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Effect of deletion of the prostaglandin EP4 receptor on stimulation of calcium release from cultured mouse calvariae: Impaired responsiveness in heterozygotes

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Abstract

The ability of prostaglandin E_2 (PGE_2), selective receptor agonists for EP2 and EP4 receptors (EP2A and EP4A) and parathyroid hormone (PTH) to stimulate calcium release from cultured fetal mouse calvariae was compared in wild type (WT) mice and in mice heterozygous (HET) or homozygous (KO) for deletion of the EP4 receptor. Calvariae from 19 day fetal mice were used in order to avoid the problem of high neonatal mortality. Calcium release was increased by PGE_2, EP4A or PTH in WT mice, but EP2A had no significant effect. There was a significant decrease in calcium release in response to PGE_2, EP4A and PTH in calvariae from HET mice compared to WT mice. The response to PGE_2 and EP4A was abrogated and the response to PTH was further diminished in EP4 receptor KO mice. These results suggest that the EP4 receptor may be rate limiting not only for PGE_2 stimulated resorption but also for resorption stimulated by other agonists, like PTH that induce PGE_2 production.

Keywords: Prostaglandin E_2; Mouse calvariae; Parathyroid hormone; Bone resorption

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1. Introduction

Prostaglandin E_2 (PGE_2) is a potent stimulator of bone resorption [1]. This effect is mediated by stimulation of cAMP production through two receptors, EP2R and EP4R [2]. The expression of EP4R in osteoblasts is essential for stimulation of osteoclastogenesis by PGE_2 [3]. Deletion of EP4R results in a high neonatal mortality due to failure to close the ductus arteriosis [4]. Surviving animals do not show a marked skeletal phenotype, but develop osteopenia and impaired fracture healing with aging [5]. Surviving EP4R knockout animals also show diminished resorptive responses to lipopolysaccharide and PGE_2 in vivo and in organ culture [6,7]. Studies using selective agonists and antagonists have confirmed the critical role for EP4R in stimulation of bone resorption and formation [7–10].

In the present study, we examined the ability of PGE_2 and selective receptor agonists (EP2A, EP4A), as well as parathyroid hormone (PTH), to increase calcium release from fetal mouse calvariae from EP4R wild-type (WT) mice and mice with heterozygous (HET) or homozygous (KO) deletion of EP4R. Fetal mice were used in order to avoid the problem of high neonatal mortality. Stimulation of calcium release was abrogated in response to PGE_2 or EP4A in EP4R KO animals. There was also a significant decrease in the calcium release response of calvariae from HET mice. Moreover, PTH stimulated calcium release responses were diminished in calvariae from HET mice and further diminished in KO mice.

2. Materials and methods

2.1. Animals

We have established EP4R HET breeding colonies from founder mice produced by Dr. Mathew Breyer (Vanderbilt University). These mice possess an EP4 receptor allele lacking Exon2, resulting from Cre mediated deletion of the floxed ER4 receptor Exon2 allele in single cell embryos [11]. We established EP4R HET mice in a pure C57B16 background by back-crossing the EP4R HET mice to C57B16 females from the same source (Harlan) for nine generations. Animals were housed in the Center for Laboratory Animal Care at the University of Connecticut Health Center. The Animal Care Committee of University of Connecticut Health Center approved all animal protocols. We mated EP4R HET mice for one night, and at 19 day of gestation sacrificed the pregnant female mice, removed the fetuses and dissected the calvariae free of connective tissue.

2.2. Materials

PGE_2 and PTH (PTH, 1-34) were purchased from Sigma (St. Louis, MO). EP4 receptor agonist (ONO-AE1-329, EP4A) and EP2 receptor agonist (ONO-AE1-259-01, EP2A) were gifts from ONO pharmaceutical. Co. Ltd. (Osaka, Japan).

2.3. Genotyping of mice

DNA was extracted from 19-day fetal tails for analysis by PCR. To genotype mice, two PCR analyses were done. In the first, we used the following primers: sense (1S) 5′-GGA