impulses is to use the random nature of ion channel kinetics. This is possible if the random openings or closures of a small number of ion channels can trigger impulses. A small number of channels is needed for randomness, to avoid the averaging effect of large populations of channels. Several reports have been presented indicating that current events originating from single ion channels may induce impulses in some secretory cell types (10–12) and in olfactory receptor cells (13–15).

In this study, we present evidence suggesting that individual openings or closures of single ion channels can induce impulses in mammalian central neurons. The evidence was obtained by tight-seal recordings from a subpopulation of small cultured neurons from rat hippocampus. These cells have a high input resistance (several gigahms) and a very low current threshold for impulse generation (down to 3 pA) (16). The present work is based on whole-cell recordings of spontaneous plateau potentials with associated impulses and on cell-attached recordings of single-channel currents with associated impulse currents.

The data presented thus suggest a mechanism for spontaneous impulse generation in central neurons. In addition, it is proposed that the impulse-generating single-channel events may provide a basis for a cellular random generator. Preliminary versions of this work have appeared as part of a thesis (17) and in abstract form (18).

METHODS

Cell Culture Conditions. The cells were prepared from embryonic (embryonic day 18–21) rat hippocampi, using the techniques described by Johansson et al. (16). The electrophysiological recordings were performed after 4–8 days in culture. Only cells with a soma diameter <10 μm were used.

The subpopulation of cells showing the phenomena described in the present work did not differ morphologically, as seen in light microscope, from those described by Johansson et al. (16).

Electrophysiological Recordings. The spontaneous potential events were recorded in the whole-cell configuration with the tight-seal technique (19) under current-clamp conditions. Single-channel currents and associated impulse currents were recorded from cell-attached membrane patches under voltage-clamp conditions (20). Borosilicate glass pipettes (GC 120F or 150TF, Clark Electromedical Instruments, Pangbourne, U.K.) were used. For the whole-cell recordings, the pipettes had a resistance of 2–8 MΩ when filled with an artificial intracellular solution and immersed in the bathing solution (see below). For the cell-attached recordings, the corresponding resistance was 2–25 MΩ. The pipette–cell membrane seal had a resistance higher than 5 GΩ.

The signals were recorded using an EPC-7 electrometer (List Electronics, Darmstadt, F.R.G.) and stored on an FM tape recorder (Racal Store 4DS, Racal Recorders, Sarasota, FL; bandwidth, 0–5 kHz). For analysis, the signals recorded were transferred to a computer using a TL-1 DMA interface (Labmaster, Axon Instruments, Burlingame, CA) and the pClamp software (version 5.5.1; Axon Instruments). All experiments were performed at room temperature (21–23°C).

Solutions. The bathing solution surrounding the cells contained (in mM) 137 NaCl, 5.0 KCl, 1.0 CaCl₂, 1.2 MgCl₂, and 10 Hepes (pH 7.4). The recording pipette was filled with either the bathing solution (solution I), a solution containing (in mM) 140 KCl, 3.0 NaCl, 1.2 MgCl₂, 1.0 EGTA, and 10 Hepes (pH 7.2) (solution II), or a solution containing 140 KOH/KH₂PO₄, 3.0 NaCl, 1.2 MgCl₂, 1.0 EGTA, and 3.0 Na₂ATP (pH 7.2) (solution III).
RESULTS

Whole-Cell Recordings. It was noted earlier that many small cultured hippocampal neurons generate action potentials spontaneously (16). The majority of these action potentials were associated with potential fluctuations of presumably synaptic origin. Action potentials that arose smoothly from the resting potential were also observed (17). Here, we describe a third type of spontaneous action potential, associated with plateau potentials. These action potentials were observed in a subpopulation of four cells among about 50 cells studied in the whole-cell configuration. However, all cells examined for spontaneous activity were not studied during periods longer than a few minutes and it is possible that this type of impulse is more frequent than suggested from the figures above. In all cases, the impulses occurred with irregular, apparently random intervals at overall frequencies (as measured over >1 min) lower than 1 Hz.

Plateau Potentials. The type of spontaneous plateau potentials associated with the impulses showed roughly exponential rise (most clearly seen in Fig. 1B) and decay phases (Fig. 1A and C–F). The time constants (as a rule shorter for the rise than for the decay) were within the same range as the membrane time constant (range about 10–60 ms for different cells, mean value about 30 ms; cf. ref. 16). The plateau phases showed a highly variable duration of apparently stochastic nature (compare Fig. 1A and C–F), also when recorded from the same cell. Sometimes, the potential events started to decay before a plateau was reached (Fig. 1F). However, often the plateau appeared to continue after an impulse was induced, and, in some cases, the duration exceeded several hundred milliseconds. The amplitude of the plateau varied only little within the same cell; only one or a few amplitudes were seen repeatedly (see, for example, Fig. 1 C–E). However, between different cells the amplitude varied from about 2 mV to >30 mV. Plateau potentials of small amplitudes were observed without impulses (Fig. 1E). Similar small plateau potentials were also recorded from cells that did not generate spontaneous impulses.

The simplest explanation for these plateau potentials is, due to the features described above, induction by single ion-channel currents. The reasons are further clarified in Discussion.

Correlation Between Plateau Potentials and Impulses. A clear positive correlation between regenerative impulses and plateau potentials was noted: In three of the four cells analyzed, impulses were generated exclusively in association with plateau potentials and each time the plateau amplitude exceeded about 30 mV. (The fourth cell showed, in addition, some impulses of apparent synaptic origin.) This implies that the impulses were most likely caused by the plateau potentials.

Cell-Attached Recordings. The cell-attached configuration provides an alternative way of recording spontaneous impulses. The currents associated with cellular impulse generation can be recorded under voltage-clamp conditions applied to the patch pipette (16, 19). In addition, currents from single ion channels in the patch can be recorded. Thus, a possible correlation between single-channel currents and impulse generation may in principle be detected. Further, if the current flowing through an open channel causes a change of the cellular membrane potential, this will be reflected by the current time course. Below, the latter effect is described first. The recordings were made from >100 cell-attached patches.

Current Relaxations. In most of the patches that showed large current steps due to ion channel openings, the steps were followed by roughly exponential relaxations (Fig. 2). Such relaxations are expected when the channel current causes a change of the cellular membrane potential (which cannot be clamped in the cell-attached configuration) as a consequence of a high cellular membrane resistance (10, 22).

Single exponentials were fitted to a number of current relaxations in two different cells. For channel opening, the mean time constants were 42 ± 19 ms (±SD, n = 27) and 10

![Fig. 1. Spontaneous potential events in whole-cell configuration. (A–D) Impulses associated with plateau potentials. Note the roughly exponential rise (B), the plateau (A–C), and the roughly exponential decay (C and D). (E and F) Spontaneous potential fluctuations from the same cell as used for C and D.](image-url)
Fig. 2. Relaxations of single-channel currents from cell-attached patches on small hippocampal neurons. Current relaxations following channel opening are shown. In B, the relaxation after closure is also shown. In C, the relaxation was fitted by an exponential curve with time constant 33 ms (superimposed). Pipette solution, pipette potential, and filter (–3 dB): solution II, –100 mV, 1 kHz (A); solution I, –100 mV, 500 Hz (B); solution I, –100 mV, 1 kHz (C).

± 5 ms (n = 23) in the two cells, and for channel closure the time constants were 60 ± 20 ms (n = 9) and 17 ± 4 ms (n = 8). The amplitude of the current change during the relaxation after channel opening was 45% ± 6% (n = 18) and 57% ± 7% (n = 16) of the initial current amplitude. After channel closure the relaxation amplitude was 11% ± 4% (n = 12) and 8.8% ± 3.4% (n = 13) of the initial open-channel current amplitude.

Impulse Currents. Further evidence indicated that currents of the type described induced action potentials. In the cell-attached configuration, spontaneous impulses were detected as bi- or monophasic current deflections (21). To directly detect a channel causing such an impulse, as suggested from the whole-cell recordings above, it is, however, required to place the patch pipette on the very membrane spot containing a channel of the required type active at the moment of recording. The success of such an operation seems rather unlikely in light of the small patch size (about 1 μm²) and a frequency of impulse-inducing channel openings of <1 per s in the whole-cell membrane (see above). However, the use of an artificial intracellular solution in the pipette or non-zero pipette potentials may increase the currents flowing through other channels, more likely to be present in the patch. Under such conditions we found a clear correlation between inward current steps and impulse currents in 5 of 23 cells that showed spontaneous impulses (Fig. 3).

In four of these cells the pipette was filled with an artificial intracellular solution (see Methods) and kept at potentials in the range 0 to –30 mV, whereas in one cell extracellular solution and potentials in the range –75 to –100 mV were used. In the former cases the impulses appeared after opening of channels causing inward currents, whereas in the latter case the impulses appeared after closures of a channel causing outward currents. The impulse currents appearing after channel closure were small with respect to the current changes associated with channel gating. However, the impulse currents could be distinguished from the channel currents by (i) gradual rise and decay phases, (ii) a return to current levels below the extrapolated baseline (not the case for channel current events of similar duration), and (iii) a roughly constant latency after channel closure (Fig. 4).

In four of the five cells analyzed, impulse currents were only seen after a preceding inward current step and every time after such a current step that was preceded by a silent interval (with no channel events) longer than 100 ms. It should be noted that for these patches the channel appeared to be closed most of the time. The correlation implies that the impulses were most likely induced by the single-channel currents.

DISCUSSION

The present paper presents evidence suggesting that individual openings of single ion channels may induce action potentials in small hippocampal neurons. The evidence is based on whole-cell recordings, showing spontaneous impulses correlated with plateau potentials, as well as cell-attached recordings, showing impulse currents correlated with single-channel currents.

Interpretation of Plateau Potentials. The properties of the plateau potentials recorded could most easily be explained by the effects of individual ion channels for the following reasons. (i) The exponential rise and decay phases, with time constants close to the membrane time constant, suggest steplike changes in underlying currents. (ii) Spontaneous transitions to and from the plateau and (iii) a highly variable duration of the plateau phase are further what would be expected from the random nature of ion channel kinetics (23).

Fig. 3. Spontaneous impulse currents from cell-attached membrane patches. The impulses were correlated to inward (shown downward) current steps. The latency from the current step to the peak of the impulse was roughly constant for each cell and pipette potential. Recordings were with pipette potential –26 mV (A) and –19 mV (B) and pipette solution III (A) and solution II (B). Filter (–3 dB) 500 Hz (A) and 1 kHz (B).
They are the cellular channels that cannot be clamped to the cellular membrane potential in small cells with high input resistance, the potential will change significantly and thereby in turn affect the electrical driving force for the channel current. As a consequence, the current declines exponentially. The time constants of the relaxations associated with openings ($\tau_{on}$) and with closures ($\tau_{off}$) are related to the membrane parameters $R_m$ and $C_m$ by the equations

$$\tau_{on} = \frac{C_m R_m}{1 + R_m \gamma}$$

and

$$\tau_{off} = \frac{C_m R_m}{1 + R_m \gamma},$$

where $R_p$ is the resistance of the patch membrane with the channel closed and $R_m$, $C_m$, and $\gamma$ are as described above. Considering that the terms $R_m/R_p$ and $R_m \gamma$ are expected to be small, both time constants are expected to be of the same order of magnitude as the membrane time constant, as experimentally observed. ($R_m/R_p$ is small due to the small ratio between the area of the patch membrane and the remainder of the cell membrane. To obtain a term $R_m \gamma$ larger than 1, even for a large-conductance channel of 100 pS, the membrane resistance has to be higher than 10 GΩ.)

The recorded current relaxations thus provide further support for the idea that single-channel currents can change the cellular membrane potential considerably.

**Single-Channel-Induced Impulses.** Spontaneous impulses were clearly correlated to plateau potentials (in the whole-cell mode) and to current steps of the type described (in the cell-attached mode). The plateau potentials and the current steps were interpreted as caused by individual openings (and/or closures) of single ion channels. Thus, the recordings provide strong evidence that, in the studied small hippocampal neurons, individual openings or closures of ion channels can lead to impulse generation.

It has earlier been noted that single-channel events may induce impulses in three kinds of secretory cells: chromaffin cells of the adrenal medulla (10), pancreatic $\beta$ cells (11), and pituitary melanotrophs (12). Similar phenomena have also been seen in high-resistance olfactory receptor cells (13–15). However, to our knowledge, this has not been reported for central neurons.

**Types of Channels.** The events inducing the impulses recorded in the whole-cell mode were relatively infrequent (i.e., impulses occurred at $<1$ Hz). Due to this low frequency and the limited number of cells, the data are as yet insufficient for a characterization of the channel type(s) involved. However, it is clear that, due to the high input resistance (several gigaohms), currents of only a few picoamperes are required. Thus, in principle, several different channel types may cause phenomena of the type described. The 80-pS $K^+$ channels described for these cells (32) may cause current steps of about 1 pA at a resting potential of $\sim$50 mV (cf. ref. 16) and could possibly account for the potential changes recorded. Alternatively, for instance, a nonselective channel of 50–100 pS would contribute currents of 2.5–5.0 pA at $\sim$50 mV. This is clearly large enough to account for the phenomena described.

**Consequences for Information Processing.** According to prevalent theory, openings and closures of individual ion channels occur randomly (23). The impulses induced by the single-channel events thus provide a basis for a cellular random generator. It seems probable that individual random impulses may play a minor role in systems where a large number of cells perform the same function—for example, secretion of a hormone. However, it is not necessarily so for the communication between nerve cells, where more precise processing of information may be expected. It is known that, in the human nervous system, individual impulses may be functionally significant. Several groups have shown that single impulses from touch receptors (of the “Meissner
type") can give rise to conscious sensations in human subjects (33, 34). Thus, it seems possible that random individual channel-induced impulses may be functionally important. They may play a role during development; for the establishment of synaptic connections or in maintaining a minimum activity level necessary for cellular survival (6, 35–37). It may here be noted that cell size is usually smaller and, consequently, input resistance is larger, early in development. Therefore, single-channel currents may be expected to have larger effects on membrane potential during early developmental stages, at a time when many synaptic connections are being formed, than at later stages, when major connections are already established.

Another possibility arises from the theoretical insight that noise, earlier often merely regarded as an unavoidable nuisance, in fact provides an essential functional advantage for neuronal information processing (7–9). Randomly generated impulses may contribute to such noise.

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