

relation to glucose metabolism by closure of mutant  $K_{ATP}$  channels; it also amplified the effect of incretins levels that are stimulated by nutrient ingestion.

In an accompanying editorial, Sperling<sup>1</sup> recommended that a test for this genetic mutation be included as part of routine newborn screening programs. In all cases, newborns with this disease should be tested for activating mutations affecting *Kir6.2*, an approach facilitated by the one exon structure of the gene. Furthermore extensive familial studies are needed and other phenotypes may be expected as a consequence of mutations with milder activity. Another cause of permanent neonatal diabetes

was also reported by Babenko et al.<sup>2</sup> A careful history is needed in all patients with the onset of diabetes in infancy. It is remarkable that some, but not all, adult patients were responsive to the treatment switch from insulin to sulfonylureas. More information is needed regarding the failures observed in about 10% of the patients with the same genetic mutations.

Raphaël Rappaport, MD

## References

1. Sperling MA. N Engl J Med. 2006;355:507–510.
2. Babenko AP, Polak M, Cave H, et al. New Eng J Med. 2006; 355:456–466.

## Acidosis and Protein Kinase: A Novel Mechanism of Growth Failure

Chronic acidosis is known to cause growth failure by an effect on the bone end-organ, but the exact mechanism has remained elusive. Goldberg and colleagues have recreated growth retardation of endochondrial ossification centers *ex vivo* by culturing murine mandibular condyles in medium with 2.4 mM HCl, to lower the pH to 7.1 – 7.15. In previous studies, this group found that acidosis led to decreased expression of both insulin-like growth factor (IGF)-I and its receptor (IGF-IR) as well as markers of differentiation like type II collagen and cartilage proteoglycans. They also found that the acidotic growth inhibition could be prevented by local application of low concentrations ( $10^{-10}$  M) of parathyroid hormone (PTH).

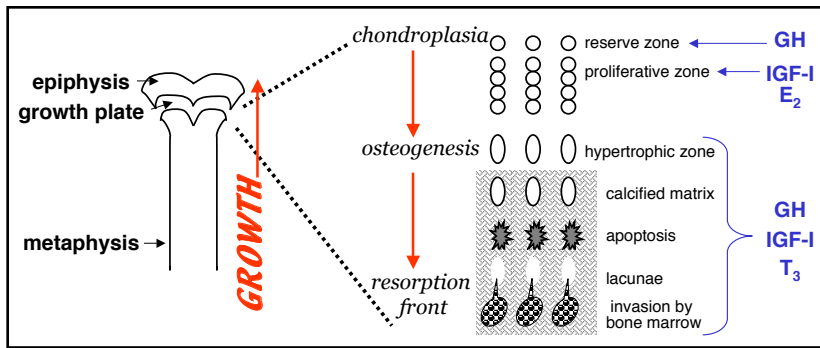
PTH works through 2 main signaling pathways: Gq protein/protein kinase C (PKC) and Gs protein/adenylate cyclase/protein kinase A (PKA) pathway. Goldberg and colleagues previously showed that acidosis represses PKC expression, an effect partially inhibited by PTH. However, the PKC agonist PMA succeeded in protecting condyles against acidotic differentiation arrest (increased expression of type II collagen and proteoglycans) but not the acidotic suppression of IGF-I and IGF-IR expression.<sup>1</sup> Therefore, the researchers sought to examine the possible role of the PKA pathway in acidosis-induced growth retardation.

In contrast to the reduction in PKC levels, PKA $\alpha$  protein levels were increased by acidosis; both levels were normalized by adding PTH to the acidotic cultures. A specific PKA inhibitor, H89, prevented the acidosis-induced reductions in expression of IGF-I, IGF-IR, and aggrecan (the core protein of cartilage-specific proteoglycans in chondrocytes). Using the converse approach, the cAMP regulating factors 8Br-cAMP (a cAMP analog), iso-butyl methyl xanthine ([IBMX] a phosphodiesterase inhibitor), and forskolin (an adenylyl cyclase analog), all reproduced the morphologic changes seen in acidotic growth plates: decreased condylar length with loss of the chondroblast population, leaving the mature hypertrophic cell layer adjacent to the chondroprogenitor zone which is

itself wider due to differentiation arrest. Chondrocyte proliferation was also reduced by acidosis, IBMX, and forskolin, as evidenced by decreased expression of proliferating cell nuclear antigen (PCNA), a cell cycle marker. Acidosis and IBMX also decreased expression of IGF-IR in the chondroblasts and chondrocytes. Using mandibular condyle-derived primary cultures of chondrocyte (MCDC) cells, the temporal cascade of endochondrial ossification was reproduced. When grown in acidotic conditions for one week, MCDC cells showed less proliferation and developed fewer cartilaginous nodules. The possibility of toxic effects of acidosis acidifying the intracellular cytoplasm was neatly ruled out by comparing the fluorescence pattern of a pH-dependent fluorescent dye, acridine orange; intracellular pH looked normal despite acidotic culture conditions, but reflected intracellular acidosis with the addition of nigericin (an ionophore that equalizes intracellular and extracellular proton concentrations). Involvement of the entire PKA pathway by acidosis was demonstrated by the increased ratio of phosphorylated-(activated) to total cAMP-responsive element binding protein ([CREB] the transcription factor which is a major PKA substrate) at one end, and the increased expression of Gs $\alpha$  protein at the other. Despite acidosis, the Gs inhibitor GDP $\beta$ S allowed normal condyle development.

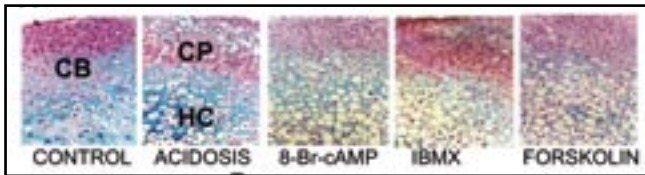
Thus, the authors developed a model of growth plate chondrocytes whereby acidosis induces Gs $\alpha$  protein, which in turn activates adenylyl cyclase and hence PKA, leading to phosphorylated CREB and altered gene expression. Both genes of differentiation and IGF-IR are ultimately down-regulated. PTH inhibits the acidotic growth retardation through both its PKA and PKC signaling pathways. The authors speculated that the activation of Gs protein by acidosis was mediated through proton-sensing receptors rather than ligand binding.

Goldberg R, Reshef-Bankai E, Coleman R, Green J, Maor G. Chronic acidosis-induced growth retardation is mediated by proton-induced expression of Gs protein. J Bone Miner Res. 2006; 21:703–713.



**Figure 1. Growth plate and process of growth.** Reprinted with permission from: Grimberg A, De Leon D. Disorders of Growth. In Moshang T, Ed., Requisites in Pediatrics - Pediatric Endocrinology, Elsevier, Inc., Philadelphia, 2005; 127–167. Copyright © Elsevier. 2005. All rights reserved.

**Editor's Comment:** I am always delighted when the underlying mechanisms of long-standing clinical observations are finally elucidated. To remind our readers, the anatomy of the growth plate and the growth process are depicted in the schematic<sup>2</sup> of Figure 1. The



**Figure 2. Effects of 8Br-cAMP, IBMX, and forskolin (cAMPPrf) on the development of the mandibular condyle.** Condyles derived from 6-day-old ICR mice were cultured for 72 h under normal, acidic condition (2.4 mM HCl) or treated with the following cAMP-inducing factors (cAMPPrf): 0.05 mM 8Br-cAMP (a cAMP analog), 0.05 mM IBMX (a phosphodiesterase inhibitor), or 1  $\mu$ M forskolin (adenylyl cyclase analog). Chondroblastic cell layer (CB) is absent in the acidosis and cAMPPrf cultures, leaving the hypertrophic cells (HC) adjacent to the chondroperichondrium zone (CP), which is larger than that of the control. Reprinted with permission from: Goldberg, et al. J Bone Min Res. 2006;21:703–713. Copyright © American Society for Bone and Mineral Research 2006. All rights reserved.

chondroblasts absent in the condyle cultures with acidosis or cAMP regulating factors in the current paper (Figure 2) correspond to the proliferative zone in Figure 1. This zone is regulated primarily by IGF-I and estradiol. Instead, the hypertrophic zone was seen adjacent to chondroprogenitors (Figure 2) in an expanded reserve zone (Figure 1) that failed to fully differentiate.

Although showing a role of PKA in acidosis-induced growth retardation in the growth plate is certainly novel, this is not the first paper to demonstrate regulation of the IGF axis by PKA. cAMP/PKA induced IGF-I expression in primary rat osteoblasts<sup>3</sup> and cultured embryonic mouse mandibular mesenchymal cells,<sup>4</sup> IGF binding protein (IGFBP)-1 in hepatocytes,<sup>5</sup> and IGFBP-3, -4 and -5 in periosteal and osteoblast bone cell cultures.<sup>6</sup> PKA inhibitors interfered with the induction of IGF-I and IGFBP-3 by growth hormone in porcine ovarian granulosa cells,<sup>7</sup> IGFBP-3 by FSH also in porcine ovarian granulosa cells,<sup>8</sup> and IGFBP-4 by platelet-derived growth factor-BB in fetal rat lung fibroblasts.<sup>9</sup>

Adda Grimberg, MD

## References

- Green J, Goldberg R, Maor G. Kidney Int. 2003;63:487–500.
- Grimberg A, De Leon D. Disorders of Growth. In Moshang T, Ed., Requisites in Pediatrics - Pediatric Endocrinology, Elsevier, Inc., Philadelphia, 2005; 127–167.
- Thomas MJ, Umayahara Y, Shu H, Centrella M, Rotwein P, McCarthy TL. J Biol Chem. 1996;271:21835–21841.
- Lambert HW, Weiss ER, Lauder JM. Dev. Neurosci. 2001;23:70–77.
- Suwanichkul A, DePaolis LA, Lee PD, Powell DR. J Biol Chem. 1993;268:9730–9736.
- Chen Y, Shu H, Ji C, et al. J Cell Biochem. 1998;71:351–362.
- Sirotkin AV, Makarevich AV. Anim Reprod Sci. 2002;70:111–126.
- Ongeri EM, Verderame MF, Hammond JM. Mol Endocrinol. 2005;19:1837–1848.
- Price WA. Exp Lung Res. 2001; 27:655–674.

**Growth, Genetics & Hormones** is supported by an unrestricted educational grant from **Insmmed, Inc.**

To obtain a complimentary subscription, send an e-mail to: [subscribe@GGHjournal.com](mailto:subscribe@GGHjournal.com) or write to:

Fima Lifshitz, MD  
GGH Editor-in-Chief  
Sansum Diabetes Research Institute  
2219 Bath Street  
Santa Barbara, CA 93105

PRST STD  
U.S. Postage  
**PAID**  
PERMIT 161  
LANCASTER, PA