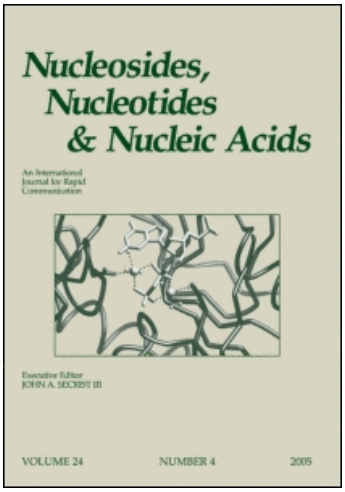

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The Neuroprotective Adenosine-Activated Signal Transduction Pathway Involves Activation of Phospholipase C

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THE NEUROPROTECTIVE ADENOSINE-ACTIVATED SIGNAL TRANSDUCTION PATHWAY INVOLVES ACTIVATION OF PHOSPHOLIPASE C

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□ *We have demonstrated before that exposure of neuronal cultures to poisoning by iodoacetic acid (IAA) followed by "reperfusion" (IAA-R insult), results in severe cytotoxicity, which could be markedly attenuated by prior activation of the adenosine A_1 receptors. We also have demonstrated that adenosine activates a signal transduction pathway (STP), which involves activation of PKCε and opening of K_{ATP} channels. Here, we provide proof for the involvement also of phospholipase C (PLC) in the neuronal protective adenosine-activated STP. R-PIA, a specific A_1 adenosine receptor agonist, was found to enhance neuronal PLC activity and protect against the IAA-R insult. The PLC inhibitor U73122, abrogated both R-PIA-induced effects. These results demonstrate that activation of PLC is a vital step in the neuronal protective adenosine-induced STP.*

Keywords Adenosine; Iodoacetic acid; Neuroprotection; Phospholipase C (PLC); Signal transduction pathway; U73122

INTRODUCTION

Previous studies in our laboratory demonstrated that exposure of neurons to IAA-R insult (poisoning by iodoacetic acid followed by a "reperfusion" period) is cytotoxic to the neurons.[1–3] Exposure of the cells to this insult resulted in ATP depletion, which occurred during the exposure to the iodoacetic acid (IAA)[3] and in generation of toxic amounts of reactive oxygen species (ROS), which occurred mainly during the "reperfusion" period.[2] We demonstrated that the neurons could be protected against the insult by presence of antioxidants[2] and by activation of adenosine A_1 receptors.[3] Investigation in our laboratory of the mechanism induced

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by adenosine to confer protection against the IAA-R insult revealed the operation of a signal transduction pathway (STP) involving activation of PKCε[4] and of K_{ATP} channels.[3] Here we provide evidence that the neuronal adenosine-induced STP against the IAA-R insult involves also activation of PLC.

METHODS

Neuronal cultures were prepared from cerebral hemispheres from rat embryos as described before.[1–3] The experimental model for the exposure of the neuronal cultures to the IAA-R insult and for evaluation of the protection conferred to the cells against this insult by exposure of the neurons to R-PIA (an established adenosine A_1 receptor agonist; Research Biochemicals International, MA, USA) and for the assessment of abrogation of the protection by the PLC inhibitor U73122 (Sigma, St. Louis, MO, USA) was as follows (Figure 1)[1]: The protocol started with change of media to fresh media, followed by a pre-insult period of 15 minutes. The insult period started by the addition of iodoacetic acid (IAA; sodium salt; Sigma, St. Louis, MO, USA) to a final concentration of 100 μM, for 150 minutes. The medium was then removed and the cultures incubated in fresh medium for a post-insult reperfusion period of 1 hour, at the end of which the injury to the cells was assessed by the trypan blue exclusion test. The adenosine-activated protection signal was induced by exposure of the cultures to R-PIA, a specific A_1 adenosine receptor agonist (1 μM; for the last 5 minutes of the pre-insult period). The capacity of U73122, a PLC inhibitor,[1] to abrogate the R-PIA-induced protection was assessed by exposure of the cultures to this compound (10 μM), during the entire pre-insult period. The concentration of the various compounds used in the experiments and the time periods of exposure to these compounds were chosen following preliminary experiments.

PLC activity was assayed by measuring the accumulation of [^{3}H]inositol phosphates in [^{3}H]inositol-prelabeled cells.[1] For the study of the effect of the PLC inhibitor U73122 on PLC activity, U73122 (10 μM) was added to the culture media 10 minutes before the addition of R-PIA.

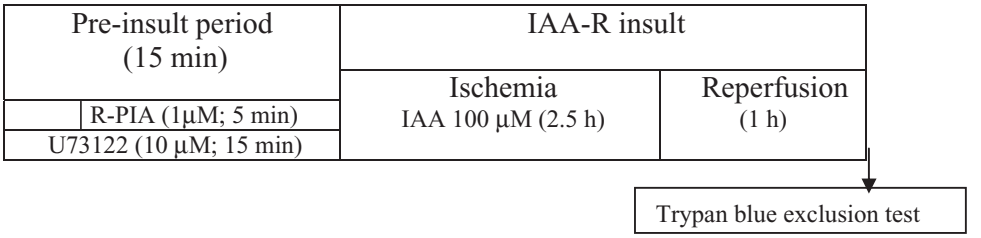

FIGURE 1 The experimental protocol.

TABLE 1 Abrogation of the R-PIA-Induced Protection by the PLC Inhibitor U73122

Conditions	% Dead Cells (Stained by Trypan Blue)
A. Control cultures	20.5 ± 1.4
B. Cell exposed to IAA-R insult	74.3 ± 5.4
C. Pre exposure to R-PIA + IAA-R Insult	37.4 ± 3.4*
D. As in C., but with presence of U73122 before and during the exposure to R-PIA	71.0 ± 2.2

*P for the difference from the insulted cells <0.001.

RESULTS

Exposure of the cultured neurons to the IAA-R insult resulted in severe cell damage that could be significantly mitigated by pre exposure of the cultures to the adenosine A_1 receptor agonist R-PIA. The PLC inhibitor U73122 abolished the R-PIA-induced protection (Table 1). R-PIA was found to enhance PLC activity by 24.3 ± 4.7% in comparison to control cultures. Addition of the PLC inhibitor U73122, abrogated the R-PIA-induced enhancement of PLC activity and furthermore even decelerated the constitutive PLC activity, resulting altogether in 15.2 ± 3.2% lower activity in comparison to control cultures (results represent means ± SEM of 4 to 10 experiments, each performed in triplicates; $P < 0.001$).[1] The duration of maintenance of PLC in its activated state following exposure of the cells to R-PIA for 5 minutes was found to be less than 10 minutes.[1]

DISCUSSION

The results of this study demonstrate that activation of PLC is involved in the adenosine-induced STP leading to protection against the IAA-R insult. Adenosine A_1 receptor is a G protein coupled receptor (GPCR).[5] Such receptors mediate the signal downstream through activation of PLC, probably PLCβ. PLC catalyzes the degradation of PIP_2 to DAG, an activator of PKC. Thus, the induction of PLC activation by R-PIA, probably mediates the protection signal from the adenosine receptors to PKCε, a component demonstrated previously in our laboratory to participate in the adenosine-induced protective STP.[4] Involvement of PLC in the adenosine-induced cardiac ischemic tolerance was demonstrated in a chick embryo ventricular myocyte culture model and in isolated rabbit hearts.[6,7] The finding that both the neuroprotective and cardioprotective adenosine-induced STP include the participation of the three components PLC, PKCε, and the K_{ATP} channels, may be taken to suggest that the two tissues contain the same adenosine-induced protective STP.

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