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Research Report

Temperature: An important experimental variable in studying PKC modulation of ligand-gated ion channels

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Abbreviations:
GABA, γ-aminobutyric acid
PKC, calcium phospholipid-dependent protein kinase
HEK, human embryonic kidney
5-HT, serotonin
PMA, phorbol 12-myristate 13-acetate
PMM, phorbol 12-mono-myristate

ABSTRACT

Amphibian oocyte and mammalian heterologous expression systems are often used to investigate the function of recombinant ion channels using electrophysiological techniques. Although both systems have yielded important information, the results obtained in these systems are sometimes conflicting. Oocytes and mammalian cells differ in their physiological temperature requirements. While room temperature is within the physiological temperature range for oocytes, this temperature is far below that required by mammalian cells. Since electrophysiological studies are often performed in both oocytes and mammalian cells at room temperature, we sought to determine if recording temperature could be a factor in some disparate results obtained in these cell types. For these studies, we examined phorbol ester modulation of $GABA_A$ and glycine receptors. Consistent with the literature, at room temperature, PMA (phorbol 12-myristate 13-acetate) produced a large reproducible decrease in the peak amplitude of GABA and glycine-gated currents in *Xenopus oocytes*. In contrast, PMA was ineffective in modulating these heterologously expressed receptors at room temperature in human embryonic kidney (HEK) 293 cells. However, when electrophysiological experiments were performed at 35 °C in HEK 293 cells, PMA decreased the function of these receptors. Our results indicate that the temperature at which electrophysiological studies are conducted is an important experimental variable. To determine the extent to which electrophysiological recordings are performed at physiological temperatures in HEK 293 cells, a PubMed search was conducted using the search terms "patch clamp" and "HEK" for the years 2003–2004. This search revealed that only 15% of the patch clamp studies were reported to have been conducted in the temperature range of 32–37 °C. The results of our study indicate that temperature is an important experimental variable that requires rational consideration in the design of electrophysiological experiments.

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1. Introduction

The *Xenopus laevis* oocyte expression system is widely used to study protein structure/function of heterologously expressed mammalian proteins. This system is particularly well suited to the investigation of recombinant ion channels since the large size of the cell allows recording of ion currents without extensive electrophysiological expertise or the expensive equipment required to conduct electrophysiological recordings in mammalian cells. Such experiments have yielded important data regarding ligand-gated ion channel gating (Newell et al., 2004), desensitization (Baur and Sigel, 2003), posttranslational regulation (Kellenberger et al., 1992; Krishek et al., 1994), pharmacology (Lippa et al., 2005), and trafficking (Chapell et al., 1998).

Members of the cysteine loop family, which include the nicotinic acetylcholine (nAch), gamma-aminobutyric acid type A ($GABA_A$), glycine and serotonin type 3A ($5\text{-}HT_{3A}$) receptors have been studied extensively in *Xenopus oocytes* (see below). Although findings in the oocyte system can often be successfully extrapolated to mammalian cells, disparate results between the two systems have been reported. Oocyte studies examining calcium, phospholipid-dependent protein kinase (PKC) regulation of some members of the cysteine loop family of channels have yielded results that are inconsistent with some findings obtained in mammalian cells. Activation of native oocyte PKC by low nanomolar concentrations of phorbol esters produces a robust and reproducible decrease in $GABA_A$ receptor function (Chapell et al., 1998; Ghansah and Weiss, 2001; Kellenberger et al., 1992; Krishek et al., 1994; Leidenheimer et al., 1992; Moran and Dascal, 1989; Sigel and Baur, 1988; Sigel et al., 1991). In contrast, the use of phorbol esters in mammalian cells to examine the function of recombinant or native receptors has yielded mixed results. In some studies, $GABA_A$ receptors expressed in HEK 293 cells are unresponsive to phorbol ester stimulation of native PKCs (Huang and Dillon, 1998; Krishek et al., 1994) and coexpression of the receptor with PKC followed by subsequent activation by phorbol esters produces limited receptor inhibition (Krishek et al., 1994). Several studies demonstrating phorbol ester inhibition of receptor function in neurons used micromolar concentrations of phorbol esters (Aguayo and Pancetti, 1994; Gillette and Dacheux, 1996; Meier et al., 2003; Tapia et al., 1997). Other studies have demonstrated a decrease in $GABA_A$ receptor function in recombinant expression systems and neurons, but the effect in mammalian cells is smaller in magnitude than that observed in oocytes (Brandon et al., 2000; Connolly et al., 1999) and is limited to a subpopulation of cells (Brandon et al., 2000). Conversely, a cell-type specific increase in $GABA_A$ receptor function has been reported following PMA (phorbol 12-myristate 13-acetate) treatment in olfactory bulb (Brunig et al., 1999).

Similar to phorbol ester modulation of $GABA_A$ receptors in oocytes, PMA treatment inhibits glycine receptor responses in oocytes (Nishizaki and Ikeuchi, 1995; Uchiyama et al., 1994; Vaello et al., 1994). In contrast, findings in mammalian cells have demonstrated both phorbol ester induced decreases (Albarran et al., 2001; Tao and Ye, 2002; Tapia et al., 1997; Ye and McArdle, 1996; Ye et al., 2002) and increases (Gu et al., 1998; Schonrock and Bormann, 1995; Xu et al., 1996) in glycine-gated chloride currents. These disparate findings between oocyte and mammalian systems remain to be explained, and the scope of conflicting findings is likely to be underappreciated since negative findings may go unreported.

Given the wide range of technical approaches and biological variability that exist, it is likely that the noted discrepancies in the literature are due to a variety of factors. Because electrophysiology experiments are often conducted at room temperature, and the physiological temperature requirements for amphibian and mammalian cells are different, 19–23 °C versus 37 °C, respectively, we chose to examine whether the differential temperature requirements between the two model systems could account for conflicting data within the literature.

Here, we report that while PKC activation in oocytes produces robust and reproducible decreases in $GABA_A$ and glycine receptor function, these effects are observed in HEK 293 cells at 35 °C, but not at room temperature. Because of these striking results, we performed a literature analysis to assess the percentage of electrophysiological experiments that were reported to be performed at physiological temperatures in HEK 293 cells. Surprisingly, within the context of our PubMed search parameters, only 15% of patch clamp studies performed in 2003–2004 in HEK 293 cells were reported to be conducted at or near physiological temperatures. Thus, our findings are not only important for reconciling the specific literature regarding phorbol ester effects on ligand-gated chloride channels but also have fundamental implications in general for electrophysiological experiments performed in mammalian cells at room temperature.

2. Results

To determine if temperature plays a role in experimental outcome when studying the effect of phorbol esters on ligand-gated ion channel function in heterologous expression systems, we conducted experiments in *X. oocytes* and HEK 293 cells expressing recombinant $GABA_A$, glycine or 5-HT3 receptors. For each recombinant receptor, we performed experiments in HEK 293 cells at both room temperature and 35 °C. Since room temperature is within physiological range for oocytes and higher temperatures lead to oocyte deterioration, we performed oocyte experiments only at room temperature.

As previously reported in *X. oocytes* (Chapell et al., 1998; Kellenberger et al., 1992; Krishek et al., 1994; Leidenheimer et al., 1992; Moran and Dascal, 1989; Sigel and Baur, 1988; Sigel et al., 1991), the PKC activator PMA, but not the inactive analog PMM, produced a large decrease in GABA-gated chloride currents (Fig. 1). This decrease persisted for several hours (data not shown). Although a decrease in $GABA_A$ receptor function has been reported by many laboratories using the oocyte expression system, these results have been inconsistent in mammalian cells (see Introduction). To examine whether the difficulty in observing PMA effects on $GABA_A$ receptors expressed in mammalian cells was due to the absence of physiological recording temperatures, we performed experiments in HEK 293 cell expressing $\alpha1\beta2\gamma2S$ $GABA_A$ receptors with PMA at both room temperature and 35 °C (Fig. 2). PMA experiments performed at room temperature did not