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Physiological roles for butyrylcholinesterase: A BChE-ghrelin axis

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ABSTRACT

Butyrylcholinesterase (BChE) has long been regarded as an "orphan enzyme" with no specific physiological role other than to metabolize exogenous bioactive esters in the diet or in medicines. Human beings with genetic mutations that eliminate all BChE activity appear completely normal, and BChE-knockout mice have been described as "lacking a phenotype" except for faster weight gain on high-fat diets. However, our recent studies with viral gene transfer of BChE in mice reveal that BChE hydrolyzes the so-called "hunger hormone," ghrelin, at a rate which strongly affects the circulating levels of this peptide hormone. This action has important consequences for weight gain and fat metabolism. Surprisingly, it also impacts emotional behaviors such as aggression. Overexpression of BChE leads to low ghrelin levels in the blood stream and reduces aggression and social stress in mice. Under certain circumstances these combined effects contribute to increased life-span in group-housed animals. These findings may generalize to humans, as recent clinical studies by multiple investigators indicate that, among patients with severe cardiovascular disease, longevity correlates with increasing levels of plasma BChE activity.

1. Introduction

Butyrylcholinesterase (BChE. E.C.3.1.1.8) is well recognized for its role in metabolizing bioactive esters in food and medications. It is best known for inactivating succinylcholine, a muscle relaxant used to facilitate abdominal surgery but endangers patients with "atypical BChE" that hydrolyzes it poorly. Although some BChE genetic variants are associated with elevated risk of cardiac death [1,2] or early-onset Alzheimer's disease [3], this enzyme was not known to play a direct role in mammalian physiology. But in 2004, De Vriese reported that BChE is capable of hydrolyzing the acylated peptide known as "ghrelin," which stimulates hunger and food-seeking [4]. This deacylation reaction cleaves the octanoyl group essential for ghrelin activity at the growth hormone secretagogue receptor, "GHSR1a," which drives growth hormone release from the pituitary gland [5,6]. The importance of the finding was underappreciated at the time, for two likely reasons. First, the reaction seemed too slow for physiological impact. Second, the small size of BChE's active site pocket made it hard to see how the enzyme could accommodate a substrate more than an order of magnitude larger than butyrylcholine.

2. BChE is a ghrelin hydrolase

Our own views of BChE's catalytic interactions with ghrelin changed very recently, after accidentally observing that mice with high blood levels of BChE generated by viral gene transfer showed dose-dependent reductions in plasma ghrelin. Thus, after a moderate viral dose (e.g., 10^{10} particles per mouse), there was a marginal decline (~10%). A 10-fold higher dose caused a ~50% decline, and a 100-fold higher dose reduced ghrelin levels 90% or more. These effects arose within ten days and lasted for as long as high BChE levels were sustained (months to years). In fact, ghrelin is an unusual peptide hormone with a biological activity that requires octanoylation of its Ser3 residue [5,7]. BChE, an esterase with wide substrate specificity, does cleave this ester bond and transforms ghrelin into desacyl-ghrelin, which is inactive at GHSR1a although it may have other physiological targets [8,9]. Because gene transfer of irrelevant luciferase did not alter plasma ghrelin, it seemed likely that the effects of high BChE were specific. To test that conclusion we gave vector-treated mice an i.p. injection of the selective BChE inhibitor, iso-OMPA. This treatment returned ghrelin levels to near normal for 12–24 h in mice with excess BChE [10]. Furthermore we observed that plasma ghrelin levels in untreated, BChE-null C57BL/

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6 mice were consistently 40% higher than in wild type animals of the same strain [10].

The summarized findings left little doubt that BChE is a regulator of circulating ghrelin, but did not explain how the enzyme can deacylate such a large substrate. We addressed that issue by molecular dynamics simulations of ghrelin and BChE. These showed most of the peptide backbone excluded from the active site, but the crucial ester bond was in an orientation conducive to hydrolysis. In fact, the consensus structure [10] had ghrelin's carbonyl ester carbon positioned closer to the catalytic serine hydroxyl than was previously seen with the equivalent atoms in BChE-cocaine simulations [11].

Further studies using commercial immunoassays for ghrelin and desacyl-ghrelin yielded identical rates for the disappearance of intact peptide and the appearance of its breakdown product, confirming the original findings of ghrelin hydrolysis by BChE. Similar results were obtained by quantitative mass spectrometry in collaboration with Schopfer et al., [12], and again with a new assay developed to track ^{3}H-ghrelin deacylation by quantitative transfer of free octanoic acid from aqueous media into toluene [13]. Although BChE hydrolyzes ghrelin less efficiently than smaller substrates, with a K_m of 3.6 ± 0.3 μM and k_{cat} of 2.2 ± 0.1 min^{-1} it can exert a strong influence on circulating levels of ghrelin. BChE is highly concentrated in the amygdala, hypothalamus, and pituitary gland [14,15], where ghrelin and its receptor are prominently localized [16]. This spatial arrangement may facilitate a central BChE-ghrelin regulatory circuit that impacts emotional behaviors. In short, past and present observations provide good grounds for concluding that BChE has evolved in part to serve as a natural ghrelin hydrolase. The next logical issue is to determine fully how it affects ghrelin-driven events in live organisms.

3. Physiological role of BChE

It is now reasonable to ask this question: does BChE serve as an important ghrelin modulator or is its hydrolytic activity a mere curiosity? Existing data support the idea that BChE does impact ghrelin-driven events. In 2008, Lockridge's group reported that BChE$^{-/-}$ S129 mice showed excessive weight gain on a high fat diet under parameters that did not promote obesity in mice with normal BChE expression [17]. That same year we found excess weight gain in rats given daily exposures to low doses of chlorpyrifos [18]. This irreversible organophosphate inhibitor impacts BChE more readily than acetylcholinesterase [19]. We have now repeated Lockridge's finding in a second BChE$^{-/-}$mouse strain (C57BL/6) and also have shown that a BChE gene transfer can restore resistance to obesity in genetically BChE-deficient mice (unpublished data). These observations are in line with the idea that ghrelin arouses hunger and stimulates eating, and that BChE's ability to deacylate ghrelin contributes to the regulation of these phenomena. Oddly, however, neither we nor Lockridge saw increased *food consumption* in BChE-null animals. Other apparent paradoxes have come to light as well. For example, plasma ghrelin levels in obese BChE$^{-/-}$ mice and humans are actually lower than in lean individuals [20]. One possible reason is that ghrelin as a hormone is influenced by many factors, only one of which is diet. We hypothesize that it may affect fat deposition more directly via actions within the adipocyte, yet to be discovered. In fact, although ghrelin is a documented regulator of food intake and energy metabolism, its essential function is still obscure. For example, eliminating the gene encoding the ghrelin peptide or its activating enzyme, ghrelin octanoyl acyl transferase (GOAT), does not seem to produce any severe phenotype in mice, such as altered body weight, length, and composition, or even a change in daily food intake [21–24]. Thus, the BChE-ghrelin axis now appears far more complex than was originally envisioned, and the intertwined roles of BChE and ghrelin in obesity progression clearly deserve intensive further investigation.

Emotional behaviors represent another complex arena in which BChE can exert a major impact by deacylating ghrelin. Our awareness of this link was prompted by cage-side observations revealing that group-housed male Balb/c mice accumulated fewer bite wounds over time if they and their cage mates had ever received BChE gene transfer. As time continued, the BChE vector recipients outlived the untreated or luciferase vector treated controls (Fig. 1). These observations were not made under conditions that met standards for unbiased analysis of mouse longevity, and they were certainly influenced by ongoing cage-fighting and unequal housing conditions. Thus they have no claim to be definitive, but they strongly indicate that administration of BChE vector can alleviate psychosocial stress.

For quantitative determinations of aggressive behaviors we turned to Klaus Miczek's rigorous “resident-intruder paradigm.” This model actively provokes aggression in a host male (residing in a cage where he had mated with a female), when another male is introduced [25]. Bites and fights were videotaped and scored by a blinded observer. Under this procedure a distinctly different behavior pattern emerged between mice that had undergone BChE gene transfer versus those that had not [10]. The chief difference was in the rate at which aggressive acts per session rose during repeated daily encounters. The rise in aggression was fastest in untreated mice or mice given a different vector that did not decrease circulating levels of ghrelin (Fig. 2A), i.e., vector encoding luciferase or a mutated version of BChE (CocH-6 ΔT) that readily hydrolyzes cocaine but has poor catalytic efficiency with ghrelin [10]. The impact was all the more surprising because ghrelin had not been recognized as a factor in aggression although widely known to affect fear and anxiety [26]. Therefore we used the same model to test the effect of sustaining modestly elevated ghrelin levels by means of double gene transfer with cDNA encoding a) ghrelin peptide and b) its activator enzyme, GOAT. This combined treatment did raise aggression scores, but not when BChE gene transfer was added to prevent the rise in plasma ghrelin (Fig. 2B).

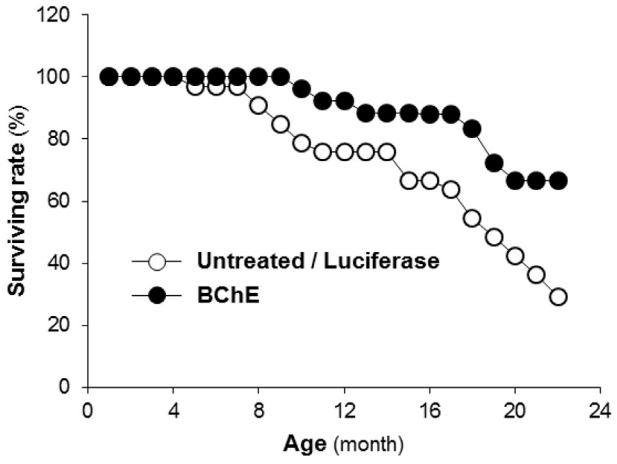

Fig. 1. Unpublished findings from long-term daily observations of group-housed male mice (32 pooled controls and 30 mice transduced at six weeks with adeno-associated virus (AAV) or helper dependent virus vector encoding mutated mouse BChE, “mBChE mut”). Plasma BChE levels in the vectors treated group were ~100-fold above normal and were sustained for the following 2 years. Initially, mice were housed 5 per cage, until signs of fighting arose (4–5 months), when they moved to single cage housing. The varied housing conditions prevent a definitive judgment of true lifespan although ANOVA showed a significant main effect of treatment (controls vs pooled vector-treated groups): $F_{1,60} = 13.64$, $P < 0.001$. No post-hoc testing was performed.