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but their peak levels decreased by about 18%.

3. Removal of CaCl₂ from the perfusing 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 hypotheses: 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 perfused preparations of mouse pancreatic islets.

2. Increasing glucose concentration of perfusing 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
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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 \(\text{poly(ADP-ribose)} \text{ synthetase (PARS)}\), 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 
\([\text{Ca}^{2+}]_c\) dynamics. This possibility was ex-
amined by using 3-aminobenzamide (3-AB), a 
PARS inhibitor. In the presence of 3-AB (1 
\(\text{mM}\)), 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 im-
plicated 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 \(\text{Ca}^{2+}\) channel blockers 
on \(\text{Ca}^{2+}\) currents and 60 mM \(K^{+}\)-induced 
catecholamine release were examined to investi-
gate the subtypes of \(\text{Ca}^{2+}\) 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 \(\text{Ca}^{2+}\) channel, \(\omega\)-conotoxin 
GVIA (1 \(\mu\)M), an inhibitor of N-type \(\text{Ca}^{2+}\) 
channel, \(\omega\)-agatoxin IVA (0.1 \(\mu\)M), an inhibitor of 
P-type \(\text{Ca}^{2+}\) channel and \(\omega\)-conotoxin MVIIC (3 
\(\mu\)M), an inhibitor of N/P/Q-type \(\text{Ca}^{2+}\) channel, 
inhibited peak amplitude of \(\text{Ca}^{2+}\) 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, \(\text{Ca}^{2+}\) 
current was inhibited additively. This result 
suggests that guinea pig adrenal chromaffin cells 
possess at least L-, N-, P- and Q-type \(\text{Ca}^{2+}\) 
channels.

4. Even after L-, N-, P- and Q-type \(\text{Ca}^{2+}\) 
currents were inhibited by selective \(\text{Ca}^{2+}\) 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), \(\text{Ca}^{2+}\) 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 \(\text{Ca}^{2+}\) current insensitive to these \(\text{Ca}^{2+}\) 
channel blockers was considered to be mediated 
through R-type \(\text{Ca}^{2+}\) channel (one of high voltage 
activated \(\text{Ca}^{2+}\) channels) or T-type \(\text{Ca}^{2+}\) channel 
(typical low voltage activated \(\text{Ca}^{2+}\) channel).