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Thirst deficits in aged rats are reversed by dietary omega-3 fatty acid supplementation

Denovan P. Begg[a,b,*], Andrew J. Sinclair[b,c], Richard S. Weisinger[a]

[a] School of Psychological Science, La Trobe University, Melbourne, Victoria, Australia
[b] School of Exercise and Nutrition Sciences, Deakin University, Burwood, Victoria, Australia
[c] School of Medicine, Deakin University, Geelong, Victoria, Australia

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Abstract

During heat waves many elderly individuals die as a consequence of dehydration. This is partially due to deficits in mechanisms controlling thirst. Reduced thirst following dipsogenic stimuli is well documented in aged humans and rodents. Low in vivo long-chain omega-3 fatty acid levels, as can occur in aging, have been shown to alter body fluid and sodium homeostasis. Therefore, the effect of dietary omega-3 fatty acid supplementation on drinking responses in aged rats was examined. Omega-3 fatty acids reversed thirst deficits in aged rats following dehydration and hypertonic stimuli; angiotensin (ANG) II induced drinking was unaffected in aged rats. Plasma atrial natriuretic peptide (ANP) and arginine vasopressin (AVP) were altered with age, but not affected by diet. Aged omega-3 fatty acid deficient animals displayed increased hypothalamic cytosolic phospholipase A_2 ($cPLA_2$), cyclooxygenase (COX)-2, and prostaglandin E (PGE) synthase messenger (m)RNA expression, compared with animals that received omega-3 fatty acids. The aged low omega-3 fatty acid fed animals had significantly elevated hypothalamic PGE_2 compared with all other groups. Hypothalamic PGE_2 was negatively correlated with drinking induced by both dehydration and hypertonicity. The results indicate that PGE_2 may be the underlying mechanism of the reduced thirst observed in aging.

Keywords: Thirst; Fluid intake; Dehydration; Omega-3 polyunsaturated fatty acids; Cyclooxygenase; Prostaglandin E; Aging; Docosahexaenoic acid; Arachidonic acid

1. Introduction

During the European heat wave of 2003 there was an estimated increase of 30,000 deaths (McMichael et al., 2006). In Paris, 82% of these deaths were people older than 75 years of age (Grynszpan, 2003). In general, failure to consume fluid in response to dehydration is a major problem for the elderly (Mackenbach et al., 1997; O'Neill et al., 1997). With aging there is a reduced capacity to maintain fluid and electrolyte homeostasis under basal conditions, primarily due to decreased ability to concentrate urine (Ledingham et al., 1987). Therefore, aged individuals generally have impaired, albeit adequate, hydration (Hodgkinson et al., 2003). However, when these individuals are challenged with stimuli that would normally induce thirst (e.g., dehydration) they have a reduced behavioral fluid intake response, as has been reported in both elderly humans (Crowe et al., 1987; Farrell et al., 2008; Phillips et al., 1984, 1991) and rodent models (McKinley et al., 2006; Silver et al., 1991; Thunhorst and Johnson, 2003; Whyte et al., 2004).

The reduction in thirst with aging has been observed in response to osmotic stimuli (Phillips et al., 1991; Thunhorst and Johnson, 2003), fluid deprivation (McKinley et al., 2006; Phillips et al., 1984, 1993; Rolls and Phillips, 1990; Thunhorst and Johnson, 2003) and thermal dehydration (Whyte et al., 2004). Importantly, a reduction in fluid intake is not usually observed in basal levels of water intake. In aging rats, daily spontaneous water intakes are often greater

* Corresponding author at: Metabolic Diseases Institute, University of Cincinnati ML0503, 2170 East Galbraith Road, Cincinnati, OH 45237, USA. Tel.: +1 513 558 5866; fax: +1 513 297 0966.
E-mail address: denovan.begg@uc.edu (D.P. Begg).

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than, or not different from, younger controls (McKinley et al., 2006; Thunhorst and Johnson, 2003). Any observation of greater basal intake is probably due to an increased urine output in aging (Chun et al., 1988).

Aged rats show dysfunction of a number of systems involved in body fluid homeostasis, including reduced arginine vasopressin (AVP) secretion under conditions of dehydration compared with young adult rats (Kaysen and Myers, 1985). This results in higher urinary output, accentuating the effects of dehydration (Kobayashi et al., 1986), so is unlikely to be involved in the reduced thirst. With aging there are also elevated levels of circulating atrial natriuretic peptide (ANP), a hormone that inhibits thirst (Wu et al., 1997). This increase in ANP levels has been observed in both elderly humans (Davis et al., 1996) and rodents (McKinley et al., 2006), and has been suggested as a possible mechanism for the reduction of thirst in aging (McKinley et al., 2006). Aging also results in diminished renin-angiotensin system (RAS) activity and decreased angiotensin II receptor density in the brain (Rowland et al., 1997; Thunhorst and Johnson, 2003), indicating possible involvement of this system in the reduced thirst with aging.

There are no epidemiological data regarding heat wave deaths that have controlled for dietary fatty acid intake; however, increased death rate during heat waves, compared with seasonal norms, appears to be less severe in regions where fish is consumed in greater quantities. For example, the increase in deaths in Hokkaido, Japan during the 1999 heat wave was approximately 10% (Qiu et al., 2002), whereas in locations where fish consumption is lower, greater increases have been observed, for example Chicago 1995 (147% increase) (Whitman et al., 1997), France 2003 (130% increase) (Grynszpan, 2003), and Australia 2009 (62% increase) (D.H.S., 2009). Furthermore, in countries where fish consumption is high, excess deaths during a heat wave are more likely to occur across different age groups and in the young (e.g. Korea in 1994; Kysely and Kim, 2009). In lower fish consumption areas, death during a heat wave occurs predominantly in those aged 75 or older (Grynszpan, 2003; Whitman et al., 1997). It has previously been observed that omega-3 fatty acids affect body fluid and sodium homeostasis (Weisinger et al., 2010), and it has been reported that omega-3 fatty acid levels can become depleted with aging (Hrelia et al., 1989), indicating that they may be involved in reduced thirst in aging.

This study examined the effect of dietary omega-3 fatty acids on thirst in aged animals. Specifically, it was determined whether the thirst deficits seen in aged Brown Norway rats is due to omega-3 fatty acid deficiency such that the thirst deficits could be abolished through omega-3 fatty acid supplementation. The omega-3 fatty acid deficient ($<$ 0.05% α-linolenic acid; ALA) diet has been demonstrated to produce brain tissue with significantly reduced levels of the omega-3 fatty acids (Jayasooriya et al., 2005) and is similar in omega-3 fatty acid content to a standard chow diet. Furthermore, the underlying mechanisms involved in loss of thirst in aging were examined, including changes in fatty acid metabolites including prostanoid synthesis and levels of prostaglandin E_2 (PGE_2) in the hypothalamus.

2. Methods

2.1. Animals and diet

Male Brown Norway rats were purchased from the Animal Resource Center (Canning Vale, WA, USA), after the aged animals were imported from the National Institute of Aging (Baltimore, MD, USA). Animals were at 22–23 (n = 38) and 2–3 (n = 36) months of age upon arrival. Aged animals (at 22 months of age) were confirmed to have a diminished thirst response when compared with younger adult animals (2-month-old) using a 24-hour fluid deprivation challenge (see Supplementary Data). Following this initial challenge, animals were placed on 1 of 2 synthetic diets that were identical in all aspects, other than fat source, for 4 months. The omega-3 fatty acid deficient diet (DEF) contained 7% safflower oil. The omega-3 fatty acid supplemented diet (SUP) contained 5.5% safflower oil, 1% flaxseed oil and 0.5% fish oil (see Supplementary Data for full diet composition). Diets included vitamin E, were packed under nitrogen and kept frozen prior to use to prevent oxidation of fatty acids. Animals in the SUP groups consumed approximately 85 mg of standard fish oil per day (15.3 mg eicosapentaenoic acid [EPA] and 10.2 mg docosahexaenoic acid [DHA]); in a 70 kg human this would equate to 4 g per day of concentrated fish oil, disease treatments with fish oils commonly use more than 12 g per day (Donadio et al., 1994). Food and water were available ad libitum except during challenges. Animals were single housed for the duration of the experiment and were maintained on a 12-hour light/dark cycle. The procedures were approved by the La Trobe University Animal Ethics Committee (AEC0616P).

2.2. Ingestive behavior measures

Basal data were collected daily over a consecutive 7-day period and averaged; it was collected after animals had been maintained on the experimental diets for 2 months. Animals received each of the experimental challenges with a minimum of 5 days between each challenge. Challenges commenced after animals had been maintained on the experimental diets for 3 months. All ingestive behavior challenges were performed with animals in metabolic cages attached to an automated drinking system; measurement of intake was recorded by computer as previously described (Begg and Weisinger, 2008).

2.2.1. Twenty-four hour fluid deprivation

Rats had fluid removed on the morning of Day 1 then, 24 hours later, drinking water was returned; water intake was