Nicotine (tobacco) addiction is a serious health issue and a leading cause of death. However, the mechanism of nicotine addiction is not fully understood. Previous reports suggest that nicotine induced activation of orexin neurons in the lateral hypothalamus (LH) region of the brain may play an important role in nicotine addiction. Adenosine is implicated to inhibit orexin neurons. Does nicotine reduce adenosine release in the LH and thereby causing disinhibition/activation of orexin neurons? To address this issue, adult male Sprague-Dawley rats (N=4) were surgically implanted with guide cannula in the LH region followed by insertion of microdialysis probe. The inlet and the outlet of the probes were connected to the microdialysis pump and the collection vial, via micro-tubing. Artificial cerebrospinal fluid perfusion was initiated (flow rate=0.7ul/min). The experiment was begun at the dark onset with saline administration (ip) and 6x20 min samples were collected. This was followed by nicotine (1 mg/kg; i.p.) administration and collection of 6x20 min samples. 10 ul from each sample was injected into the HPLC for adenosine measurements. On completion of the experiment, the animals were euthanized, brains removed and sectioned. Cresyl violet staining was performed on coronal sections containing LH for probe localization. Our initial results suggest that systemic administration of nicotine caused a reduction in extracellular adenosine in the LH. Based on our results, we conclude that nicotine inhibits adenosine release in the LH which may be responsible for disinhibition/activation of LH orexin neurons.