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BRAIN RESEARCH

Research Report

Cellular thiol pools are responsible for sequestration of cytotoxic reactive aldehydes: Central role of free cysteine and cysteamine

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ABSTRACT

Cellular thiol pools have been shown to be important in the regulation of the redox status of cells, providing a large antioxidant pool consisting of free thiols, thiols bound in the disulfide form and thiols bound to proteins. However, experimental studies with the thiol cysteamine and its disulfide cystamine have demonstrated dramatic cytoprotection in experimental models where antioxidants provide only minor protection. These data suggest that an alternate action of thiols is important in their cytoprotective actions. A common feature of the *in vitro* and *in vivo* models, where these thiol agents demonstrate cytoprotection, is the generation of cytotoxic aldehydes. We therefore studied the actions of cystamine, cysteamine and several reference thiol agents as cytoprotectants against cell death induced by increased "aldehyde load". We found that all the thiol agents examined provided dramatic protection against aldehyde-induced cell death in SN56 cholinergic neurons, under conditions in which acrolein induced 100% cell death. With regard to mechanism of action, the reference thiols cysteine, *N*-acetylcysteine, 2-mercaptoethanesulfonic acid, mercapto-propionyglycine, and cysteamine can directly sequester aldehydes. In addition, these thiols were all found to augment intracellular cysteine levels via disulfide interchange reactions. Cysteamine and cystamine also augmented basal intracellular cysteamine levels. Our data, for the first time, demonstrate the importance of intracellular thiols in sequestering toxic reactive aldehyde products of lipid peroxidation and polyamine metabolism. In addition it appears that pharmacological manipulation of intracellular thiol pools might offer a new approach in the design of neuroprotective drug candidates.

1. Introduction

The thiol agent cysteamine and its disulfide, cystamine, have been shown to be neuroprotective in a number of cell culture and animal models. *In vitro*, these include protection from glutamate toxicity in rat primary astroglial cultures (Ientile et al., 2003), from 3-nitropropionic acid toxicity in Huntington's disease knock-in murine striatal cells (Mao et al., 2006), and from toxicity in cellular models of polyglutamine aggregation (Fox et al., 2004). *In vivo*, neuroprotection has been demonstrated in the R6/2 murine model of Huntington's disease (Dedeoglu et al., 2002); in the YAC128 murine model

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of Huntington's disease (Pinto et al., 2005); against striatal lesions induced by parenteral injection of the mitochondrial toxin 3-nitropropionic acid (Fox et al., 2004) and in the MPTP murine model of Parkinson's disease (Tremblay et al., 2006). There is also a large historical database demonstrating cytoprotection with cysteamine and cystamine in animal models of chemically induced hepatotoxicity (Nagiel-Ostaszewski and Lau-Cam, 1990) and as radioprotectants (Stoklasova et al., 1980; Zheng et al., 1988).

These cytoprotective properties of cystamine and cysteamine are dramatic; however, the mechanism of action remains controversial. It is clear that cysteamine is most likely the active principal since cystamine is rapidly metabolized to cysteamine (Pinto et al., 2005). With this in mind, a number of molecular mechanisms have been studied. Inhibition of transglutaminases by cystamine led to its evaluation in murine models of Huntington's disease; however, while neuroprotection was demonstrated, studies of R6/2 mice expressing and not expressing transglutaminase concluded that transglutaminase inhibition is not the neuroprotective mechanism of cystamine (Bailey and Johnson, 2006). In contrast, dramatic and sustained increases in cellular cysteine and glutathione levels in human T4 lymphoblastoid cells (Jokay et al., 1998); in human neuroblastoma cells over-expressing transglutaminase (Lesort et al., 2003) and in PC12 cells transfected with huntingtin (Fox et al., 2004), suggest that augmentation of cellular thiol pools by cystamine and cysteamine may be important in their neuroprotective and cytoprotective actions. In this regard, *in vivo* studies have demonstrated that cystamine and cysteamine increase brain levels of cysteine (Fox et al., 2004; Pinto et al., 2005). Cystamine has also been shown to augment the non-protein thiol pool in bone marrow and small intestine (Stoklasova et al., 1980). While the intracellular thiol pool, including cysteine and

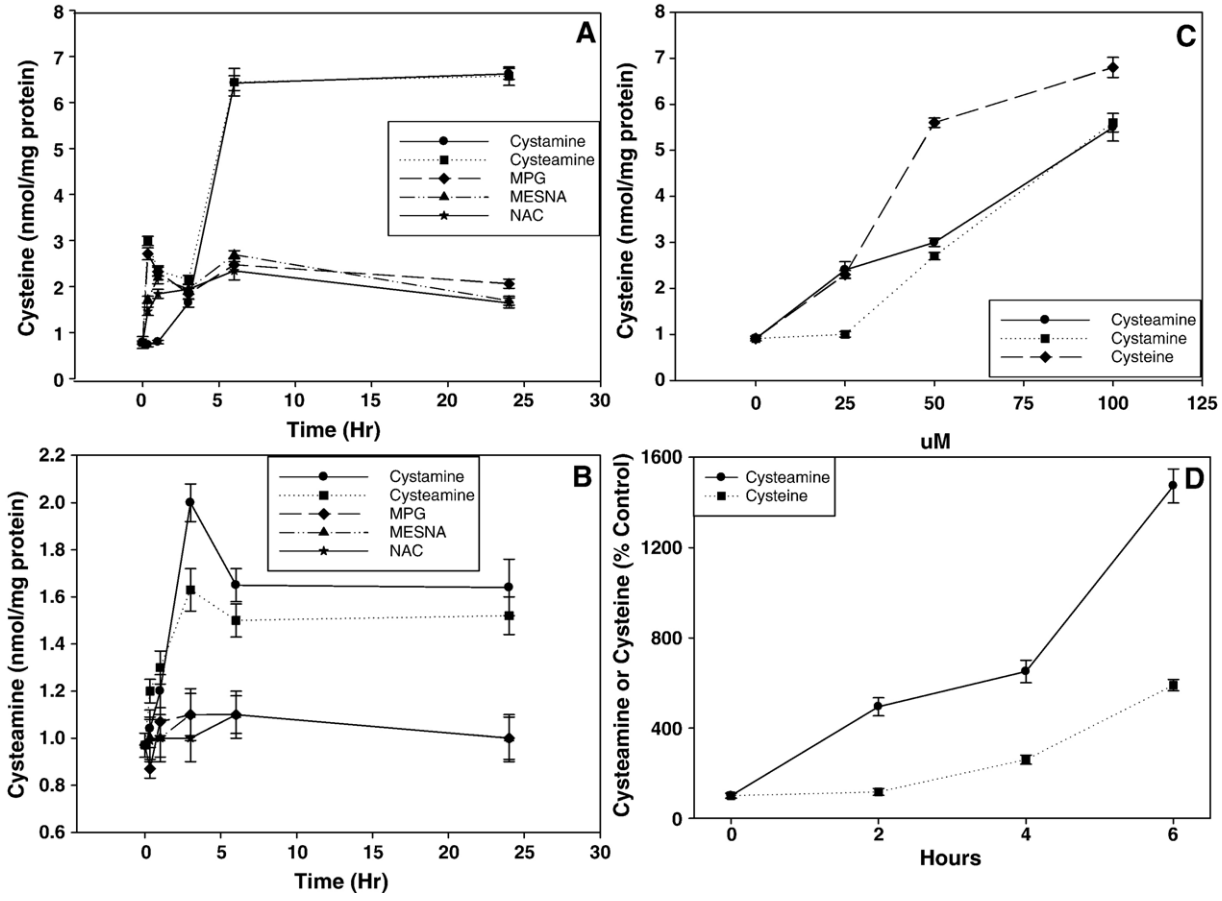

Fig. 1 – Time course for increases in cellular cysteine (A) and cysteamine (B) levels in SN56 cholinergic neurons incubated with 100 μM cystamine, cysteamine, mercaptopropionylglycine (MPG), 2-mercaptoethanesulfonic acid (MESNA), or *N*-acetylcysteine (NAC) for 20 min to 24 h. These increases in cysteine were concentration dependent after 6-h incubations with 25 to 100 μM cystamine, cysteamine or cysteine (C). The cysteamine precursor, pantethine (300 μM) also resulted in rapid increases in cellular cysteamine levels which in turn resulted in delayed increases in cellular cysteine levels (D). Data are presented as mean ± SEM (n=6 wells). *Statistics*: (A) All cysteine levels were significantly greater than controls (p<0.05) except with cystamine treatment at 0.5 and 1 h. (B) Cysteamine levels were significantly greater than controls (p<0.05) for cysteamine treatment starting at 0.5 h and starting at 1 h for cystamine treatment. (C) Cysteine levels were significantly greater than controls (p<0.05) at all points except for cystamine at 25 μM. (D) Cysteamine levels were significantly greater than controls (p<0.05) at all points while cysteine levels were significantly increased starting at 4 h.