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Research Article

Cytosolic phospholipase A_2 and lipoxygenase are involved in cell cycle progression in neuroblastoma cells

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Abstract. Arachidonic acid has been implicated in regulating cellular proliferation, and is preferentially released by the 85-kDa cytosolic phospholipase A_2 ($cPLA_2$). Recently, we demonstrated that $cPLA_2$ is activated at distinct periods during the ongoing cell cycle of neuroblastoma cells. The purpose of the present study was to establish the role of these $cPLA_2$ activity peaks in cell cycle progression. Inhibition of $cPLA_2$ activity with arachidonyl trifluoromethylketone (ATK) in early G1 phase reduced DNA synthesis markedly. A 24-h incubation with ATK revealed no significant difference in cell number compared to untreated cells, although $cPLA_2$ activity was still inhibited. This suggests redundancy of different PLA_2 enzymes. Lipoxygenase inhibition in early G1 resulted in G1 phase arrest, whereas inhibitors for cyclooxygenase had no effect. Furthermore, cells stopped progressing through S phase when lipoxygenase was inhibited in early S phase, demonstrating the requirement of lipoxygenase products for S phase progression.

Key words. Phospholipase A_2; lipoxygenase; cyclooxygenase; G1 phase; cell cycle; DNA synthesis; S phase.

The factors that determine whether cells continue to proliferate, arrest growth or differentiate are activated by signals from the extracellular environment and operate predominantly during the G1 phase of the cell cycle. Progression through the cell cycle is regulated by cyclin-dependent kinases (Cdks) that are activated upon binding with their cyclins, and by multiple phosphorylation and dephosphorylation steps [1–3]. The activity of Cdks is negatively regulated by Cdk inhibitory proteins that comprise two families of which the INK4 family specifically inhibits cyclin D/Cdk4,6 complexes, while the Kip/Cip family inhibits most cyclin/Cdk2,4 and 6 complexes [4, 5]. The first cyclin/Cdk complex in the G1 phase is activated by growth factors and consists of cyclin D and Cdk4 or 6 [6]. This results in retinoblastoma phosphorylation and the subsequent activation of cyclin E/Cdk2. Furthermore, activated $p42/44^{MAPK}$ was shown to induce cyclin D expression and down-regulation of $p27^{Kip}$ [7, 8]. In addition, sustained activation of $p42/44^{MAPK}$ is required to pass the restriction point, whereas inhibition of $p42/44^{MAPK}$ blocks DNA synthesis and proliferation [9–11]. Furthermore, $p42/44^{MAPK}$ overexpression has been observed in human breast cancer cells [12], showing the importance of $p42/44^{MAPK}$ activity in cell proliferation.

Recently, cytosolic phospholipase A_2 ($cPLA_2$) activity was demonstrated to be cell cycle dependent and, furthermore, $cPLA_2$ phosphorylation in these periods was mediated by $p42/44^{MAPK}$ [13]. $cPLA_2$ preferentially releases arachidonic acid from membrane phospholipids. The released arachidonic acid can be metabolised by cyctochrome p450s, cyclooxygenases (COXs) or by

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lipoxygenases (LOs) to produce eicosanoids. Arachidonic acid and/or its metabolites appear to play an important role in growth-dependent signalling pathways and are involved in mitogenic signalling, cell migration and cytotoxicity [14–16]. Furthermore, most tumour cells produce elevated levels of eicosanoids that result in induced growth and invasiveness of the tumours [17]. Accordingly, in oncogenic ras-transformed lung cancer cells, $cPLA_2$ expression was found to be constitutively high [18].

To gain more insight into the mode of action of $cPLA_2$ in cell proliferation, we studied the role of $cPLA_2$ activity in cell cycle progression. We showed that the activity of $cPLA_2$ in G1 phase was necessary for progression into S phase in contrast to Ca^{2+}-independent PLA_2 ($iPLA_2$) or secreted PLA_2 ($sPLA_2$). $cPLA_2$ inhibition for 24 h resulted in a comparable cell number of arachidonyl trifluoromethylketone (ATK)-treated versus untreated cells, suggesting redundancy of the different PLA_2 enzymes. By using inhibitors, we assessed the involvement of LO but not COX, in cell cycle progression into S phase. Moreover, LO inhibition in early G1 resulted in G1 phase arrest. DNA synthesis and S phase progression were blocked when LO was inhibited in early S phase, demonstrating that LO metabolites are required for S phase progression.

Materials and methods

Materials

Tissue culture nutrients, Dulbecco's modified Eagle's medium supplemented with 25 mM HEPES (DMEM-HEPES) and fetal bovine serum (FBS) were purchased from Gibco BRL (UK). Methyl-[^{3}H]-thymidine (2 mCi/mmol) was obtained from Amersham (Arlington Heights, Ill). ATK, bromoenol lactone (BEL), 5(S)-HpETE and 12(S)-HpETE were acquired from Cayman Chemical (Ann Arbor, Mich.). Manoalide was from Biomol (Plymouth Meeting, Mass.) and NS-398 and caffeic acid were obtained from Calbiochem (La Jolla, Calif.). 4-Bromophenacyl bromide (4-BPB), indomethacin, nordihydroguaiaretic acid (NDGA) and arachidonic acid were purchased from Sigma-Aldrich (Steinheim, Germany). All other chemicals were either from Sigma or Merck (Darmstadt, Germany).

Cell culture and cell synchronisation

Neuroblastoma (N2A) cells were grown in DMEM-HEPES supplemented with 7.5% FBS. Cells were maintained at 37 °C in a humidified atmosphere. One day prior to shake off, cells were cultured at 5×10^6 cells per 175-cm^2 flask. Each hour, flasks, containing asynchronous cells, were shaken for 1 min at 37 °C to obtain mitotic cells as described previously [19].

[^{3}H]-thymidine incorporation

Mitotic cells were plated in 24-well plates at a density of 3×10^4 cells per well in DMEM-HEPES containing 7.5% FBS and 1 µCi [^{3}H]-thymidine/well. At the indicated times, the cells were washed twice with phosphate-buffered saline (PBS), whereafter they were dissolved in 0.1 M NaOH and the incorporated [^{3}H]-thymidine was counted in a scintillation counter (LS 6000 SE; Beckman Instruments, Fullerton, Calif.). In other experiments, as indicated, [^{3}H]-thymidine incorporation was started 3 h ahead of the actual experiment as a control. Then, at the indicated times after mitosis, half of the cells were incubated with ATK (10 µM) to inhibit $cPLA_2$ or with NDGA (10 µM) to inhibit lipoxygenase. After 30 min, [^{3}H]-thymidine was added to both control and ATK- or NDGA-treated cells. Fifteen minutes later, the cells were washed twice with PBS, then washed with 10% trichloroacetic acid, washed again twice with PBS, and then dissolved in 0.1 M NaOH; [^{3}H]-thymidine incorporation was then measured.

Western blot analysis

Mitotic cells were replated and at the indicated times after mitosis, the medium was removed and the cells were washed twice with ice-cold PBS. Subsequently, the cells were scraped in homogenisation buffer (50 mM Hepes/NaOH pH 7.4, 0.25 M sucrose, 50 mM NaF, 250 µM Na_3VO_4, 1 mM EGTA, 10 µM leupeptin, 1 µM pepstatin and 1 mM PMSF). Cells were homogenised by 15 strokes through a 26G needle and sonicated for 3×10 s. Proteins of 1×10^5 cells per time point were separated on 10% SDS-PAGE and transferred to polyvinylidene difluoride membrane by semi-dry blotting using a BioRad trans-blot SD. The membrane was blocked for 1 h at room temperature with 2% milk powder in PBST [PBS containing 0.1% (v/v) Tween-20] following primary antibody incubations overnight at 4 °C in 0.2% milk powder in PBST. Cyclin A was detected with a monoclonal antibody (Calbiochem. Cambridge, UK) at a concentration of 2.5 µg/ml. Subsequently, the membrane was washed and primary antibodies were detected with rabbit anti-mouse IgG conjugated to horseradish peroxidase and the bands were visualised with enhanced chemiluminescence (NEN Life Science Products, Boston, Mass.).

Results

$cPLA_2$ activity in G1 phase is required for cell cycle progression

We previously demonstrated that $cPLA_2$ activity is cell cycle dependent in neuroblastoma (N2A) and Chinese hamster ovary (CHO) cells [13], being high at mitosis and thereafter decreasing in early G1. A small increase in activity was measured during mid/late G1, and a strong in-

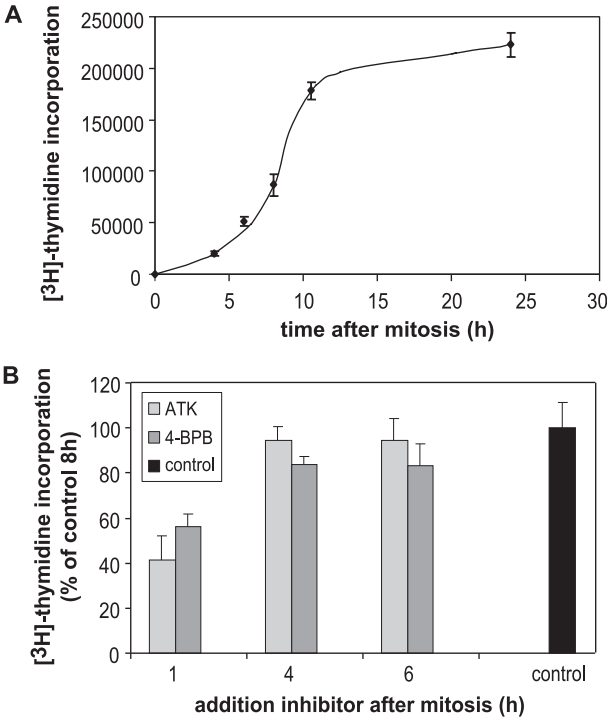

Figure 1. [^{3}H]-thymidine incorporation (*A*) and the effect of ATK and 4-BPB on DNA synthesis (*B*) in N2A cells, synchronised by mitotic shake off. (*A*) N2A cells were synchronised using the mitotic shake-off method. [^{3}H]-thymidine (1 µCi/well) was added to the mitotic cells and at the indicated times after mitosis the incorporated radiolabel was quantified using a liquid scintillation counter. (*B*) ATK (10 µM) and 4-BPB (10 µM) were added to synchronous cells at 1, 4 and 6 h after mitosis and left to incubate until 8 h after mitosis, whereafter the incorporated [^{3}H]-thymidine into the DNA was measured. Data are means ± SD (n=3).

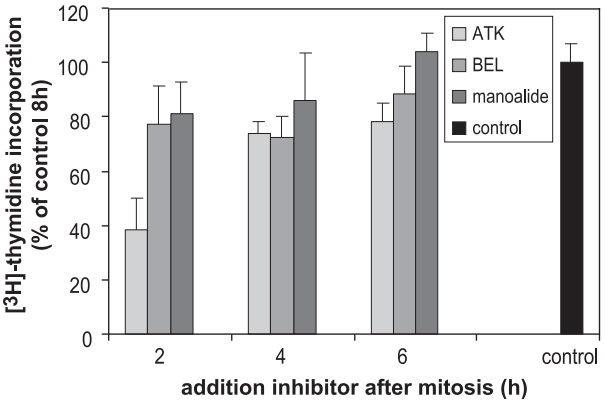

Figure 2. Effects of PLA_2 inhibitors on cell cycle progression into S phase in N2A cells. Synchronous N2A cells were left untreated, or incubated with ATK (10 µM), BEL (2.5 µM) or manoalide (0.1 µM) from 2, 4 and 6 h after mitosis. [^{3}H]-thymidine incorporation was measured at 8 h after mitosis. Data are the means ± SD (n=3).

crease was measured following the G1/S transition. To investigate whether $cPLA_2$ activity is required for cell cycle progression to S phase, the activity of $cPLA_2$ was inhibited at different time points in G1 phase in N2A cells, which were synchronised by using the mitotic shake-off method [19]. Therefore, a non-specific PLA_2 (4-BPB) [20] and a potent reversible $cPLA_2$ (ATK) inhibitor [21, 22] were used. Synchronous cells were either left untreated or were incubated with ATK (10 µM) or 4-BPB (10 µM) from 1, 4 and 6 h after mitosis. At 8 h, at which most cells have entered S phase (fig. 1A), DNA synthesis was determined by measuring [^{3}H]-thymidine incorporation of the cells as described in Materials and methods. As shown in figure 1B, [^{3}H]-thymidine incorporation was reduced approximately 60% compared to control cells if ATK was added 1 h after mitosis, while no reduction was measured when ATK was added at later time points. Apart from the effect on DNA synthesis, inhibition of $cPLA_2$ activity did not influence the total number of cells, indicating that the inhibition of DNA synthesis was not due to cytotoxic effects of ATK (data not shown). In addition, a similar pattern of [^{3}H]-thymidine incorporation was obtained in synchronous N2A cells treated with 4-BPB. These results indicate the involvement of $cPLA_2$ activity in G1 for cell cycle progression.

However, ATK has also been reported to be able to inhibit the Ca^{2+}-independent PLA_2 ($iPLA_2$) in macrophages, although at higher concentrations [23]. We therefore investigated whether the reduction in [^{3}H]-thymidine incorporation was solely due to $cPLA_2$ or to other PLA_2 isoforms as well. To discriminate between $cPLA_2$ and $iPLA_2$, BEL was used, which is a potent irreversible inhibitor of $iPLA_2$, but not of $cPLA_2$ [23, 24]. The involvement of $sPLA_2$ was investigated using manoalide [25]. ATK, BEL (2.5 µM) or manoalide (0.1 µM) were added to synchronously growing cells at 2, 4 and 6 h after mitosis, or the cells were left untreated. Addition of ATK at 2 h after mitosis resulted in a reduced [^{3}H]-thymidine incorporation of approximately 60% compared to control cells (fig. 2). A small reduction was measured when ATK was added at 4 h and no effect was measured when added at 6 h after mitosis. Due to variations in the length of the G1 phase [26], the small inhibition in [^{3}H]-thymidine incorporation of ATK added at 4 h was absent in figure 1. The [^{3}H]-thymidine incorporation measured at 8 h was only slightly reduced when BEL was added at 2 and 4 h after mitosis compared to control cells. No effect was observed in cells incubated with BEL from 6 h after mitosis. Manoalide only marginally affected the [^{3}H]-thymidine incorporation of the cells when added at 2, but not at 4 or 6 h compared to control cells. These results show again the involvement of $cPLA_2$ activity in G1 for cell cycle progression into S phase. Furthermore, they indicate that $sPLA_2$ is not and $iPLA_2$ is at best only marginally involved in cell cycle progression to S phase.

Not clear, however is whether $cPLA_2$ inhibition results in cell cycle arrest or whether it is just delayed. Therefore,

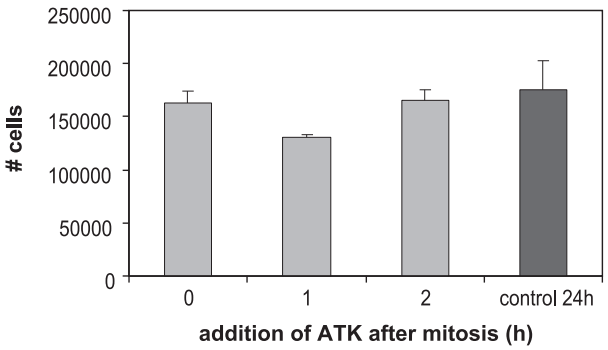

Figure 3. Long-term cPLA$_2$ inhibition does not result in cell cycle arrest. To synchronous N2A cells, ATK (10 µM) was added at 0, 1 and 2 h after mitosis and cells were left to incubate until 24 h after mitosis. The total cell number was then counted. Data are means ± SD (n=2).

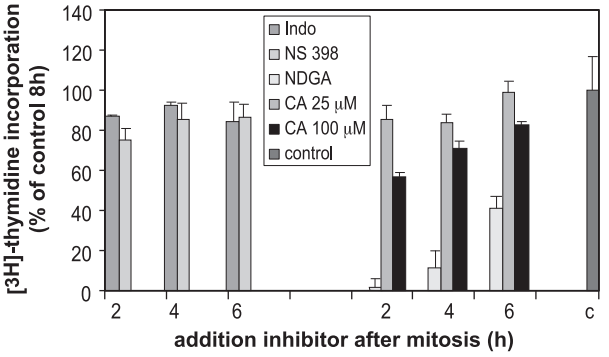

Figure 4. Effects of COX and LO inhibitors on cell cycle progression. Synchronous N2A cells were left untreated, or incubated with the COX inhibitors Indo (10 µM) and NS 398 (10 µM), and the LO inhibitors NDGA (10 µM) and caffeic acid (CA) (25 and 100 µM) from 2, 4 and 6 h after mitosis. The amount of [^{3}H]-thymidine incorporated into the DNA was measured at 8 h after mitosis. Data are means ± SD (n=3).

cell count experiments were performed in which cells were left untreated, or to which ATK was added at 0, 1 or 2 h after mitosis. At 24 h after mitosis, the total number of cells in ATK-treated cells was similar to untreated cells (fig. 3) and, moreover, was increased compared to the total number of cells present at 8 h, showing that cells have completed their cell cycle. In addition, ATK was still functional, since cPLA$_2$ activity in synchronous cells treated for 8 or 24 h with ATK was inhibited compared to untreated cells (data not shown).

Collectively, these results show the involvement of cPLA$_2$, but most likely not of iPLA$_2$ or sPLA$_2$, in cell cycle progression, since 4-BPB did not reduce DNA synthesis any further than ATK, whereas inhibitors for iPLA$_2$ and sPLA$_2$ displayed only a small effect. In addition, cPLA$_2$ inhibition in early G1 results in a temporal inhibition of cell cycle progression.

LO, but not COX, is involved in cell cycle progression

The arachidonic acid released by cPLA$_2$ can be further metabolised by cyclooxygenases or lipoxygenases into a large family of eicosanoids that have been implicated, amongst others, in mitogenic signalling, cytotoxicity and cancers [27–29]. To examine the role of COX and LO on cell cycle progression, we evaluated the effects of COX and LO inhibitors on DNA synthesis. Indomethacin (Indo, 10 µM), a non-selective COX inhibitor [30], and NS 398 (10 µM), a selective COX-2 inhibitor [31], were added at 2, 4 and 6 h after mitosis and [^{3}H]-thymidine incorporation was measured at 8 h after mitosis. These inhibitors did not significantly reduce the [^{3}H]-thymidine incorporation relative to control cells (fig. 4), demonstrating that neither COX-1 nor COX-2 plays a role in G1/S phase progression. On the other hand, complete inhibition of [^{3}H]-thymidine incorporation was measured at 8 h when LO was inhibited from 2 or 4 h after mitosis with 10 µM NDGA, a potent inhibitor of LOs [32, 33]. An inhibition of approximately 60% was still observed upon addition of NDGA at 6 h after mitosis. These inhibitory effects of NDGA were not due to cytotoxic effects, because the total number of cells was similar to untreated cells (data not shown). In contrast, 25 µM caffeic acid (CA), which predominantly inhibits 5- and 12-LO but at higher IC$_{50}$ values than the common LO inhibitor NDGA [34, 35], was not effective in inhibiting [^{3}H]-thymidine incorporation. A significant inhibition of about 40 and 30%, respectively, was measured when 100 µM CA was added only at 2 and 4 h. These results show that LO is involved in cell cycle progression to S phase and that inhibition of LO in early G1 may result in cell cycle arrest.

We further investigated whether LO inhibition in G1 results in G1 phase arrest by determining cyclin A expression, since cyclin A is expressed in late G1 prior to DNA synthesis [36]. The Western blot in figure 5A is of a representative experiment showing that during the ongoing cell cycle, cyclin A is expressed at a low level at 4 h after mitosis, which represents mid/late G1 (fig. 1A), while at 6 h, in early S phase, and at 8 h, in S phase, a significant increase in cyclin A is observed. These results were evaluated by quantification of the bands and presented as a percentage of cyclin A expressed at 8 h after mitosis (fig. 5B). Incubating synchronous N2A cells from 0 or 2 h after mitosis with NDGA until 8 h, reduced cyclin A expression compared to control cells at 8 h (fig. 6A). Densitometrical evaluations of the blots showed that cyclin A expression of NDGA-treated cells at 0 or 2 h did not reach the expression present at 8 h after mitosis (fig. 6B), but remained at the level present at 4 h after mitosis of normal cycling cells (fig. 5B), thus demonstrating that the cells were arrested in G1. Similarly, treating

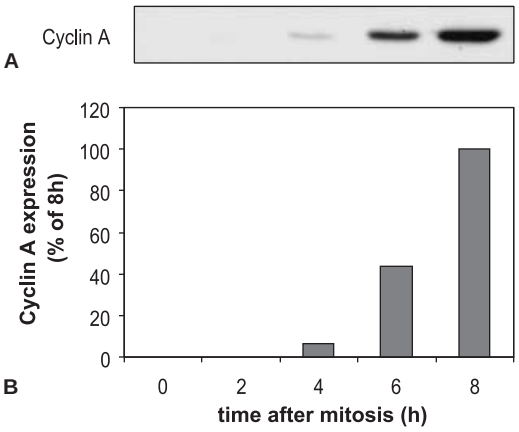

Figure 5. Expression of cyclin A during the ongoing cell cycle. Cells were synchronised via mitotic shake off, replated and harvested at the indicated times after mitosis as described in Materials and methods. Cell lysates of 1×10^5 cells were separated on SDS-PAGE, whereafter cyclin A was analysed on Western blot (*A*) and its expression quantified (*B*). Results shown are representative of three different experiments.

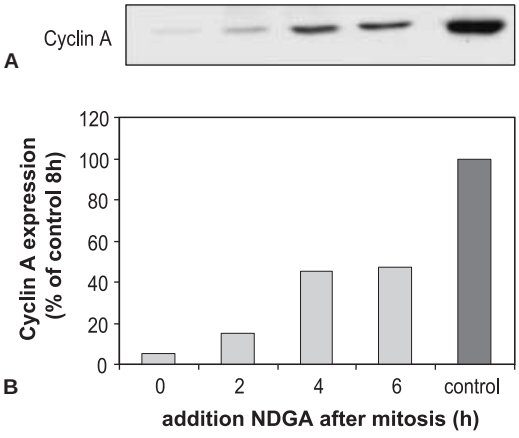

Figure 6. LO inhibition results in cell cycle arrest. Synchronised N2A cells were left untreated or were incubated with NDGA (10 µM) from 0, 2, 4 and 6 h after mitosis and harvested at 8 h after mitosis. Thereafter, 1×10^5 cells were used for electrophoresis and cyclin A was analysed on western blot (*A*). Cyclin A expression of cells incubated with NDGA was quantified as percentage of untreated cells at 8 h after mitosis (*B*). Results shown are representative of three different experiments.

cells from 4 or 6 h after mitosis with NDGA and analysing cyclin A at 8 h did not result in full expression compared to control cells at 8 h (fig. 6). Cyclin A expression remained at the level present at 6 h of untreated cycling cells, as can also be surmised from the densitometrical evaluations (fig. 6B versus 5B). These data demonstrate that LO inhibition in early G1 results in G1 phase arrest, and that LO may also be involved in S phase progression.

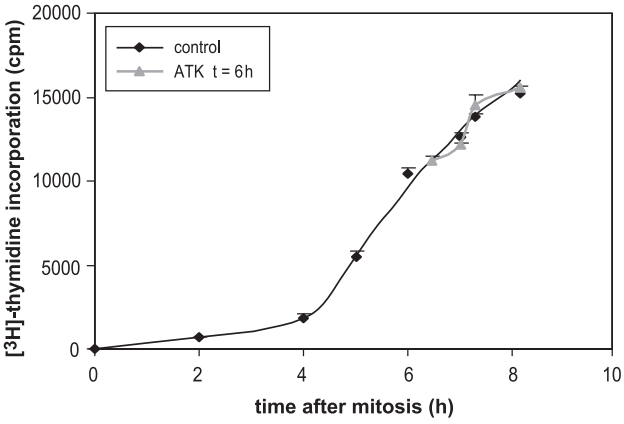

Figure 7. $cPLA_2$ activity is not necessary for S phase progression. ATK (10 µM) was added to half of the cells at 6 h (triangles) after mitosis. After 30 min, [^{3}H]-thymidine (1 µCi/well) was added to both untreated and ATK-treated cells, and 15 min later, [^{3}H]-thymidine incorporation was measured at the indicated times after mitosis as described in Materials and methods. Data are represented as means ± SD (n=3).

Effect of $cPLA_2$ and LO inhibition on S phase progression

Since $cPLA_2$ activity during the cell cycle is high in S phase, we examined the possible role of $cPLA_2$ during the S phase. However, from figures 1 and 2, no large inhibitory effect on [^{3}H]-thymidine incorporation was measured at 8 h when ATK was added at 4 or 6 h after mitosis, which is in late G1 or early S phase. To investigate the effect of $cPLA_2$ inhibition on S phase progression we needed to be sure that the cells were in S phase. Therefore, each hour after mitosis, [^{3}H]-thymidine incorporation was measured as a control, and at 6 h after mitosis, ATK was added, in S phase, to inhibit $cPLA_2$ activity. No inhibition in [^{3}H]-thymidine incorporation was observed (fig. 7, triangles), as compared to control cells (fig. 7, diamonds), showing that $cPLA_2$ activity in S phase in not necessary for DNA synthesis.

Next, we investigated whether LO products play a role in S phase progression. NDGA was added at 3 and 5.5 h after mitosis, and [^{3}H]-thymidine incorporation measurements were started for different time periods. As shown in figure 8, 3 h after mitosis is still in the G1 phase (triangles) of the cell cycle, while at 5.5 h, the cells are starting to progress through S phase (squares). When inhibiting lipoxygenase at 3 h, full inhibition in the [^{3}H]-thymidine incorporation was observed, showing that the cells were no longer able to progress into S phase. These data are in agreement with the previous results of figure 6, in that inhibition of LO in early G1 resulted in G1 phase arrest. Furthermore, the addition of NDGA at 5.5 h completely inhibited DNA synthesis, that was also confirmed by cyclin A expression (fig. 6), thus demonstrating the importance of LO products in the progression through S phase.

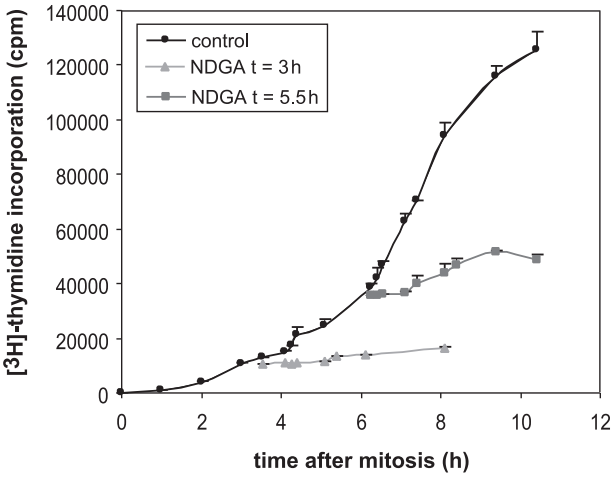

Figure 8. LO is required for S phase progression. At 3 h (triangles) and 5.5 h (squares) after mitosis, NDGA (10 µM) was added to the cells. After 30 min, [^{3}H]-thymidine (1 µCi/well) was added to both untreated and NDGA-treated cells, and 15 min later, [^{3}H]-thymidine incorporation was measured at the indicated times after mitosis as described in Materials and methods. Data are represented as means ± SD (n=3).

Discussion

Although previous studies demonstrated that PLA$_2$ inhibition reduced cell proliferation [37–39], the present study shows that cPLA$_2$ activity at a distinct period in the cell cycle is involved in cell cycle progression. By using [^{3}H]-thymidine incorporation as a marker for DNA synthesis and progression from G1 to S phase, inhibition of cPLA$_2$ in early G1, using ATK, resulted in reduced DNA synthesis. This reduction was measured only when cPLA$_2$ was inhibited until 2–3 h after mitosis. Interestingly, after 2–3 h, an increase in cPLA$_2$ activity in the G1 phase occurs [13]. Inhibition of cPLA$_2$ at 4 or at 6 h, in G1 or S phase, respectively, did not reduce DNA synthesis. In addition, similar results were obtained with the non-specific PLA$_2$ inhibitor, 4-BPB. Additionally, cells in which cPLA$_2$ was inhibited by ATK from 0 or 2 h after mitosis showed a lower expression of cyclin A compared to control cells at 8 h after mitosis (data not shown). No difference in cyclin A expression was measured when ATK was added at 4 or 6 h after mitosis. This is in agreement with the [^{3}H]-thymidine incorporations measured. Manoalide only slightly reduced DNA synthesis at 2 h but not at 4 or 6 h, indicating that sPLA$_2$ does not play a role in cell cycle progression to S phase. However, whether manoalide inhibits all the known sPLA$_2$ types is not established. Although we could not detect sPLA$_2$ IIA and V in these cells, we cannot completely rule out the possible involvement of sPLA$_2$ in cell cycle progression, since it might also be (inducibly) expressed in another phase of the cell cycle. Indeed, sPLA$_2$ is present in other neuroblastoma cell types [40]. iPLA$_2$ inhibition by BEL at 2 and 4 h but not at 6 h resulted in a small reduction in [^{3}H]-thymidine incorporation. Although iPLA$_2$ is present in these cells (data not shown), this reduction might be the result of phosphatidate phosphohydrolase inhibition [41]. These data show that in N2A cells, the activity of cPLA$_2$ in mid/late G1 phase is important for cell cycle progression into S phase, while cPLA$_2$ activity in S phase is not required for DNA synthesis.

Because cPLA$_2$ activity was involved in G1/S phase progression, we evaluated whether this inhibition resulted in cell cycle arrest. The total cell number of synchronised N2A cells after a 24 h treatment with ATK was comparable to untreated cells and, moreover, was increased to the total number of cells present at 8 h. This shows that the cells have completed their cell cycle, and may result in an increased doubling time of the ATK-treated cells. Proliferation of asynchronous human coronary artery vascular smooth muscle cells growing to confluency was previously shown to be inhibited by ATK, but no phase-specific arrest of the cell cycle was observed [42]. We also found that although cPLA$_2$ activity is required for G1/S phase progression, the cells do complete their cell cycle, while cPLA$_2$ activity was still inhibited after 24 h treatment with ATK. However, cPLA$_2$ function is also possibly taken over by other enzymes, like the sPLA$_2$s or iPLA$_2$, which then become active to finally generate the arachidonic acid necessary for progression through the cell cycle. Redundancy of PLA$_2$ enzymes has been observed in P388D$_1$ macrophages in which a transient accumulation of arachidonic acid produced by cPLA$_2$ resulted in phospholipid hydrolysis which was probably mediated by activated sPLA$_2$ [43]. iPLA$_2$ has also recently been shown capable of releasing arachidonic acid in macrophases and urothelial carcinoma cells [44, 45].

The mechanism by which cPLA$_2$ influences cell cycle progression remains to be determined. COX inhibitors had no effect on cell cycle progression into S phase, indicating that the cPLA$_2$-dependent progression is not mediated by arachidonic acid metabolites converted by COX However, our findings demonstrate that LO is involved in cell cycle progression. The LO inhibitors, CA and NDGA, partially and completely, respectively, inhibited DNA synthesis when added in early G1, which was not due to cytotoxic effects of the inhibitors (data not shown). Additionally, cells in which LO was inhibited from 3 h after mitosis did not synthesise DNA (fig. 8). This was also confirmed by the low expression of cyclin A in cells incubated with NDGA from 0 or 2 h after mitosis compared to control cells. These data show an arrest in the G1 phase of LO-inhibited cells. In line with this are the results of Korystov et al. [39] who demonstrated a suppression of proliferation of lympholeukaemic cells with NDGA, also by blocking the G1/S transition. Additionally, LOs are present in neuroblastoma cells [46] and are involved in cell growth [47, 48]. However, the mechanism

by which NDGA blocks the G1/S transition is still unknown. Readdition of arachidonic acid or the LO metabolites 5(S)-, 12(S)- or 15(S)-HpETE, or 15(S)-HETE at various times and concentrations after mitosis could not overcome the inhibitory effect of ATK or NDGA. NDGA might inhibit a protein through the inhibition of LO, which is required for S phase entry. LO has been reported to be involved in mediating arachidonic acid-induced $p42/44^{MAPK}$ activation [49, 50]. However, LO inhibition by NDGA did not affect $p42/44^{MAPK}$ phosphorylation in N2A cells (data not shown), indicating that LO exerts its effects downstream of $p42/44^{MAPK}$.

LO inhibition in early S phase also blocked DNA synthesis. Furthermore, cells in which LO was inhibited from 4 or 6 h after mitosis were arrested in early S phase as judged by cyclin A expression. Taken together, $cPLA_2$ activity is involved in cell cycle progression from G1 into S phase, while $cPLA_2$ activity in S phase is not involved in DNA synthesis. Furthermore, LO is required for both G1/S and S phase progression.

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