Extensive heterogeneous dye-coupling among cells derived from human induced-pluripotent-stem cells

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Cells derived from induced pluripotent stem cells (IPSCs) is a fast developing field with promising results for development of therapies for human conditions (Qiang *et al.*, 2013). There is limited information about morphological and functional properties of these cells. Here we characterise extensive dye-coupling properties of these cells in culture environment. Sixty to 90 days after induction, the cells were plated on to coverslips and studied by electrophysiological and dye-filling methods using 2% Neurobiotin (Kanjhan & Vaney, 2008). Neuronal and non-neuronal cells were electrophysiologically characterized: the cells with action potential firing or displaying fast sodium currents were identified as neurons; while cells without action potential firing or no fast sodium currents were considered as non-neuronal. Some neurons also displayed low frequency (<1Hz) and small amplitude (<50 pA) excitatory synaptic inputs at holding potential of −70 mV. Such excitatory synaptic inputs were confirmed by immunocytochemistry: pre-synaptic VGLUT-2 terminals apposing PSD-95 labelled post-synaptic sites of dye-filled neurons. Both neuronal and non-neuronal cell types were individually filled with 2% Neurobiotin^{TM} (MW = 323) using current pulses of 350 to 400 pA amplitude (500 ms duration) at 1 Hz for 4 minutes (Kanjhan & Vaney, 2008). This amount of total current pulses usually fills single neurons and detects dye-coupling in whole-mount retina or in brain slices obtained from mice. Individually filled neuronal and non-neuronal cells derived from human IPSCs were dye-coupled heterogeneously to many hundreds of cells (mean ~1000 cells) surrounding them (n = 12). Not all cells displayed dye-coupling despite being in the vicinity of the dye-filled soma that was dye-coupled to many other cells; such as a subpopulation of cells expressing $GABA_A$ α-1 subunit immuno-reactivity in their small to medium sized somas. Pulling out the primary-filled cell from the cover slip with the electrode tip upon completion of dye electroporation did not prevent the extent of dye-coupling (n = 5), suggesting that the dye-coupling and passage of Neurobiotin among cells happens unopposed and immediately. Pre-incubation of cells in carbenoxolone (1-100 µmolar; 15 min), a blocker of connexins and pannexins that form gap junctions, completely prevented dye-coupling (n = 16). Carbenoxolone also significantly hyperpolarized the resting membrane potential of cells from a mean of −36 mV to −49 mV (n = 7 each group); and changed current responses to voltage steps and ramps. Cells incubated in carbenoxolone remained viable for a longer time (up to 4 h), with reduced signs of degeneration such as vacuolization, granulation, swelling and disintegration of the membranes usually seen in an hour under control conditions. These observations suggest that heterogeneous dye-coupling *via* carbenoxolone-sensitive gap junctions is extensive among cells derived from human IPSCs. Gap junctional coupling likely plays significant roles in determining intrinsic membrane properties and intercellular signalling during cell proliferation, differentiation, and prior to maturation of synaptic transmission and formation of neural networks.

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