Astrocyte Volume Regulation and ATP and Phosphocreatine Concentrations After Exposure to Salicylate, Ammonium, and Fatty Acids

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Cellular volume regulation following swelling in hypo-osmotic phosphate-buffered saline (PBS) and ATP and phosphocreatine concentrations of cells incubated in iso-osmotic or hypo-osmotic PBS were measured in primary cultured rat cerebral astrocytes exposed for 30 min to NH₄Cl, salicylate, hexanoate, octanoate, and/or dodecanoate. These compounds have been implicated in the pathogenesis of cerebral edema in Reye's Syndrome. NH₄Cl (0.10 - 10 mM) had no effect on astrocyte volume regulation or ATP concentration. Salicylate significantly reduced ATP concentrations at 3.0 mM and 10 mM but had no effect on volume regulation. Hexanoate (10 mM and 30 mM) decreased astrocyte ATP content by over 80% while octanoate (10 mM) reduced ATP content by more than 50%. Concentrations of these fatty acids at or below 3.0 mM had no effect on ATP content. Volume regulation was inhibited by 3.0 mM hexanoate and 3.0 mM octanoate but not lower concentrations. Dodecanoate (0.1 - 3.0 mM) decreased cellular ATP content by 33-51% in iso-osmotic PBS solutions. Phosphocreatine content was reduced by exposure to salicylate or octanoate at concentrations which had no effect on ATP content. These results indicate that astrocyte energy metabolism and volume regulation may be compromised by agents associated with cerebral edema in Reye's Syndrome. Analysis of the dose-dependence of these effects suggests that inhibition of astrocyte energy metabolism is not sufficient to affect volume regulation.

KEY WORDS: hexanoate; octanoate; dodecanoate; cell volume regulation; ATP; phosphocreatine; Reye's Syndrome.

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INTRODUCTION

The pathogenesis of cerebral edema in Reye's Syndrome may result from circulating endogenous or exogenous compounds (Trauner, 1982; DeLong and Glick, 1982; Heubi et al., 1987). Increased serum levels of saturated fatty acids such as octanoate (Mamunes et al., 1975), dicarboxylic acids (Tonsgard, 1985), unsaturated fatty acids (Ogbum et al., 1982), and salicylate used during the prodromal illness (Starko et al., 1980; Waldman et al., 1982) have been associated with this process. In addition, serum ammonium levels in the acute phase of Reye’s Syndrome have been shown to be a prognostic indicator of neurological outcome (Lovejoy et al., 1974; Dezateux et al., 1986). Uric acid and fatty acids, commonly elevated in the serum of Reye's Syndrome patients (Mamunes et al., 1975; Aprille et al., 1980; Tonsgard, 1985), uncouple brain mitochondrial oxidative phosphorylation (Asimakis and Aprille, 1977; Ansevin, 1980; Parker et al., 1983; Tonsgard and Getz, 1985). Exogenous octanoic acid, unsaturated fatty acids, naturally occurring oils, and other compounds can produce blood chemistry changes and neurological manifestations similar to those observed in Reye’s Syndrome (Glasgow and Chase, 1975; Trauner and Adams, 1982; Kang et al., 1984; Sinniah et al., 1985).

For most mammalian cells, cellular volume is maintained in iso-osmotic conditions by a gradient of sodium ions across the plasma membrane which opposes the osmotic gradient caused by intracellular impermeant molecules (Macknight, 1987). In aniso-osmotic conditions, various transport systems for ions, amino acids, and other molecules may be activated to regulate cellular volume toward normal levels (MacKnight and Leaf, 1985; Eveloff and Warnock, 1987; Chamberlin and Strange, 1989). Following rapid hypo-osmolar swelling of cultured astrocytes, cell volume returns towards normal over the next 10-15 min in an energy-dependent process which can be blocked by inhibitors of the (Na,K)-dependent ATPase (Olson et al., 1986, 1989). This regulatory volume decrease (RVD) is due to an efflux of osmolytes including potassium (Kimelberg and Frangakis, 1985) and amino acids, (Pasantes-Morales and Schousboe, 1988).

Astrocytes are observed to swell in situ under a variety of pathological conditions including Reye’s Syndrome (Walker and Schenker, 1970; Partin et al., 1978). Mechanisms underlying this cell swelling have not been completely elucidated; however, the persistence of the swollen state suggests that regulatory processes responsible for RVD in hypo-osmotic conditions are not functional in this pathological state. To understand the cellular mechanisms of brain water homeostasis and the perturbation of these processes in Reye’s Syndrome, we have studied cell volume regulation of rat cerebral astrocytes from primary culture (Olson et al., 1986, 1989, 1990). In the present study we test the ability of agents which have been implicated in the pathogenesis of cerebral edema in Reye’s Syndrome to interfere with astrocyte volume regulation following hypo-osmotic swelling. Because our previous data indicated that cellular energy was necessary for volume regulation, we also determined changes in astrocyte energy state induced by these agents. Our present data suggest that medium chain-length fatty acids and salicylate, but not ammonium, may compromise astrocyte energy metabolism and/or volume regulatory functions at concentrations found in the serum of Reye’s Syndrome patients. We also find that inhibition of energy metabolism alone may not be sufficient to block astrocyte volume regulation.