but their peak levels decreased by about 18%.
3. Removal of $CaCl_2$ from the perifusing solution almost completely abolished CCK-8-induced increases in $[Ca^{2+}]c$. This result suggests that the presence of $CaCl_2$ in extracellular space of the islet cells is essential for $[Ca^{2+}]c$ dynamics induced by CCK-8. This phenomenon may be explained by one of two separate hypothesis ; 1) Ca^{2+} influx is important for CCK-8-induced $[Ca^{2+}]c$ increase or 2) refilling of Ca^{2+} in intracellular Ca^{2+} stores is mandatory for $[Ca^{2+}]c$ increase.
4. Addition of low concentration of $NiCl_2$ which is shown to selectively block T-type Ca^{2+} channel strongly inhibited CCK-8-induced $[Ca^{2+}]c$ increase, suggesting that Ca^{2+} influx via T-type Ca^{2+} 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 Ca^{2+} channel blocker, ω-conotoxin GVIA, a selective N-type Ca^{2+} channel blocker, ω-conotoxin MVIIC, a selective Q-type Ca^{2+} channel blocker, and ω-agatoxin IVA, a selective P-type Ca^{2+} channel blocker, were all without effect on CCK-8-induced $[Ca^{2+}]c$ increase.
6. Possible involvement of Ca^{2+} influx by CCK-8 stimulation was also supported by following evidence. U73122, a PLC inhibitor, had no effect on CCK-8-induced $[Ca^{2+}]c$ increase, suggesting that PLC-IP_3-Ca^{2+} release cascade is not functioning.
7. It was concluded that CCK may physiologically participate in regulation of pancreatic endocrine secretion by modulating cytosolic Ca^{2+} dynamics which are brought about by possible activation of T-type Ca^{2+} channel but not by L-type, N-type, Q-type, and P-type Ca^{2+} channels.

Effects of nitric oxide on cytosolic Ca^{2+} 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 Ca^{2+} concentration ($[Ca^{2+}]_c$) 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 Ca^{2+} 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 $[Ca^{2+}]_c$, the first transient rise (first phase) followed by a continuous $[Ca^{2+}]_c$ increase on which oscillatory fluctuation was often superimposed (second phase). The first $[Ca^{2+}]_c$ 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 $[Ca^{2+}]_c$ 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 $O_2^{\cdot -}$, and resultant peroxynitrite tended to reduce the second phase of $[Ca^{2+}]_c$ increase induced by 15 mM glucose. Superoxide dismutase which scavenges produced $O_2^{\cdot -}$ 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 $[Ca^{2+}]_c$ dynamics.

4. A K^+_{ATP} channel inhibitor, tolbutamide (300 μM), caused a $[Ca^{2+}]_c$ rise and this increase was also inhibited by NOR3 (200 μM). The inhibition by NOR3 was restored by oxyhemoglobin. A high K^+ (50 mM)-induced transient $[Ca^{2+}]_c$ rise was not influenced by NOR3 (400 μM). These results suggest that NO has no direct action on voltage-dependent Ca^{2+} channels, but it opens K^+_{ATP} channels directly or indirectly, resulting in cessation of glucose-induced $[Ca^{2+}]_c$ 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 ($[ATP]_c$). There would be a possibility that this reduction of $[ATP]_c$ might be related to NO-induced inhibition of $[Ca^{2+}]_c$ 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 $[Ca^{2+}]_c$ 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^+_{ATP} channels probably via reduction of mitochondrial ATP production in mouse pancreatic β cells.

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 μM) for 4 min, an inhibitor of L-type Ca^{2+} channel, ω-conotoxin GVIA (1 μM), an inhibitor of N-type Ca^{2+} channel, ω-agatoxin IVA (0.1 μM), an inhibitor of P-type Ca^{2+} channel and ω-conotoxin MVIIC (3 μM), 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, ω-conotoxin GVIA, ω-agatoxin IVA and ω-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 μM), ω-conotoxin GVIA (1 μM), ω-agatoxin IVA (0.1 μM) and ω-conotoxin MVIIC (3 μM), Ca^{2+} currents, with the amplitude of about 23% of control curents, 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).