but their peak levels decreased by about 18%.

3. Removal of CaCl₂ from the perifusing solution almost completely abolished CCK-8-induced increases in [Ca²⁺]c. This result suggests that the presence of CaCl₂ in extracellular space of the islet cells is essential for [Ca²⁺]c dynamics induced by CCK-8. This phenomenon may be explained by one of two separate hypothesis; 1) Ca²⁺ influx is important for CCK-8-induced [Ca²⁺]c increase or 2) refilling of Ca²⁺ in intracellular Ca²⁺ stores is mandatory for [Ca²⁺]c increase.

4. Addition of low concentration of NiCl₂ which is shown to selectively block T-type Ca²⁺ channel strongly inhibited CCK-8-induced [Ca²⁺]c increase, suggesting that Ca²⁺ influx via T-type Ca²⁺ channel is occurring during CCK stimulation in this type of cells.

5. This idea was further supported by following evidence. Nifedipine, a selective L-type Ca²⁺ channel blocker, ω-conotoxin GVIA, a selective N-type Ca²⁺ channel blocker, ω-conotoxin MVIIIC, a selective Q-type Ca²⁺ channel blocker, and ω-agatoxin IVA, a selective P-type Ca²⁺ channel blocker, were all without effect on CCK-8-induced [Ca²⁺]c increase.

6. Possible involvement of Ca²⁺ influx by CCK-8 stimulation was also supported by following evidence. U73122, a PLC inhibitor, had no effect on CCK-8-induced [Ca²⁺]c increase, suggesting that PLC-IP₃-Ca²⁺ release cascade is not functioning.

7. It was concluded that CCK may physiologically participate in regulation of pancreatic endocrine secretion by modulating cytosolic Ca²⁺ dynamics which are brought about by possible activation of T-type Ca²⁺ channel but not by L-type, N-type, Q-type, and P-type Ca²⁺ channels.

Effects of nitric oxide on cytosolic Ca²⁺ dynamics in mouse pancreatic islets.

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1. The purpose of the present study is to explore effects of nitric oxide (NO) on changes in cytosolic Ca²⁺ concentration ([Ca²⁺]c) and to clarify possible sites of action of NO in mouse pancreatic β cells. A microfluorometric method was applied by using Fura-2, a fluorescent Ca²⁺ indicator, in the isolated perifused preparations of mouse pancreatic islets.

2. Increasing glucose concentration of perifusing solution from 3 mM to 10 mM caused biphasic increases in [Ca²⁺]c, the first transient rise (first phase) followed by a continuous [Ca²⁺]c increase on which oscillatory fluctuation was often superimposed (second phase). The first [Ca²⁺]c increase was completely abolished by the addition of 400 μM NOR3, a spontaneous NO donor. The second phase was also calmed by NOR3 (200 μM). This inhibitory effect by NOR3 on glucose-induced [Ca²⁺]c rises was restored by pretreatment with 10 μM oxyhemoglobin, a NO scavenger.

3. The addition of SIN-1, which is known to produce NO and O₂⁻, and resultant peroxynitrite tended to reduce the second phase of [Ca²⁺]c increase induced by 15 mM glucose. Superoxide dismutase which scavenges produced O₂⁻ and thus reduces peroxynitrite production amplified the inhibitory effect by SIN-1. Based on these results, it is suggested that NO but not peroxynitrite plays a major role in the inhibition
by NO donors of glucose-induced $[\text{Ca}^{2+}]_c$ dynamics.

4. A $K^+_{\text{ATP}}$ channel inhibitor, tolbutamide (300 
$\mu$M), caused a $[\text{Ca}^{2+}]_c$ rise and this increase was also inhibited by NOR3 (200 $\mu$M). The inhibition by NOR3 was restored by oxyhemoglobin. A high $K^+$ (50 mM)-induced transient $[\text{Ca}^{2+}]_c$ rise was not influenced by NOR3 (400 $\mu$M). These results suggest that NO has no direct action on voltage-dependent $\text{Ca}^{2+}$ channels, but it opens $K^+_{\text{ATP}}$ channels directly or indirectly, resulting in cessation of glucose-induced $[\text{Ca}^{2+}]_c$ dynamics in mouse pancreatic islet cells.

5. It has been shown that NO causes damage on DNA strands, which initiates an ATP-consuming repair process by activation of poly(ADP-ribose) synthetase (PARS), causing a reduction of cytosolic ATP concentration ($[\text{ATP}]_c$). There would be a possibility that this reduction of $[\text{ATP}]_c$ might be related to NO-induced inhibition of $[\text{Ca}^{2+}]_c$ dynamics. This possibility was examined by using 3-aminobenzamide (3-AB), a PARS inhibitor. In the presence of 3-AB (1 mM), the inhibitory effect by NOR3 on glucose-induced $[\text{Ca}^{2+}]_c$ dynamics was not affected. This result suggests that the ATP-consuming PARS cascade is not directly involved in the NO-induced inhibition. In conclusion, it is implied that NO but not peroxynitrite interferes with glucose-induced closure of $K^+_{\text{ATP}}$ channels probably via reduction of mitochondrial ATP production in mouse pancreatic $\beta$ cells.

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Ca$^{2+}$ CHANNEL SUBTYPES IN GUINEA PIG ADRENAL CHROMAFFIN CELLS

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1. The effects of selective Ca$^{2+}$ channel blockers on Ca$^{2+}$ currents and 60 mM $K^+$-induced catecholamine release were examined to investigate the subtypes of Ca$^{2+}$ channels and their contribution to catecholamine release in isolated guinea pig adrenal chromaffin cells.

2. Application of nifedipine (3 $\mu$M) for 4 min, an inhibitor of L-type Ca$^{2+}$ channel, $\omega$-conotoxin GVIA (1 $\mu$M), an inhibitor of N-type Ca$^{2+}$ channel, $\omega$-agatoxin IVA (0.1 $\mu$M), an inhibitor of P-type Ca$^{2+}$ channel and $\omega$-conotoxin MVIIC (3 $\mu$M), an inhibitor of N/P/Q-type Ca$^{2+}$ channel, inhibited peak amplitude of Ca$^{2+}$ current by 33%, 15%, 23%, 33%, respectively.

3. When nifedipine, $\omega$-conotoxin GVIA, $\omega$-agatoxin IVA and $\omega$-conotoxin MVIIC were applied sequentially onto the same cell, Ca$^{2+}$ current was inhibited additively. This result suggests that guinea pig adrenal chromaffin cells possess at least L-, N-, P- and Q-type Ca$^{2+}$ channels.

4. Even after L-, N-, P- and Q-type Ca$^{2+}$ currents were inhibited by selective Ca$^{2+}$ channel blockers (nifedipine (3 $\mu$M), $\omega$-conotoxin GVIA (1 $\mu$M), $\omega$-agatoxin IVA (0.1 $\mu$M) and $\omega$-conotoxin MVIIC (3 $\mu$M), Ca$^{2+}$ currents, with the amplitude of about 23% of control cur rents, were evoked by the depolarizing pulses to $+10$ mV for 50 ms from a holding potential $-70$ mV.

5. The Ca$^{2+}$ current insensitive to these Ca$^{2+}$ channel blockers was considered to be mediated through R-type Ca$^{2+}$ channel (one of high voltage activated Ca$^{2+}$ channels) or T-type Ca$^{2+}$ channel (typical low voltage activated Ca$^{2+}$ channel).