Effects of 2-phenyl-4, 4, 5, 5-tetramethylimidazolineoxyl-1-oxyl-3-oxide (PTIO) on intracellular Ca^{2+} dynamics in mouse pancreatic islets.

Ryouki Yamanaka

Laboratory of Physiology,
Department of Biomedical Sciences,
School of Veterinary Medicine,
Hokkaido University, Sapporo 060-0818, Japan

1. The present study was carried out to examine the effects of a NO scavenger, 2-phenyl-4, 4, 5, 5-tetramethylimidazolineoxyl-1-oxyl-3-oxide (PTIO) on changes in intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) and to clarify possible sites of action of PTIO in mouse pancreatic β cells. A microfluorometric method was applied by using Fura-2, a fluorescent Ca^{2+} indicator, in the isolated perifused preparations of mouse pancreatic islets. A confocal imaging analysis combined with NO imaging with diaminofluorescein-2 (DAF-2) was adopted to investigate changes in intracellular NO concentration ($[NO]_i$).

2. Stimulation of isolated pancreatic islets with 15 mM glucose caused a biphasic increase in $[Ca^{2+}]_i$, the first transient rise (first phase) followed by a continuous $[Ca^{2+}]_i$ increase (second phase). The second phase was inhibited by PTIO (≥10 μM) dose-dependently. The first $[Ca^{2+}]_i$ rise was completely abolished by the addition of PTIO (100 μM).

3. A $[Ca^{2+}]_i$ increase induced by high K^+ (20 mM) or K^+_{ATP} channel inhibitor, tolbutamide (300 μM), was not influenced by PTIO (100 μM) which inhibited the glucose-induced $[Ca^{2+}]_i$ increase. These results suggest that NO has no direct action on voltage-dependent Ca^{2+} channels and K^+_{ATP} channels. PTIO (100 μM) did not prevent a $[Ca^{2+}]_i$ rise caused by carbachol. A high concentration of PTIO (500 μM) reduced the $[Ca^{2+}]_i$ increase by each stimulus but it is assumed that a high dose of PTIO elicits non-specific inhibitory actions on mechanisms which lead to the $[Ca^{2+}]_i$ rise.

4. A transient $[NO]_i$ rise was induced by a high glucose stimulus. The time course of this rise is almost consistent with that of the first phase in the glucose-induced $[Ca^{2+}]_i$ increase. PTIO, a NO scavenger, abolished the first phase of glucose-induced $[Ca^{2+}]_i$ rise. Consequently these results indicate that NO correlates with the mechanism of the first phase in a glucose-induced $[Ca^{2+}]_i$ rise.

5. The present study did not completely clarify the site of the inhibitory action of PTIO on the glucose-induced $[Ca^{2+}]_i$ increase. However, this study suggested the possibility of a new mechanism of insulin secretion which may be related with NO.