1?,25(OH)2-VITAMIN D3 CONTRIBUTES TO GLUCOSE HOMEOSTASIS IN AN IN VITRO APPROACH[1]

Ana Karla Bittencourt Mendes[2], Heloisa Wilbert Schlindwein[3], Fátima Regina Mena Barreto Silva[4], Paola Miranda Sulis[5]

[1] Pesquisa Institucional desenvolvida no Grupo de Pesquisa do Laboratório de Hormônios e Transdução de Sinais, Programa de Pós-Graduação em Farmácia da Universidade Federal de Santa Catarina.
[2] Aluna de Doutorado do Programa de Pós graduação em Farmácia UFSC, akmendes29@hotmail.com, Florianópolis, SC, Brasil.
[3] Aluna do Curso de Graduação em Farmácia da UFSC, helowilbert@gmail.com, Florianópolis, SC, Brasil.
[4] Professora Orientadora, Doutora em Bioquímica, Curso de Farmácia (UFSC), mena.barreto@ufsc.br, Florianópolis, SC, Brasil.
[5] Aluna de Doutorado do Programa de Pós graduação em Bioquímica da UFSC, pasulis@hotmail.com, Florianópolis, SC, Brasil.

Introduction: Diabetes is a chronic disease which results in failure on insulin action or secretion. Vitamin D has been described as an adjuvant in diabetes prevention and treatment by acting on pancreatic β cells by non-genomic pathways and contributing to the exocytosis of the insulin granules. **Aim:** To study the rapid response of 1α,25(OH)$_2$-vitamin D$_3$ (1,25D$_3$) on $^{45}Ca^{2+}$ influx to insulin secretion from rat pancreatic islets mediated by K^+ and Ca^{2+} channels, as well as, protein kinases pathways. **Methodology:** Male Wistar rats (180–200 g) were used. For islet isolation the bile duct was clamped at the tip of the duodenum and cannulated. Krebs Ringer-bicarbonate (KRb) buffer was introduced into the bile duct by syringe. The pancreas was removed by dissection. Secretion of static insulin was dosed by ELISA. For calcium influx, isolates of pancreatic islets were incubated in KRbâ€?HEPES buffer containing 5 mM glucose, 0.1 µCi/ml $^{45}Ca^{2+}$ for 60 min, without (control) or treated with 1,25-D$_3$ (1 nM – 1 minute). The channel activator/blocker or receptor agonist/antagonist were added at 45 min of incubation and maintained throughout the incubation period. Lanthanum chloride buffer (10 mM) was added at 2°C to stop Ca^{2+} flux. Aliquots were taken from each sample for radioactivity measurement in scintillation liquid using a LKB rack beta liquid scintillation spectrometer. Proteins were quantified by the Lowry method. (Protocol CEUA/UFSC/2119280317). **Results:** 1,25D$_3$ was able to stimulate calcium influx and increase insulin secretion in isolated pancreatic islets. The stimulatory effect of the 1,25D$_3$ was decreased by apamin and glibenclamide, a Ca^{2+}-dependent K^+ channel and K^+ channel antagonist, respectively. Similarly, diazoxide and TEA, a K^+ channel and voltage-dependent K^+ channel (VDCC) agonist, respectively, totally blocked the 1,25D$_3$ effect. On the other hand, the effect of 1,25D$_3$ was enhanced in the presence of N-ethylmaleimide, a vesicular transport blocker. The effect of 1,25D$_3$ was decreased by thapsigargin, a sarco/endoplasmic reticulum Ca^{2+}-ATPase (SERCA) inhibitor

and totally blocked by nifedipine, a voltage-dependent Ca^{+2} channel antagonist. Instead, the effect of $1,25D_3$ were not blocked by BAPTA-AM, an intracellular Ca^{+2} chelator. Likewise, dantrolene, a ryanodine receptor antagonist and 2-APB, an endoplasmic reticulum Ca^{+} release blocker, reduced the effect of $1,25D_3$. When KT 5720 (PKA inhibitor) and Ro 31-0432 (PKC inhibitor) were used, the effect of 1,25D3 was abolished. **Conclusion:** The stimulatory effect of $1,25D_3$ on calcium influx in isolated pancreatic islets is dependent upon K^{+}â€?ATP and VDCC, since the effect of $1,25D_3$ was inhibited by diazoxide and TEA. In addition, the effect of nifedipine, 2-APB, and dantrolene exerted similar decreases in $1,25D_3$â€?induced calcium influx, indicating that the Lâ€?type calcium channels and the release of calcium from intracellular stores take part of the mechanism of action of this compound. As well as PKA and PKC pathways are also involved in this effect. **Financial support:** CNPq; PGFAR-CAPES; PPG-BQA PROAP/UFSC.

Key words: Pancreatic islets, $1,25D_3$, calcium influx, insulin secretion.