Cyclic adenosine 3',5'-monophosphate (cAMP) agonist prevents latency reversal

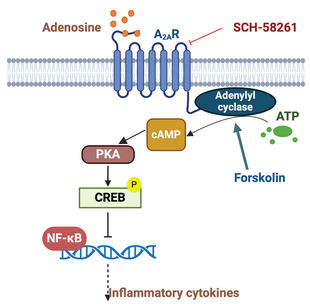
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BACKGROUND

- cAMP is a secondary messenger involved in diverse cellular and metabolic processes.
- The levels of intracellular cAMP are critical in determining the balance between activation and suppression of multiple immunological processes.
- Some *ex vivo* and *in vitro* studies have shown that cAMP levels are elevated in the setting of HIV infection with T cells from PLWH displaying twice as much cAMP as compared to PLWoH.
- In our previous studies, we have identified the adenosine signaling pathway, which leads to production of cAMP, as a determinant of HIV latency[1].
- The role of cAMP signaling in HIV latency is still unknown. Here, we determined the impact of cAMP modulation on HIV latency in primary CD4⁺ T cells and cell lines using forskolin, a cAMP agonist.



OBJECTIVE

To determine the impact of cAMP modulation on HIV latency.

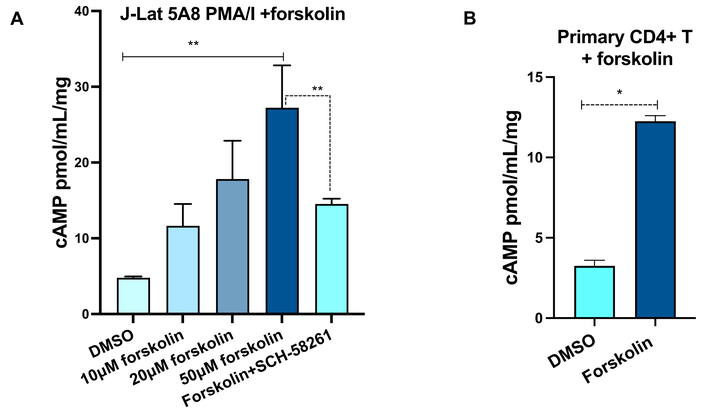
METHODS

- Intracellular cAMP was quantified using a colorimetric assay-based ELISA.
- J-Lat CD4+ T cells and HC69.5 microglia harboring GFP reporter HIV constructs were pretreated with various doses of forskolin followed by phytohaemagglutinin (PHA) or PMA/ionomycin treatment.
- CD4+ T cells from PLWH on ART were treated with forskolin followed by CD3/CD28 stimulation.
- Viral reactivation was measured by flow cytometry (the frequency of GFP-positive cells or qPCR measurement of *tat-rev* transcripts).
- The effect of cAMP agonism on establishment of HIV latency was studied in an *ex vivo* infection model using the Duo Fluo II dual reporter virus enabling isolation and enumeration of latently- and productively-infected cells by flow cytometry.

cAMP agonism blocks NF-κB-mediated reversal of HIV latency. cAMP agonists can be explored in block-and-lock HIV cure strategies.

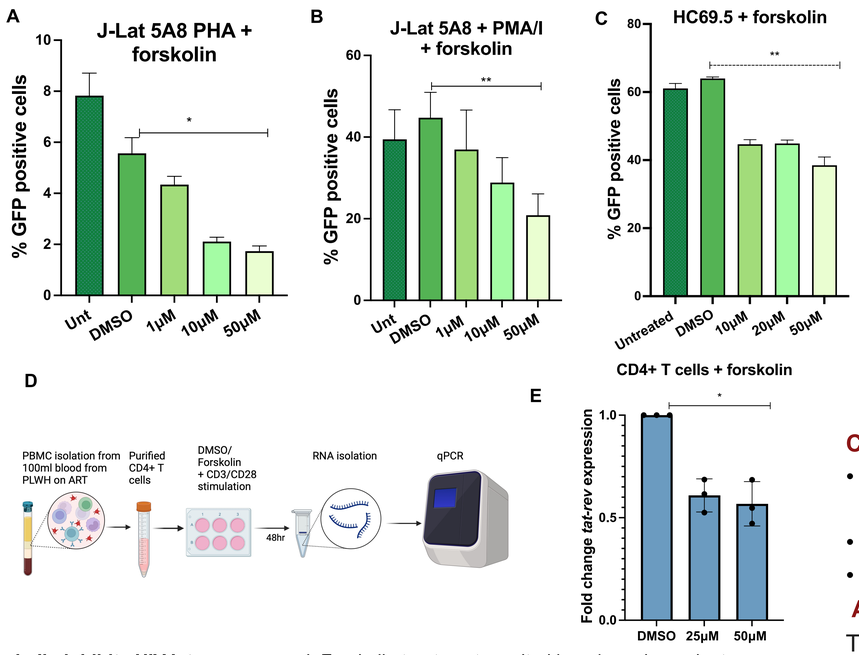
RESULTS

1) Forskolin elevates cAMP levels in J-Lat and primary CD4+T cells in an adenosine 2A receptor-dependent manner



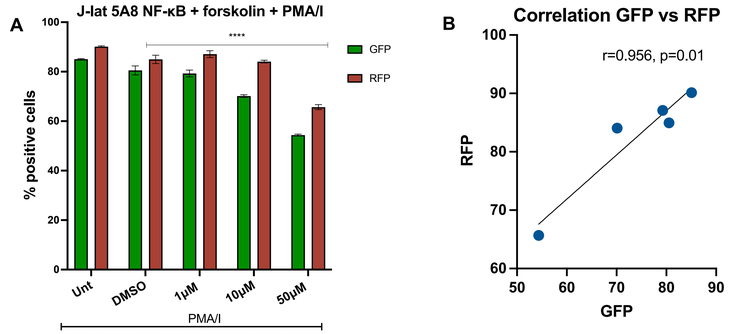
Forskolin elevates cAMP levels. J-lat 5A8 and primary CD4+ T cells were treated with forskolin for 24 hours. ELISA was performed to measure cAMP levels in cell culture supernatant. Forskolin treatment resulted in a significant increase in cAMP levels in and **A**.J-lat 5A8 (p=0.0071) and **B.**CD4+ T (p=0.02) cells. SCH-58261, an $A_{2A}R$ antagonist, reversed forskolin-mediated elevation of cAMP in J-Lat 5A8 cells. P values were calculates using a Student's T test or Mann-Whitney test.

2) Increase in cAMP levels blocks latency reversal in HIV-infected cells



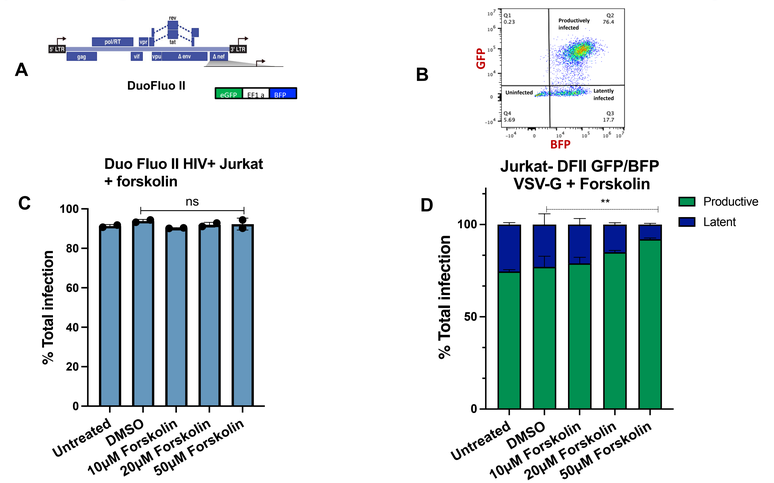
Forskolin inhibits HIV latency reversal. Forskolin treatment resulted in a dose-dependent inhibition of HIV latency reversal in (**A**. and **B.**)J-Lat 5A8 cells (PHA; p=0.0286, PMA/I=0.0011) and dose-dependent inhibition of HIV expression in **C.**HC69.5 cells (p=0.0046). **D**. Schematic describing forskolin treatment. **E.** Fold change in *tat-rev* transcript levels following forskolin treatment in CD4+ T cells from PLWH on ART. Forskolin treatment resulted in a significant decrease in latency reversal in PLWH (p=0.03).

3) Forskolin inhibits NF-κB-mediated HIV latency reversal



Forskolin inhibits NF-κB activation. J-Lat 5A8.NF-κB.RFP cells containing a NF-kB-driven RFP reporter were used to assess the effect of forskolin on the NF-κB pathway. **A**.Forskolin resulted in a decrease in the frequency of RFP-positive cells (p<0.0001). **B.** Correlation plot of GFP vs RFP showing suppression following forskolin treatment.

4) cAMP agonism inhibits the establishment of HIV latency



Forskolin reduces the frequency of HIV latently-infected cells in an *ex vivo* infection model. The effect of forskolin on establishment of latency was studied in Jurkat cells using the Duo Fluo II dual reporter virus construct**. A**. Genome map of dual reporter virus B. Fow cytometry plot. C and D. Forskolin reduced the ration of latently infected cells to productively infected cells(p=0.0036) with no change on total infection.

CONCLUSIONS

- cAMP agonism results in inhibition of HIV latency reversal and viral expression in CD4+ T cells and microglia.
- cAMP agonism prevents the initial establishment of HIV latency.
- cAMP modulators can be explored in HIV cure strategies.

ADDITIONAL KEY INFORMATION

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Reference

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