The effects of phospholipase C on the voltage-gated Ca current in *Lymnaea stagnalis* mollusc neurons

Abraham R. Akopyan, Nikolai K. Chemeris*, Victor I. Iljin*†, Fuat E. Ilyasov* and Alevtina A. Selishcheva+

*Institute of Biochemistry, Academy of Sciences of the Armenian SSR, Yerevan 375044, *Institute of Biological Physics, Academy of Sciences of the USSR, Pushchino, Moscow Region 142292* and *+Moscow State University, Moscow 117234, USSR*

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Application of phospholipase C to isolated voltage-clamped molluscan neurons substantially increased the voltage-gated Ca current (I_{Ca}) in most of the cells studied. In contrast, phosphoinositide-specific phospholipase C inhibited I_{Ca} in all neurons. The mechanism of phospholipase C-induced alterations of I_{Ca} is poorly understood at present, but they may have some relevance to plasma membrane phosphoinositide-coupled changes in cytosolic Ca^{2+} and to α-adrenergic neurotransmitter control of neuronal I_{Ca}.

(Mollusc) Neuron Voltage-gated Ca^{2+} current Phospholipase C Phorbol ester Voltage clamp Intracellular dialysis

1. INTRODUCTION

The role of plasma membrane phospholipid content in specific cell functions in various animal species is well documented [1]. Much attention has been focused on the phosphoinositides (PI) and, in particular, on the polyphosphoinositides (PPI), the membrane content of which is altered by the activation of a wide variety of membrane surface receptors [2–4]. This membrane response is assumed to be mediated by stimulation of the activity of cellular phosphoinositide phosphodiesterase (phospholipase C) [4]. In parallel with PPI hydrolysis an increase in cytosolic free Ca^{2+} is observed [5], which may be due to release of Ca^{2+} from intracellular stores or to enhancement of Ca influx across the plasma membrane. Indeed, the major product of PPI hydrolysis, i.e. diacylglycerol (DAG), is known to activate C-kinase [6] which in turn may phosphorylate membrane proteins thus leading to additional Ca influx. Also, DAG may be phosphorylated to phosphatidic acid (PA) which has been proposed as a Ca ionophore [7,8].

Possible coupling between transmembrane Ca^{2+} transport and PPI metabolism is also of great importance because of the following reasons. Voltage-dependent Ca channels form one of the main pathways for Ca^{2+} entry into both vertebrate and invertebrate neurons. The size of the Ca currents (I_{Ca}) is affected by various neurotransmitters and, in particular, by biogenous amines (review [9]). The latter seem to inhibit I_{Ca} due to activation of α-adrenoreceptors [10], although the mechanism of inhibition remains unclear [11]. According to biochemical data, α-adrenergic agents

Abbreviations: PI, phosphoinositides; PPI, polyphosphoinositides; DAG, diacylglycerol; PA, phosphatidic acid; I_{Ca}, voltage-gated Ca current; PLC, phospholipase C; PLC_s, phosphoinositide-specific phospholipase C

† To whom correspondence should be addressed

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are capable of stimulating endogenous phospholipases which results in PI hydrolysis [1].

At present biochemical techniques cannot easily resolve the question of whether regulation of membrane Ca permeability by some neurotransmitters, such as dopamine and serotonin, and the neurotransmitter-induced hydrolysis of PI are parallel and independent mechanisms. The aim of the present study was to elucidate whether exogenous phospholipase C influences the functioning of voltage-gated Ca channels in the neuronal membrane.

2. MATERIALS AND METHODS

Completely isolated [12] nonidentified neurons from the circumesophageal ganglia of *Lymnaea stagnalis* were used for the experiments under intracellular dialysis [13] and voltage-clamp conditions. I_{Ca} was separated from other voltage-sensitive currents by equimolar substitution of external Na^+ by Tris and blockage of the voltage-dependent K^+ conductances by Cs^+. The external physiological solution contained 1.6 mM CsCl, 4 mM $CaCl_2$, 1.5 mM $MgCl_2$, 86 mM Tris (pH 7.5). The solution for intracellular dialysis contained 80 mM CsCl, 1 mM glucose, 20 mM Tris (pH 7.2), and 0.2 mM EGTA to maintain free Ca^{2+} at a low level. Phospholipase C from *Bacillus cereus* (4000 U/mg protein) was obtained from Boehringer (FRG); phospholipase C specific to PI from *B. cereus* was a gift from V.I. Kulene (NPO Ferment, Vilnius, Lithuanian SSR). 12-*O*-Tetradecanoylphorbol-13-acetate (TPA) was obtained from P-L Biochemicals, Milwaukee, WI. Enzymes were dissolved in external physiological solution immediately before use and introduced directly into the external compartment of the experimental chamber. All experiments were done at room temperature (18–22°C).

3. RESULTS AND DISCUSSION

After the beginning of the intracellular dialysis, I_{Ca} manifested two kinds of behavior with time: either the amplitude of I_{Ca} elicited by standard electrical stimulation (see fig.1 legend) remained stable for at least 2 h, or a rapid 'spontaneous' decline of I_{Ca} was observed (fig.1a) (see also [14]). Irrespective of whether I_{Ca} was stable, in 16 neurons out of 23 PLC caused qualitatively the same effect: the amplitude of I_{Ca} increased. The magnitude of this potentiating effect was larger for non-stable I_{Ca} than for stable currents: after substantial spontaneous decay of I_{Ca}, addition of PLC led to a 2–3-fold increase of the amplitude of the current so that I_{Ca} could almost recover to the initial value (fig.1A). The potentiating effect per-

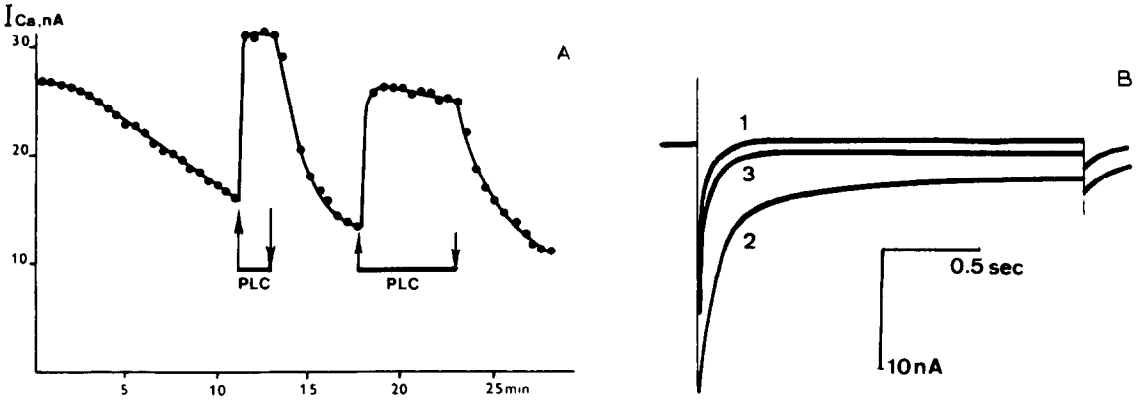

Fig.1. (A) The potentiating effect of 4 $\mu g \cdot ml^{-1}$ PLC on I_{Ca} at two successive applications. The envelope of peak values of maximal I_{Ca} is shown; I_{Ca} is elicited by pulsing the membrane potential from −90 mV (holding potential) to 0 mV for 100 ms every 30 s. Leakage current was subtracted. In this graph as in fig.2A the inward current is plotted upwards. (B) Oscillograms of I_{Ca} in control (1), at application of 4 $\mu g \cdot ml^{-1}$ PLC (2) and after 10 min washout (3). Here and in fig.2B holding potential −90 mV, step depolarization to 0 mV; the voltage pulse duration is increased to achieve practically complete inactivation of I_{Ca}.

sisted during the entire period of time of application of PLC, but disappeared rapidly upon washout; it could be reproduced several times on the same neuron. A reliable potentiation of 10–15% was obtained by using PLC at concentrations of about 0.2 $\mu g \cdot ml^{-1}$. In the other seven neurons PLC reduced I_{Ca}.

In all 23 experiments, PLC led to apparent retardation of I_{Ca} inactivation (fig.1B). Computer analysis of I_{Ca} inactivation was unsuccessful because the decay of a single current recording with time could not be fitted unambiguously by the sum of the exponential components.

Since PLC splits practically all membrane phospholipids, the different effects of enzyme action that we observed may be due to variations in membrane phospholipid content from cell to cell and related differences in the ratio of breakdown products. This is why it was of interest to study the effect of a phospholipase C, which specifically hydrolyses PI. We found that in all 25 neurons which were treated with PLC_s I_{Ca} decreased. Thereby, in the same neuron PLC could increase I_{Ca} while PLC_s decreased it (fig.2A). In contrast to PLC, PLC_s caused no apparent alteration of the inactivation kinetics of I_{Ca} (fig.2B).

At present we cannot explain how phospholipase C acts on I_{Ca}. PLC_s is known to split PPI into DAG and into inositol 1,2,4-triphosphate. Could DAG-dependent phosphorylation be involved in the inhibition of I_{Ca} observed here? According to our results such phosphorylation ought to result in a decrease of I_{Ca}. However, it has been shown recently that injection of C-kinase also leads to an increase of I_{Ca} in molluscan neurons [15]. Moreover, our results (on 6 cells) with C-kinase activator, TPA, showed that when applied extracellularly, it affected neither the size nor the kinetics of I_{Ca} even at concentrations as high as 0.16 mM. This important finding indicates that the activity of C-kinase in *Lymnaea* neurons seems to be low and its activation by DAG unlikely.

DAG can be phosphorylated into PA, which alleged Ca ionophoric properties [7,8] may induce a decrease of I_{Ca} by the Ca-dependent inactivation process [16]. However internal administration of 10 mM EGTA did not prevent PLC_s-induced inhibition of I_{Ca} (7 cells), whereas this concentration of EGTA is more than sufficient for complete elimination of Ca-dependent inactivation [17]. The same experiments show that a release of intracellular Ca^{2+} by inositol 1,4,5-triphosphate is not involved either in the present I_{Ca} reduction. Finally, one can suggest that the enzymes induce changes in phospholipid content of the neuronal membrane in a manner which disturbs protein-lipid interactions in the vicinity of the Ca channels. A different ratio of breakdown products may determine the sign of changes of I_{Ca}: inhibition or potentiation.

In conclusion, although the mechanism of phospholipase C action on I_{Ca} has still not been elucidated, it is of interest to know that such an action does exist. This indicates that perturbations in membrane phospholipid content and, in particular, in phosphoinositide content, can strongly affect the efficiency of the voltage-gated Ca permeability and opens the possibility of neurotransmitter or hormone control of neuronal Ca channel functioning through a mechanism differing from the cyclic AMP-dependent one [18].

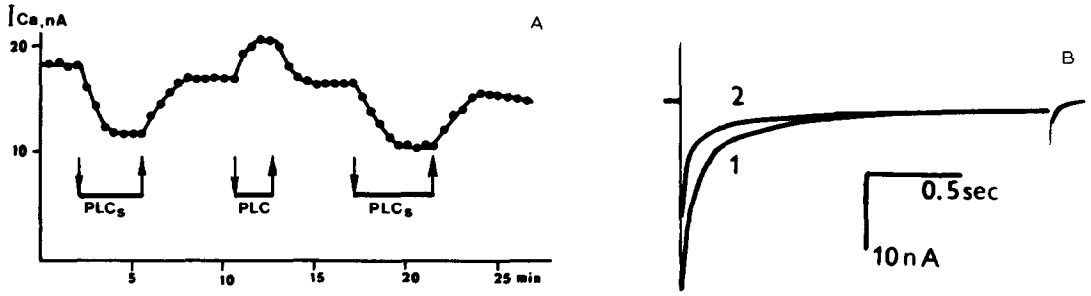

Fig.2. (A) Inhibition of I_{Ca} by 1 $\mu g \cdot ml^{-1}$ PLC_s and potentiation of I_{Ca} by 2 $\mu g \cdot ml^{-1}$ PLC in one neuron. The electrical stimulation parameters are the same as in fig.1A. (B) Oscillograms of I_{Ca} in control (1) and during application of 1 $\mu g \cdot ml^{-1}$ PLC_s (2).

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