Conditional Intrinsic Voltage Oscillations in Mature Vertebrate Neurons Undergo Specific Changes in Culture

Pierre A. Guertin and Jørn Hounsgaard
Division of Neurophysiology, Department of Medical Physiology, Panum Institute, University of Copenhagen, Copenhagen N, Denmark

Submitted 8 August 2005; accepted in final form 3 October 2005

Guertin, Pierre A. and Jørn Hounsgaard. Conditional intrinsic voltage oscillations in mature vertebrate neurons undergo specific changes in culture. J Neurophysiol 95: 2024–2027, 2006. First published October 19, 2005; doi:10.1152/jn.00832.2005. Although intrinsic neuronal properties in invertebrates are well known to undergo specific adaptive changes in culture, long-term adaptation of similar properties in mature vertebrate neurons remain poorly understood. To investigate this, we used an organotypic slice preparation from the spinal cord of adult turtles maintainable for several weeks in culture conditions. N-methyl-D-aspartate (NMDA)-induced-tetradotoxin (TTX)-resistant voltage oscillations in motoneurons were ~10 times faster in culture than in acute preparations. Oscillations in culture were abolished by NMDA receptor antagonists or by high extracellular Mg²⁺ concentrations. However, in contrast with results from motoneurons in the acute slice, NMDA-induced oscillations in culture did not depend on CaV1.3 channel activation as they still remained after nifedipine application. Other CaV1.3 channel-mediated properties such as metabotropic receptor-induced oscillations and plateau potentials failed to be induced in culture. This study shows that changes specifically affecting CaV1.3 channel contribution to intrinsic oscillatory property expression may occur in culture. The results contribute also to understanding further the potential for plasticity of mature vertebrate neurons.

INTRODUCTION

Seminal observations first made in the invertebrate stomatogastric system have contributed to establish that intrinsic cellular properties such as voltage oscillations can specifically change in culture isolation presumably to maintain homeostasis and stable activity in absence of normal synaptic events (Turrigiano et al. 1994). Generally, long-term adaptation is believed to involve modifications of intracellular calcium, gene expression, and transcription levels that rely, in some cases, on N-methyl-D-aspartate (NMDA) and CaV1.3 channel activation (Bading et al. 1993). Yet, long-term changes of intrinsic (i.e., TTX-resistant) voltage oscillatory properties that critically depend on both NMDA and CaV1.3 channels (Guertin and Hounsgaard 1998) have surprisingly never been examined in mature vertebrate neurons. To investigate this question, the present study aimed at examining the long-term effects of culture isolation on the intrinsic oscillatory properties of mature spinal motoneurons.

METHODS

For that purpose, we used an organotypic preparation from adult turtles developed in this laboratory that can be stably maintained for several weeks in culture (for rational, surgical, and methodological details, see Perrier et al. 2000). In brief, 0.8- to 1.0-mm-thick transverse slices were obtained from the hindlimb enlargement spinal cord segments (D₈–S₁) of adult turtles (Pseudemys scripta). Slices were cultured at 37°C in roller tubes with culture medium for 1–3 wk and ciliary neurotrophic factor (CNTF, n = 6), neurotrophin-3 (NT3, n = 5), or glial cell line-derived neurotrophic factor (GDNF, n = 11; Alomone, Jerusalem, Israel). GDNF and CNTF were combined in three cases (n = 3). For acute preparations, slices were placed instead in oxygenated physiological solution at room temperature until recording (<24 h). Incubated preparations consisted of acutely prepared slices kept for 4–24 h in oxygenated physiological solution containing also GDNF (n = 3), CNTF (n = 3), or NT-3 (n = 1). For electrophysiological details, see Perrier et al. 2000. Oscillation frequencies were calculated for 1 min from stable activity. Bath-applied drugs included 10–80 μM NMDA (Tocris), 25–100 μM 2-amino-5-phosphonopentanoic acid (AP5, Tocris), 1–2 μM TTX (Sigma), 50–100 μM 5-hydroxytryptamine (5-HT), 10–20 μM muscarine, 5–10 μM BayK 8644 (RB1), and 1–10 μM nifedipine (Sigma). Data are presented as means ± SE. Statistical significance was determined using Student’s t-test with P < 0.05 considered as significant.

RESULTS

NMDA-induced rhythmic bursts of action potentials in most cultured motoneurons studied (n = 18/25). In each case where rhythmic bursting was found (Fig. 1Ai), blocking sodium spikes with bath-applied TTX uncovered the presence of intrinsic voltage oscillations (Fig. 1Aii). We discovered that NMDA-induced intrinsic voltage oscillations in motoneurons were significantly (P < 0.05) faster (a 10-fold increase) in culture than in acute preparations (Fig. 1, A vs. B). They displayed indeed average frequencies of 1.62 ± 0.24 Hz (n = 18) and 0.13 ± 0.01 Hz (n = 18) in culture and acute preparations, respectively (Fig. 1D). We also found that concentrations of NMDA <20 μM never induced motoneuronal oscillations, whereas concentrations >60 μM typically induced irregular and rather disorganized rhythmic depolarization in both culture and acute preparations. We noticed that relatively high doses of NMDA were consistently required to induce intrinsic voltage oscillations in culture (i.e., 30 μM in culture vs. 20 μM in acute preparations, see Fig. 1D). Oscillations in culture were not only faster but also significantly (P < 0.05) smaller compared with those in acute preparations. Averaged amplitudes of 21.0 ± 2.1 and 16.8 ± 1.4 mV were found in acute and cultured motoneurons respectively (see Fig. 1, Aii vs. B).

To examine the possibility of acute and direct modulating effects induced by the trophic factors per se, oscillations were...
examined in motoneurons from acute slices incubated with trophic factors for several hours. A representative case shown in Fig. 1C illustrates that incubation for 7 h with GDNF (10 ng/ml) did not induce oscillations similar to those found in culture. On average (n = 7), motoneurons incubated with trophic factors for 4–24 h displayed relatively slow (0.23 ± 0.16 Hz) and large-amplitude (21.5 ± 2.3 mV) oscillations in the presence of NMDA not significantly different (P = 0.12 and P = 0.81, respectively) from those found in acute preparations without neurotrophic factor incubation (Fig. 1B). This demonstrates that the changes in cultured motoneurons were not mainly induced by acute neurotrophic factor effects. In turn, this may suggest the existence of long-term mechanisms in response to a lack of peripheral and descending supraspinal inputs (Turrigiano et al. 1994), although we cannot exclude the participation to these effects of long-term and chronic actions of neurotrophic factors on electrical properties by increasing for instance PI3-pathway-mediated intracellular Ca2+ levels and both inhibitory and glutamatergic excitatory synaptic transmission (Arvanian et al. 2003; Perez-Garcia et al. 2004; Schwyzer et al. 2002).

Another main finding of this study concerns a change in the sets of calcium-permeable channels normally involved in mediating these oscillations. We found that NMDA-induced oscillations in the presence of TTX (Fig. 2i) were always abolished by AP5 (Fig. 2v, n = 4/4) and blocked by high [Mg2+]o in culture (Fig. 2ii, n = 6/6). However, nifedipine (10 μM) consistently failed to abolish the voltage oscillations that returned in normal [Mg2+]o (Fig. iii and iv). Similar results were found in preparations that did not previously received AP5 or other ligands (n = 7/7). Note that the applied bias currents shown in ii and v were used mainly to ensure that the effects were not simply the result of an increased threshold for oscillatory property expression. To test whether CaV1.3 channels could still participate in mediating other active cellular properties, we examined their possible implication in mediating serotonin (5-HT)-induced plateau potentials and muscarine-induced voltage oscillations. These two conditional intrinsic cellular properties are metabotropic receptor-activated and essentially mediated by CaV1.3 channels in turtle motoneurons from acute slice preparations (Guertin and Hounsgaard 1999; Hounsgaard and Kiehn 1989). Interestingly, we found that these intrinsic properties failed to be induced in cultured motoneurons. Plateau potentials normally expressed in acute preparations in the presence of 5-HT after intracellular injection of a depolarizing square pulse (Fig. 3A) could not be induced in any of the cultured motoneurons tested (Fig. 3B, n = 6/6).

A similar lack of effect was found in the presence of BayK 8644 (n = 5/5), a selective agonist of the dihydropyridine-site on CaV1.3 channels that normally can potently activate plateau potential properties in motoneurons from acute preparations (Hounsgaard and Mintz 1988). Moreover, motoneuronal voltage oscillations normally induced by muscarine in acute preparations failed to be induced in culture (n = 4/4, not shown).

FIG. 3. Serotonin (5-HT)-induced effects on plateau potentials and oscillations. A: in an acute motoneuron, an intrasomatic depolarizing 0.8-nA current pulse induced a self-terminating plateau potential in the presence of 10 μM 5-HT. B: depolarizing current pulse did not induce plateau potential in a cultured motoneuron. C: rhythmic activity induced by 40 μM NMDA (i) that became intrinsic oscillations in TTX (ii) remained still with 100 μM 5-HT (iii).
Finally, NMDA-induced oscillations in cultured motoneurons were not affected by bath-applied muscarine or 5-HT. This is shown in Fig. 3C where NMDA-induced bursts (i) transformed by TTX into intrinsic voltage oscillations (ii) remained of relatively large amplitude and slow frequency after addition of 5-HT (iii). On average (n = 6), oscillation frequencies and amplitudes (1.72 ± 0.20 and 18.1 ± 1.5) were not significantly (P > 0.05) changed after 5-HT or muscarine application (1.58 ± 0.31 and 15.7 ± 2.1). Further supporting the idea that metabotropic receptor activation with 5-HT or muscarine did not affect the channels under these conditions.

Otherwise, impaled motoneurons from this organotypic culture preparation (n = 18) displayed action potentials, resting membrane potentials and input resistance values that were not significantly different (P > 0.05) compared with those normally found in acute preparations (n = 18). The average peak-to-peak action potential amplitude was smaller, although not significantly (P = 0.07), in culture compared with acute preparations (53.0 ± 3.1 vs. 63.9 ± 2.6 mV). However, the input resistance and resting membrane potential values of cultured motoneurons were of 18.1 ± 1.7 MΩ and -57.4 ± 1.5 mV, which are not significantly different (P > 0.05) compared with those found in motoneurons from acute preparations (16.8 ± 0.9 MΩ and -62.6 ± 1.2 mV, respectively).

**DISCUSSION**

These results show that NMDA generally induced fast TTX-resistant voltage oscillations in organotypic cultured motoneurons. These were insensitive to nifedipine but blocked by AP5 or high [Mg²⁺]₀. Muscarine, 5-HT, and BayK 8644 were found not to uncover or modulate oscillations and plateau potentials. Otherwise, action potential, resting potential, and input resistance values remained relatively unchanged in organotypic cultures and acute preparations.

Voltage oscillations induced by NMDA were also abnormally fast as they were ~10 times faster in culture (1.62 Hz) than in acute preparations (0.13 Hz, Fig. 1D). The high frequencies found in culture are, in fact, similar to those reported in acute preparations from neonatal preparations (i.e., 1–3 Hz) (Hochman et al. 1994; MacLean et al. 1998). Yet it is unlikely that the motoneuronal oscillation frequencies found in acute preparations were atypically slow or specific to turtles because comparable NMDA-induced frequencies have been reported in other adult vertebrate species (i.e., <0.3 Hz in rat, Durand 1991; in lampreys, Wallén and Grillner 1987).

Some of the changes in neuronal response properties during culture deviate from previous studies. For example, while we found that voltage oscillations in motoneurons are transformed in culture (i.e., faster frequencies and fewer activating systems), the ability to generate CaV1.3-dependent plateau potentials is completely abolished (Fig. 3B) (see also Perrier et al. 2000). Neurons in the lobster stomatogastric ganglion system, which normally express tonic depolarization in acute preparations, acquire oscillatory properties as an intrinsic property and become unconditional bursters after a few days in culture (Turrigiano et al. 1994). Also in contrast with our results, the bursting activity that is expressed in embryonic neurons (not identified as motoneurons) is not intrinsic (i.e., not TTX-resistant) but depends on network activity in culture (Ballerini et al. 1999; Keefer et al. 2001; Streit 1993). Altogether, this may indicate that intrinsic properties of mature vertebrate neurons adapt differently to culture conditions than invertebrate neurons and neonatal vertebrate neurons. Reasons for this are unclear, but given the differences known to exist between some of these systems (e.g., neonates vs. adults), developmentally mature systems could undergo some regression associated with a return to non-fully functional CaV1.3 channels as in neonates (Jiang et al. 1999). This hypothesis has been proposed already by Perrier and colleagues who have shown a complete loss of CaV1.3-dependent plateau potential properties in a similar preparation (Perrier et al. 2000).

Such possible down-regulation of CaV1.3 channels is supported by data showing that nifedipine (i.e., as high as 10 μM) consistently failed to abolish the fast oscillations induced by NMDA in cultured motoneurons (Fig. 2). Given that nifedipine generally completely blocks NMDA-induced oscillations in acute preparations (Guertin and Hounsgaard 1998), this strongly support the idea that CaV1.3 channels lost their capacity to mediate this property in culture. The idea is further supported by the fact that direct channel activation with BayK 8644, a highly potent agonist for CaV1.3 channels, did not induce plateau potentials, as it does normally in acute slice preparations (Hounsgaard and Kiehn 1989). Given that the plateau potential in turtle motoneurons is an intrinsic property essentially mediated by CaV1.3 channels (Hounsgaard and Kiehn 1989; Simon et al. 2003), this suggests, again, that CaV1.3 channels were functionally down-regulated. This could be the result of reduced CaV1.3 channel transcript levels in culture as reported with cultured Purkinje neurons (Gruol et al. 1992).

On the other hand, NMDA ionophores were shown to remain a key factor in mediating oscillations in cultured motoneurons as they are in acute preparations (Guertin and Hounsgaard 1998). We showed, in fact, that the NMDA receptor-channel system remained critically important because oscillations in culture were completely blocked by AP5 or high [Mg²⁺]₀ (Fig. 2). In turn, this may suggest the existence of a compensatory mechanism by which the NMDA ionophore had to increase its ionic conductance level or that a small undetectable increase of its density resulted in greater calcium entry (Turrigiano et al. 1995) to allow voltage oscillations to still be inducible despite a presumably down-regulated CaV1.3 channel system.

As mentioned earlier, we found that 5-HT lost its capacity to modulate NMDA-induced voltage oscillations (Fig. 3C) and to uncover plateau potentials (Fig. 3B). We reported also that muscarine consistently failed to induce or modulate voltage oscillations in culture. Because in acute preparations, 5-HT and muscarine can normally induce and modulate these properties (Guertin and Hounsgaard 1999), the present results show that the 5-HT and muscarine receptor systems lost their capacity to affect these properties in culture. This loss of functions may be explained by downstream changes affecting second-messenger systems or CaV1.3 channel function as mentioned earlier. Because NMDA-induced oscillations in culture were not modulated by muscarine or by 5-HT (Fig. 3C) as they should normally via functionally-coupled intracellular signaling systems between NMDA ionophores and 5-HT receptors for instance (MacLean and Schmidt 2001; MacLean et al. 1998), this could argue also for a down-regulation of metabotropic
receptor system-activated intracellular pathways or of the 5-HT and muscarine receptors themselves.

**ACKNOWLEDGMENTS**

Thanks to H. Jahnsen, J. F. Perrier, and I. Kjaer for help with cultures.

**GRANTS**

This work was supported by Medical Research Council Canada, Medical Research Council Denmark, and The Lundbeck Foundation.

**REFERENCES**


