

# Novel mechanism of increasing cerebral vascular constriction in acute hyperglycemia and diabetes through adenylyl cyclase 5-generated cyclic adenosine monophosphate

The recent increase in the global incidence of type 2 diabetes mellitus poses major threats to societies worldwide. Patients with diabetes are at heightened risk of vascular complications, such as cardiovascular diseases (CVD), including stroke. Many studies showed that glycaemic control improves cardiovascular outcome in diabetes patients; however, the relationship linking diabetes to CVD is more complex and multifaceted in nature. Although the improvement of strategy to prevent CVD induced by diabetes is required, the molecular mechanism altering vascular reactivity in diabetes has not yet been well defined. Hypertension is extremely common in patients with diabetes. In observational studies, patients with both diabetes and hypertension have approximately twice the risk of CVD as non-diabetic people with hypertension. Epidemiological and functional studies show that impaired arterial myocyte contractility contributes to vascular complications in diabetes patients and animal models of diabetes<sup>1</sup>. Therefore, to clarify the mechanism of how hyperglycemia increases vascular tone is an important subject of diabetes research.

Several types of potassium (K<sup>+</sup>) channels and L-type Ca<sup>2+</sup> channels have important roles in regulating arterial

myocyte excitability and myogenic tone. The expression and function of these channels alter diabetic hyperglycemia, which induces arterial myocyte contractility and myogenic tone.

Navedo *et al.*<sup>2</sup> reported that L-type Ca<sup>2+</sup> channel activity in arterial myocytes and myogenic tone is elevated in diabetes. This was found to be mediated by phosphorylation of the L-type Ca<sup>2+</sup> channel pore-forming Ca<sub>v</sub>1.2 subunit at serine 1928 by protein kinase A (PKA). In addition, they reported that the binding of PKA to A-kinase anchoring protein 150 is a key determinant to activate the Ca<sub>v</sub>1.2 subunit at serine 1928.

The fact that PKA induces vasoconstriction in hyperglycemia is important, because this changes the way we think of the role of PKA in the pathophysiology of artery. Historically, we thought of PKA primarily as a function of vasodilation, but now we have to consider the opposite function of PKA. This raises the possibility that regulating PKA activity could be a potential therapeutic application in vascular disease induced by diabetes. Although the prior work of Navedo *et al.*<sup>2</sup> showing that glucose could mediate vasoconstriction through a PKA pathway is very impressive, the mechanism by which elevated extracellular glucose leads to increased PKA activity is not well understood.

Syed *et al.*<sup>3</sup> recently reported convincing evidence in the *Journal of Clinical Investigation* that elevating extracellular glucose stimulates cyclic adenosine monophosphate (cAMP) production in arterial myocytes, and that this was specifically dependent on adenylyl cyclase 5 (AC5) activity.

First, the authors found that acute elevation in extracellular glucose increases myogenic tone and cAMP synthesis, which requires adenylyl cyclase (AC) activity in arterial myocytes. To evaluate cAMP synthesis in myocytes, the authors used a membrane-targeted Epac1–cAMP-based fluorescence resonance energy transfer (FRET) sensor (ICUE3-PM). FRET sensor is a well-established method to measure cAMP levels in real-time and in living cells. In ICUE3-PM-expressing arterial myocytes, extracellular high glucose induces FRET signal change, which means that high glucose increases cAMP synthesis. The authors carried out voltage ramps using patch clamp electrophysiology and showed L-type Ca<sup>2+</sup> channel activity in freshly dissociated cerebral arterial myocytes is increased after exposure to high glucose. These responses are reversed by treating with the broad AC inhibitor, 2',5'-dideoxyadenosine.

The general belief is that AC activity increases cAMP in cells, which is the key driver of PKA activation. AC has nine isoforms, and AC3, AC5 and AC6 are most abundantly expressed in arterial myocytes. Although AC6 and AC3 have been reported as key pathways of vasodilation, AC5 has not been well defined. Therefore, they focused on the function of AC5 in mediating the glucose effects on cAMP synthesis. The authors use AC5 knockout (AC5<sup>-/-</sup>) mice, and analyze arterial myocytes and arteries. In ICUE3-PM-expressing arterial myocytes from AC5<sup>-/-</sup> mice, extracellular high glucose cannot induce FRET signal change. In addition, high glucose failed to stimulate L-type Ca<sup>2+</sup> channel activity in

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arterial myocytes from AC5<sup>-/-</sup> mice by patch clamp electrophysiology. To evaluate the effects of high glucose on vascular reactivity *in vivo*, the authors used an open cranial window, which is the way to expose middle cerebral arteries in living animals. Permeation of the cranial window with high glucose solution induces a robust sustained constriction of cerebral arteries in wild-type mice, but not in AC5<sup>-/-</sup> mice. On the contrary, high glucose induces vasoconstriction to the same extent as in wild-type mice arteries in a subset of experiments using arteries from AC6<sup>-/-</sup> mice. These results suggested that AC5 is a key driver for PKA-dependent L-type Ca<sup>2+</sup> channel activity and constriction in cerebral arteries during hyperglycemia.

To further investigate the role of glucose-induced AC5 activation to regulate L-type Ca<sup>2+</sup> channel activity and constriction in the artery, the authors focused on spatial organization between AC5 and L-type Ca<sup>2+</sup> channel. They used super-resolution nanoscopy and proximity ligation assay analysis, which showed that a subpopulation of AC5 is located in close proximity to the L-type Ca<sup>2+</sup> channel pore-forming subunit Ca<sub>v</sub>1.2 in arterial myocytes. Because AC6 is not close to the Ca<sub>v</sub>1.2 in the previous report, this intimate spatial organization might be an important role for local PKA-dependent regulation of L-type Ca<sup>2+</sup> channels in arterial myocytes. In addition, their group also reported a similar arrangement between Ca<sub>v</sub>1.2 and PKA, which are closely associated with each other<sup>2</sup>. These data suggest that a close Ca<sub>v</sub>1.2–PKA–AC5 association is required for glucose-mediated potentiation of L-type Ca<sup>2+</sup> channels, and vasoconstriction in arterial myocytes.

Finally, the authors analyzed the function of AC5 in diabetes model mice. A diet-induced diabetic mouse model and the streptozotocin-induced (STZ-induced) diabetic mouse model are well-established chronic hyperglycemia models. It was reported that L-type Ca<sup>2+</sup> channels activity increased in diabetes. They used these models to evaluate the function of AC5 to regulate L-type Ca<sup>2+</sup> channels and

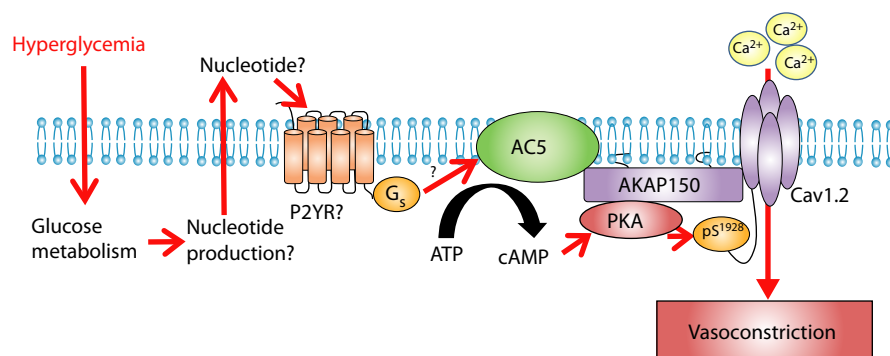
vasoconstriction in diabetes. L-type Ca<sup>2+</sup> channel activity and increases in arterial myocytes were seen in high-fat diet (HFD)-fed mice and STZ mice. Interestingly, there was an increased association between Ca<sub>v</sub>1.2 and AC5 in HFD-fed mice and STZ mice in proximity ligation assay. The myogenic tone of dissected arteries in HFD-fed mice is significantly increased, and similarly, *in vivo* imaging of cerebral arteries using cranial windows showed that myogenic tone increased in STZ mice. These changes of HFD mice and STZ mice are inhibited in AC5<sup>-/-</sup> mice, indicating that AC5 has a key function of regulating L-type Ca<sup>2+</sup> channel activity and myogenic tone induced by hyperglycemia in HFD-fed mice and STZ mice.

The research summarized here broadens our understanding of vasoconstriction in hyperglycemia (Figure 1). This gives us an insight into the pathophysiology of vascular complication in diabetes. However, further research is still required to fully clarify the underpinnings of this process.

First, it is still unclear how AC5 is regulated by hyperglycemia. In general, AC is regulated by G<sub>s</sub> protein-coupled receptors. Their group reported that G protein-coupled purinergic receptor (P2Y receptor) regulated hyperglycemia-induced Ca<sup>2+</sup> influx in the arterial myocyte<sup>4</sup>. There are eight isoforms of P2Y receptor, and

P2Y<sub>11</sub> is the only isoform that is coupled to G protein. Several studies showed that extracellular high glucose increases autocrine secretion of nucleotides. Adenosine 5'-triphosphate (ATP) can be transported to the outside of cells through ATP-binding cassettes, vesicular exocytosis, plasma membrane F1F0-ATPase, connexin hemichannels and pannexin channels. P2Y<sub>11</sub> can be activated by ATP/ATP-derived nucleotides. It is speculated that one of the secreted ATP/ATP-derived nucleotides is induced by high-glucose activated AC5, but further investigation is required to clarify the question as to how nucleotides are released and activate vasoconstriction through activation of AC5 in response to hyperglycemia.

Second, the discrepancy of canonical function of PKA is still unknown. In contrast to this report, PKA activation traditionally induces arterial myocyte dilation in response to endogenous and exogenous vasodilatory agents<sup>5</sup>. Indeed, the authors found that application of forskolin, which is an adenylyl cyclase activator, induces vasodilation, even in the condition of elevated glucose<sup>3</sup>. The authors predict that localization of cAMP signaling makes it possible to specifically stimulate a pool of PKA and determine the effect of vascular reactivity. They showed there is a close association between the L-type Ca<sup>2+</sup> channel and AC5, in contrast to less association




**Figure 1** | Schematic model of increasing vascular constriction in acute hyperglycemia through adenylyl cyclase 5 (AC5)-generated cyclic adenosine monophosphate (cAMP). Elevations in extracellular glucose might activate the purinergic receptor (P2Y receptor) through nucleotide signaling. This induces AC5 activation, which catalyzes the conversion of adenosine triphosphate (ATP) to cAMP. The localized increase of cAMP might stimulate A-kinase anchoring protein 150-anchored protein kinase A (AKAP150), which activates Ca<sup>2+</sup> channel activity. This will result in increasing arterial myocyte contraction and myogenic tone. PKA, protein kinase A.

between the L-type  $\text{Ca}^{2+}$  channel and AC6<sup>3</sup>. Together with a previous report, they consider A-kinase anchoring protein 150 as essential to anchor AC5, PKA and L-type  $\text{Ca}^{2+}$  channel, but it is still unclear why the association between AC5 and L-type  $\text{Ca}^{2+}$  channel increases in diabetic model mice<sup>3</sup>. Further investigation is required into this macromolecular complex in arterial myocytes.

In summary, the authors found a novel role of AC5 in PKA-dependent activation of L-type  $\text{Ca}^{2+}$  channel activation and vasoconstriction during acute hyperglycemia and diabetes. This report might contribute to the clinical implications and novel targets for therapeutic intervention, as they add to several mechanisms for hyperglycemia-induced vasoconstriction through this pathway in a number of pathophysiological conditions.

#### DISCLOSURE

The authors declare no conflict of interest.

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