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Propionate induces the release of granules from bovine neutrophils

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ABSTRACT

Short-chain fatty acids (SCFA) are produced by bacterial fermentation in the rumen of cattle and are the primary energy source in ruminants. Propionate is one of the main SCFA and it can exert multiple effects on the inflammatory process and neutrophil function via calcium (Ca^{2+}) release, reactive oxygen species, and intracellular pH changes. However, currently no evidence has shown whether propionate can induce granule release from bovine neutrophils. The purpose of this study was to analyze the effect of propionate on granule release and to evaluate the expression of two G-protein coupled receptors—GPR41 and GPR43—that are activated by propionate. Neutrophil degranulation was assessed by quantifying the release of the neutrophil enzymes myeloperoxidase (MPO), lactoferrin, and matrix metalloprotease-9 (MMP-9) as markers of azurophil, specific granules, and gelatinase granules, respectively. Isolated bovine neutrophils were treated with millimolar concentrations of propionate (0.3, 3 and 30 m*M*), and the cell-free supernatants were recovered. The stimulation of neutrophils with 0.3 m*M* propionate induced the release of lactoferrin and MMP-9 as revealed by ELISA and gelatin zymography, respectively. Propionate at 30 m*M* induced the release of MPO as demonstrated using an enzymatic assay. The role of intracellular Ca^{2+} influx and the signaling pathways that may regulate the propionate effect on granules release were also determined. Reverse transcription (RT)-PCR and real-time PCR were performed to analyze the expression of *GPR41* and *GPR43* mRNA in bovine neutrophils. Both mRNA were detected, whereas the expression of *GPR43* was higher than that of *GPR41*, and the synthetic agonists for this receptor, phenylacetamides 1 and 2, caused an increase in intracellular Ca^{2+}, lactoferrin, and MMP-9 release. These results support that propionate-induced granule release is mediated by intracellular Ca^{2+} influx and activation of extracellular signal–regulated kinase ERK 1/2. We also propose a potential role of GPR43 in propionate-induced granule release from bovine neutrophils that may be involved in regulatory effects of propionate in the innate immune response in cattle.
Key words: short-chain fatty acid, G-protein coupled receptor GPR43, bovine neutrophil, neutrophil granule

INTRODUCTION

Neutrophils are critical for the initial defense of the host against invading microbial pathogens in cattle (Paape et al., 2003). Two different microbicidal mechanisms occur within the neutrophils: the oxidative and the nonoxidative systems. The oxygen-dependent mechanism acts through the generation of reactive oxygen species, and the oxygen-independent mechanism acts through the production of antimicrobial peptides and proteolytic enzymes (Burvenich et al., 2003). Most of the steps in this process depend on the mobilization of cytoplasmic granules and secretory vesicles. At least 4 types of granules and vesicles have been classified in human neutrophils, including primary granules (also termed azurophilic granules), secondary and tertiary granules (also termed specific and gelatinase granules, respectively), and a group of highly mobilizable secretory vesicles (Faurschou and Borregaard, 2003; Borregaard et al., 2007). Several granule types have been characterized in bovine neutrophils, some of which are analogous to the granules found in human neutrophils, such as the specific and azurophilic granules. However, bovine neutrophils contain a unique granule (known as the large granule) that is not present in human cells (Gennaro et al., 1983). Additionally, bovine neutrophils may differ in the important functional characteristics of human neutrophils, such as their lack of receptors for *N*-formylated peptides (Brown and Roth, 1991).

The secretion of neutrophil granules can be triggered after stimulation of a receptor, usually a 7-transmembrane G protein-coupled receptor, which causes an increase in the intracellular calcium (Ca^{2+}) levels and activation of distal signaling pathways (Lacy and Eitzen, 2008). In this sense, a strict rank order of exocytosis of the 4 compartments (secretory vesicles, gelatinase granules, specific granules, and azurophil granules) has been observed when cytosolic Ca^{2+} is elevated using a

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calcium ionophore or formyl-Met-Leu-Phe (**fMLP**) in human neutrophils (Sengeløv et al., 1993). In bovine neutrophils, increasing concentrations of platelet-activating factor (**PAF**) caused an increase in the intracellular Ca^{2+} and progressively mobilized the secretory vesicles, specific granules, and at extremely high doses, azurophil granules (Swain et al., 1998). In addition to the intracellular Ca^{2+} increase, members of the mitogen-activated protein kinase (**MAPK**) family, which includes ERK1/2 and p38 MAPK, have been shown to mediate granule exocytosis (Lacy and Eitzen, 2008). In bovine neutrophils, the signaling events between the activation of G-protein-coupled receptors and the exocytosis of neutrophil granules are largely unknown.

Short-chain fatty acids (**SCFA**) are the principal byproducts of fiber fermentation in the gastrointestinal tract and are the primary energy source in ruminants (Bergman, 1990). The total SCFA concentration in the rumen is normally between 70 and 130 m*M*, and SFCA are rapidly absorbed into the bloodstream (Bergman, 1990). Propionate is one of the main SCFA and it exerts multiple effects on human neutrophil function, including Ca^{2+} release (Naccache et al., 1988), superoxide production (Nakao et al., 1998), and cytoskeletal actin distribution (Brunkhorst et al., 1992), and it induces chemotaxis in vitro (Le Poul et al., 2003; Vinolo et al., 2011a), suggesting a potential role as a modulator of the innate immune response. In cattle, propionate exerts similar effects as in human neutrophils, including intracellular acidification, Ca^{2+} release, superoxide production, and ERK1/2 activation (Sandoval et al., 2007a). However, little is known about the effect of propionate on granule release in bovine neutrophils. Recently, Wang et al. (2009) demonstrated that the bovine genome encodes functional G-protein coupled receptors GPR41 (free fatty acid receptor 3, FFA3) and GPR43 (FFA2), 2 previously described receptors that can be activated by SCFA. Activation of GPR41 and GPR43 is coupled with several intracellular signals, such as inositol trisphosphate generation, elevated intracellular Ca^{2+} release, and ERK1/2 activation (Brown et al., 2003; Le Poul et al., 2003). In addition, these genes, especially human and bovine GPR43, are highly expressed in hematopoietic tissues (spleen and bone marrow), supporting a putative role of SCFA in immune cell activation in cattle (Le Poul et al., 2003; Wang et al., 2009). In fact, a discrepancy exists in the role of SCFA in the regulation of the inflammatory response. The most common action of SCFA is their antiinflammatory effect, inhibiting the stimuli-induced expression of adhesion molecules (Zapolska-Downar and Naruszewicz, 2009) and suppressing the production of proinflammatory mediators (Cox et al., 2009). In contrast, a proinflammatory action of SCFA has been observed, mainly due to the ability of SCFA to induce neutrophil migration in different species (Vinolo et al., 2011b). In cattle, the influence of intraluminal SCFA on inflammation in the colon is well documented and is likely the mechanism that triggers the inflammatory response in the ruminal epithelium (Mortensen and Clausen, 1996). Because granule release is involved in tissue damage during migration of neutrophils, we hypothesized that propionate could induce release of granules from bovine neutrophils and modulate the inflammatory process during nutrition imbalance in ruminants.

In the present study, we demonstrated that propionate induces primary, secondary, and tertiary granule release and characterized the signaling mechanisms that regulate this process. We also showed a high expression of GPR43 receptors in bovine neutrophils and demonstrated that the propionate effects were mimicked by the synthetic GPR43 agonists phenylacetamides 1 and 2. These data can contribute to the understanding of the mechanisms of propionate in the inflammatory response in cattle.

MATERIALS AND METHODS

Animals

Five clinically healthy, nonpregnant black Friesian dairy heifers were used in all experiments. The animals were maintained in the Universidad Austral de Chile herd and routinely checked by a veterinarian. The physiological parameters monitored include respiratory rate, heart rate, rectal temperature, and body condition. The heifers were fed twice daily, and the daily ration was divided into meals of equal size of 1.0 kg/d of concentrate. Heifers grazed a naturalized pasture composed mainly of perennial grasses, the most abundant being *Holcus lanatus* (common velvetgrass) and *Agrostis capillaris* (colonial bentgrass). The contribution of forage legumes was low (<10% of DM). The experiments were conducted in accordance with the Ethical Committee of the Universidad Austral de Chile.

Study Design

Figure 1 shows an overview of the experimental study design. First, blood samples from black Friesian dairy heifers were collected and neutrophils were isolated. Then, the dose and time responses of propionate were evaluated by incubating the neutrophils with different concentrations of propionate for different times. To determine the role of Ca^{2+} and the signaling pathway on release of granules induced by propionate, the neutrophils were pretreated with Ca^{2+} blockers and signaling