Analysis of Dissociated Single Neurons by Simple and Semi-Quantitative RT-PCR (Reverse Transcription and Polymerase Chain Reaction)

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ABSTRACT—We have developed a simple and semi-quantitative method for mRNA determination in single cells using the reverse transcription and polymerase chain reaction (RT-PCR). The distinct features of this method are the highly efficient RNA harvest from whole dissociated cells and the ability to perform all RT procedures in one tube that allowed semi-quantitative determination of mRNA in dissociated cells. This method revealed that histamine H1-receptor mRNA was highly expressed in 5/28 small and 1/26 large dorsal root ganglion neurons of the mouse.

Keywords: Histamine H1-receptor mRNA, Single whole-cell PCR, Primary sensory neuron

The determination and analysis of expression of several factors in single cells may provide useful information on their physiological roles and cellular mechanisms of signal transduction. Several studies have detected mRNA in a single cell by the reverse transcription and polymerase chain reaction (RT-PCR). For example, the combination of whole-cell patch-clamp recording and PCR amplification of mRNA in a single neuron was reported (1, 2). This required two-round PCR (25–35 cycles per round), which made quantitative mRNA determination inaccurate and the comparison of the expression level among cells difficult. The necessity of so many PCR cycles may be partly due to the procedure of aspirating cytoplasmic content into the patch pipette. Furthermore, it is difficult to ascertain whether whole cytosol has been collected into the pipette. This also lowers the accuracy of comparing mRNA expression levels among cells. Therefore, if PCR can be performed on a single whole-cell, much fewer PCR cycles would be needed and quantitative comparison would become more accurate. We report here a simple and semi-quantitative method for mRNA determination in a single whole-cell using RT-PCR.

Dorsal root ganglia (DRGs) were isolated from a male ICR mouse (5-week-old) and treated with collagenase solution (25 mg/ml) at 37°C for 30 min. The DRG cells were dispersed into Dulbecco's modified Eagle's medium containing heat-inactivated bovine serum (10% v/v) and kept at 37°C in a humidified atmosphere of 90% air and 10% CO2 for 24 hr before assay. A whole target neuron was sucked into a glass capillary pipette under a phase contrast microscope and then put into a test tube. After adjusting the volume to 10 µl with RNase-free water, the sample was treated with 1 µl of proteinase K (200 µg/ml) at 37°C for 30 min, and then the enzyme was inactivated by heating at 99°C for 5 min. The sample was chilled on ice for 5 min and then treated with 1 µl of DNase I (70 units/µl) at 37°C for 20 min for the digestion of genomic DNA. RT-PCR was performed on the sample that was heated at 99°C for 5 min for the inactivation of the DNase I; we confirmed that PCR of plasmid DNA (1 µg) in the presence or absence of pre-heated DNase I gave almost the same result (data not shown). To determine the expression level of mRNA, the signal intensity was measured by an image analyzer (Kodak, New York, NY, USA).

Since histamine H1-receptors (H1R) are thought to be expressed by primary sensory neurons, we examined H1R mRNA expression in a single DRG neuron. Half of the RT product was used for the PCR assay of H1R mRNA, and the remaining half was for PCR of glyceraldehyde 3-phosphate dehydrogenase (GAPDH) to ascertain that a cell had been collected. In the case shown in Fig. 1A, H1R and GAPDH mRNAs were detectable at 38 or more and 32 or more PCR cycles, respectively. In other experiments, 70-cycle PCR was done on a template prepared from single DRG neurons without RT, which yielded no
positive band of the target genes (data not shown), suggesting the complete digestion of genomicDNAs withDNase I.

To examine the possibility of semi-quantitative analysis using PCR, serially diluted samples were assayed for mRNA by RT-PCR. As shown in Fig. 1B, the signal intensity correlated almost linearly with the amount of mRNA for H1R at least between 1/1 and 1/8 dilution. RT-PCR of four aliquots of samples from another neuron gave bands with similar signal intensity; the variation in the signal intensity of H1R was less than 10%. When this single-cell RT-PCR was performed in 54 different neurons, of which 28 were small (<25 μm) and 26 were large (≥ 25 μm), H1R mRNA was found to be expressed in 8 (15%) small and 8 (15%) large neurons (Fig. 2A). However, 5 small neurons and one large neuron were of relatively high expression (Fig. 2B). Although the signal intensity of GAPDH mRNA varied considerably in different neurons, the average level GAPDH mRNA in small and large neurons positive for H1R mRNA were 0.80±0.11 and 0.74±0.10 (×10^4, ±S.E.M. of 8 neurons), respectively. There were no significant relationships between the levels of H1R and GAPDH mRNAs (P > 0.05, Pearson product moment correlation).

The present method has a high efficiency of RNA ex-
traction from single cells because whole cytoplasmic contents are used and RNA extraction and RT procedures are performed in one tube. Tong et al. (3) demonstrated single-cell RT-PCR, in which all procedures were conducted in one tube. However, as they harvested cytoplasm into a patch pipette, it was unclear whether RNA had been completely collected. Our method collects all RNAs from single cells and could detect simultaneously at least both target and GAPDH mRNAs in a single cell. In addition, the signal intensity was almost linear with the amount of H1R mRNA, and the variation due to RT-PCR procedures was relatively small. These results taken together suggest that it is possible to determine semi-quantitatively H1R mRNA in single neural cells and to compare the expression level among cells.

Histamine produces pain and itch, when applied to the human skin (4). Signals for such sensations are thought to be mediated by small primary sensory neurons (5). Therefore, for application of the present method, we examined whether H1R mRNA would be expressed mainly by small DRG neurons in mice. Although the sample size was small, H1R mRNA was highly expressed predominantly in small DRG neurons, supporting the idea that H1R on primary sensory neurons are involved in pain and itch sensations. The expression level of H1R mRNA varied considerably in different DRG neurons. This might be due to the variation of expression level rather than to that of experimental procedures because signal intensities of GAPDH mRNA were independent of cell size, and there were no relationships between signal levels of H1R and GAPDH mRNAs. Although more than one third of the examined neurons were positive for H1R mRNA, 6 out of 54 neurons showed relatively high expression. Considering the high sensitivity of PCR (6), the latter rate is roughly similar to that reported by Kashiba et al. (7) who showed, using in situ hybridization, that about 10–20% of guinea pig DRG cells were positive for H1R mRNA.

In conclusion, this single whole-cell RT-PCR may allow the semi-quantitative determination of mRNA in single cells. This method can be applied to concurrent PCR assay of many dissociated cells after physiological and pharmacological experiments on their functions.

Fig. 2. Proportion of dorsal root ganglion neurons expressing H1-receptor mRNA and its expression level in the individual neurons. A: Percentage of neurons expressing H1-receptor mRNA. Hatched and open slices indicate neurons positive and negative for H1-receptor mRNA, respectively. The cells were classified into two groups according to their size (small, < 25 μm; large, ≥ 25 μm). Figures in parenthesis indicate percentage. B: Expression level of H1-receptor mRNA in individual neurons positive for this mRNA.

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