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Effects of Calcium Channel Blockers on Insulin Secretion and $^{45}Ca^{2+}$-Uptake of Rat Islets Stimulated by Glucose or K^+-Depolarization

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Two calcium channel antagonists, verapamil and nifedipine, have been used to explore the dependence of secretion on voltage-gated influx of calcium. Both antagonists were able to suppress the secretory response to K^+-depolarization as well as the stimulation of $^{45}Ca^{2+}$-uptake. However, they inhibited only partially the stimulation of both secretion and $^{45}Ca^{2+}$-uptake induced by glucose, alone or with palmitate. The stimulation of $^{45}Ca^{2+}$-uptake by K^+-depolarization, unlike that induced by glucose, was not sensitive to norepinephrine, starvation or fatty acid oxidation inhibitors. Therefore, it is suggested that glucose either modifies the properties of the voltage-dependent calcium channel and/or accelerates the exchange of a particular intracellular pool of calcium.

Key-words: Islets, Insulin secretion, Calcium channel antagonists, Calcium fluxes.

It is generally accepted that insulin secretagogues (glucose, aminoacids and sulfonylureas) stimulate secretion mainly by depolarizing the plasma membrane of B-cells through different mechanisms that subsequently result in the opening of voltage-dependent calcium channels and an increase of the cytoplasmic calcium concentration (9, 15). Direct depolarization of the plasma membrane with high potassium increases cytoplasmic calcium but stimulates insulin secretion only transiently (8), suggesting that other messenger systems are also probably activated by physiological secretagogues. Specific $^{45}Ca^{2+}$-channel antagonists (11) of the phenylalkylamine-(verapamil, D-600) or the dihydropyridine-type (nifedipine) have been used to test whether voltage-activat-

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ed channels are operative in B-cells and whether they may fully account for secretagogue-induced increase of cytoplasmic calcium and insulin release. It has become evident that verapamil inhibits with similar sensitivity the increase of both cytoplasmic calcium and insulin release in response to K^+-depolarization (2, 4, 6) and sulfonylureas (7). However, islet stimulation by nutrient secretagogues (2, 3, 6, 16) is less sensitive to verapamil than the response to K^+-depolarization. Recent studies of the current (barium)-voltage relationship in mouse pancreatic B-cells (10) and of the current (calcium)-channel blocker dose-response curve in hemagglutination inhibition test (HIT) cells (4), support the existence of a unique calcium channel which is voltage-dependent and dihydropyridine sensitive. Quantitative as well as qualitative differences between the effects of different calcium-channel antagonists on insulin secretion have been attributed to different sites of action (verapamil vs. nifedipine) (1) or the existence of dihydropyridine-sensitive and -insensitive channels (5).

In the present work, we have investigated whether two different types of calcium channel antagonists (verapamil and nifedipine) are able to block proportionately the initial uptake of $^{45}Ca^{2+}$ (into a lanthanum-nondisplaceable pool) and insulin secretion. Their ability was comparatively tested on isolated rat islets stimulated by both K^+-depolarization and glucose. The latest was used alone or in combination with palmitate. This fatty acid accelerates, in a dose-and metabolically-dependent manner, the islet $^{45}Ca^{2+}$-turnover already initiated by glucose (13).

Materials and Methods

Islets were collagenase-isolated from the pancreas of Wistar-Albino rats (adult males). Insulin secretion was studied in batch type incubations of isolated islets in Krebs-Ringer-bicarbonate buffer, equilibrated with O_2:CO_2 (19:1) to pH = 7.4 and containing 2 % (w/v) bovine serum albumin (fatty-acid free). Insulin was radioimmunologically measured in the incubation medium (13). $^{45}Ca^{2+}$-uptake was measured as the radioisotope content of isolated islets which, after variable periods of incubation at 37 °C, were finally washed in a medium containing lanthanum chloride ($LaCl_3$) (13). This lanthanum-wash technique allows a better discrimination between extracellular (La^{3+}-displaceable) and intracellular (La^{3+}-nondisplaceable) $^{45}Ca^{2+}$. Stock solutions of calcium-channel antagonists were freshly prepared every day and the experiments with nifedipine were performed under a yellow light for minimizing its photo-decomposition.

Results

Insulin release. — Depolarizing B-cells with 25 mmol/l K^+ increased more than twofold the basal rate of insulin secretion

Table I. *Insulin response (ng/islet × h) of isolated islets to 25 mmol/l K^+ is reduced by norepinephrine, verapamil and nifedipine.*

Values denote means ± S.E.M.; numbers of animals used in each experimental condition, 5. Statistical comparisons were performed by the non-paired Student's t test. Abbreviations: G3, 3 mmol/l glucose; 10^{-6} NE, 10^{-6} mol/l norepinephrine; 10^{-5} Y, 10^{-5} mol/l yohimbine; 5×10^{-6} V and 5×10^{-5} V, 5×10^{-6} mol/l and 5×10^{-5} mol/l verapamil, respectively; 10^{-7} Nf, 10^{-7} mol/l nifedipine.

Additives	Insulin	p
G3	4.6 ± 0.24	—
G3 + 10^{-6} NE	1.4 ± 0.13	< 0.001
G3 + 10^{-6} NE + 10^{-5} Y	4.4 ± 0.42	N.S.
G3 + 5×10^{-6} V	2.5 ± 0.24	< 0.001
G3 + 5×10^{-5} V	1.5 ± 0.19	< 0.001
G3 + 10^{-7} Nf	1.7 ± 0.08	< 0.001

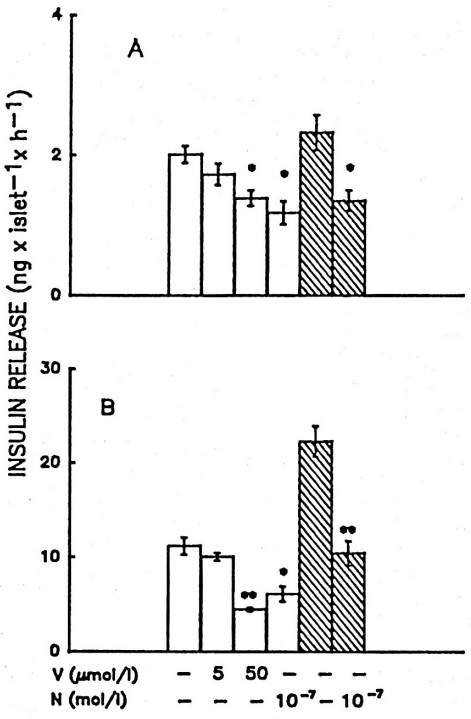

Fig. 1. *Insulin secretion at 3 (A) and 20 (B) mmol/l glucose, alone or with 1 mmol/l palmitate (hatched columns), is reduced by verapamil (V) and nifedipine (N).*
Number of experiments = 5 in all groups. * $p < 0.01$) and ** $p < 0.001$, compared with the corresponding control (first column to the left).

recorded at 3 mmol/l glucose during 60 min of incubation (4.6 ± 0.24, n = 5 vs 2.01 ± 0.12 ng/islet × h, n = 5; $p < 0.001$). This increase was completely prevented by 10^{-6} mol/l of norepinephrine, 5×10^{-5} mol/l verapamil or 10^{-7} mol/l nifedipine (table I). The effect of norepinephrine was fully antagonized by 10^{-5} mol/l yohimbine. A lower dose of verapamil (5×10^{-6} mol/l) decreased partially the secretory response to K^+-depolarization ($p < 0.02$ between the effects of the two doses). Similar results were obtained in islets isolated from 48 h starved rats (not shown).

Both calcium-channel antagonists, verapamil 5×10^{-5} mol/l) and nifedipine 10^{-7} mol/l), decreased significantly insulin secretion at 3 mmol/l glucose (fig. 1). The secretory response to 20 mmol/l glucose was reduced within 50 % by both nifedipine and the higher dose of verapamil. The lower concentration of verapamil was without effect on insulin secretion at either 3 or 20 mmol/l glucose. Nifedipine reduced also within 50 % the secretory response to glucose and palmitate (fig. 1).

$^{45}Ca^{2+}$-uptake. — Islet uptake of $^{45}Ca^{2+}$ increased linearly during the first 15 minutes of K^+-depolarization at 3 mmol/l glucose (fig. 2), tending apparently to a steady-state after 120 min. The initial rate of uptake (15 min) induced by K^+-depolarization was intermediate between that obtained at 3 mmol/l (3.5 ± 0.08, n = 5 vs 1.5 ± 0.23 pmol/islet, n = 3; $p < 0.001$) and 20 mmol/l glucose (3.5 ± 0.08, n = 5 vs. 4.7 ± 0.11 pmol/islet, n = 3; $p < 0.001$). It was completely suppressed by either nifedipine and 5×10^{-5} mol/l verapamil and norepinephrine did not induced any significant effect. Qualitatively similar results were obtained after 120 min of incubation (long-term $^{45}Ca^{2+}$-uptake) (table II). Neither the initial nor the long-

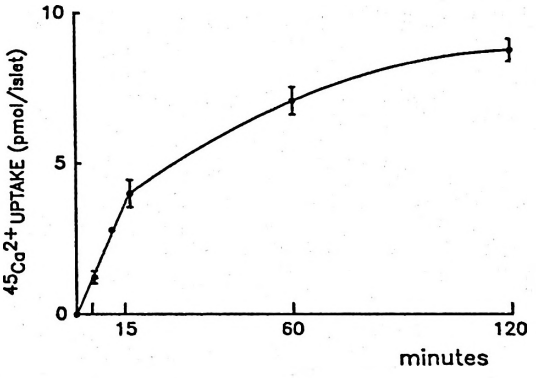

Fig. 2. *Time-kinetics of islet $^{45}Ca^{2+}$-uptake induced by K^+-depolarization in the presence of 3 mmol/l glucose (n = 4 in all time-points).*

Table II. *Short (15 min)- and long (120 min)-term $^{45}Ca^{2+}$-uptake (pmol × islet^{-1}) induced by 25 mmol/l K^+ in the presence of 3 mmol/l glucose and their modification by norepinephrine, verapamil and nifedipine.*
Values denote means ± S.E.M.; numbers of animals used in each experiment are shown in parentheses. Abbreviations: G3, 3 mmol/l glucose; 10^{-6} NE, 10^{-6} mol/l norepinephrine; 5 × 10^{-6} V and 5 × 10^{-5} V, 5 × 10^{-6} mol/l and 5 × 10^{-5} mol/l verapamil, respectively; 10^{-7} Nf, 10^{-7} mol/l nifedipine.

Additives	15 min	p	120 min	p
G3	3.5 ± 0.08 (5)	—	8.4 ± 0.95 (6)	—
G3 + 10^{-6} NE	3.3 ± 0.11 (5)	N.S.	8.3 ± 0.60 (8)	N.S.
G3 + 5 × 10^{-6} V	2.2 ± 0.13 (5)	< 0.001	3.8 ± 0.24 (9)	< 0.001
G3 + 5 × 10^{-5} V	1.1 ± 0.14 (5)	< 0.001	3.2 ± 0.10 (9)	< 0.001
G3 + 10^{-7} Nf	1.3 ± 0.06 (5)	< 0.001	2.7 ± 0.12 (7)	< 0.001

term $^{45}Ca^{2+}$-uptake was affected by 48 h starvation or addition of the fatty acid oxidation inhibitors 2-bromostearate (0.25 mmol/l) or 2-tetradecylglycidate (10 µmol/l) (not shown).

Verapamil (5 and 50 × 10^{-6} mol/l) did not modify short-term $^{45}Ca^{2+}$-uptake at 3 and 20 mmol/l glucose, either alone or with 1 mmol/l palmitate (table III). The long-term uptake was only reduced (within 50 %) by the higher dose of verapamil. Nifedipine decreased both the short- and long-term $^{45}Ca^{2+}$-uptake induced by glucose, alone or with palmitate, by approximately 50 % (fig. 3).

Discussion

The present results strongly suggest that inhibition of calcium influx by verapamil and nifedipine brings about the suppression of the secretory response to K^+-depolarization. Similar results were obtained with verapamil in isolated islets (6) and either verapamil (2, 16) or nimodipine (4)

Table III. *Short (15 min)- and long (120 min)-term $^{45}Ca^{2+}$-uptake (pmol × islet^{-1}) at 3 and 20 mmol/l glucose, alone or with 1 mmol/l palmitate and their modification by verapamil.*
Values denote means ± S.E.M. and they were statiscally compared by the nonpaired Student's t test. Numbers of animals used in each experiment are given in parentheses. Abbreviations: G3 and G20, 3 mmol/l and 20 mmol/l glucose, respectively; P1, 1 mmol/l palmitate; 5 × 10^{-6} V and 5 × 10^{-5} V, 5 × 10^{-6} mol/l and 5 × 10^{-5} mol/l verapamil, respectively.

Additives	15 min	p	120 min	p
G3	1.1 ± 0.08 (5)	—	3.4 ± 0.13 (7)	—
G3 + 5 × 10^{-6} V	1.2 ± 0.20 (6)	N.S.	3.1 ± 0.32 (5)	N.S.
G3 + 5 × 10^{-5} V	1.4 ± 0.08 (5)	N.S.	3.6 ± 0.33 (5)	N.S.
G3 + P1	1.4 ± 0.27 (4)	—	3.9 ± 0.17 (4)	—
G3 + P1 + 5 × 10^{-6} V	1.6 ± 0.24 (5)	N.S.	3.4 ± 0.33 (5)	N.S.
G3 + P1 + 5 × 10^{-5} V	1.6 ± 0.28 (5)	N.S.	3.0 ± 0.27 (5)	< 0.05
G20	4.9 ± 0.19 (6)	—	13.0 ± 0.66 (7)	—
G20 + 5 × 10^{-6} V	4.4 ± 0.14 (7)	N.S.	13.1 ± 0.87 (5)	N.S.
G20 + 5 × 10^{-5} V	5.0 ± 0.29 (5)	N.S.	4.7 ± 0.24 (5)	< 0.001
G20 + P1	10.1 ± 0.86 (5)	—	13.6 ± 1.04 (8)	—
G20 + P1 + 5 × 10^{-6} V	9.5 ± 0.61 (5)	N.S.	13.8 ± 0.68 (6)	N.S.
G20 + P1 + 5 × 10^{-5} V	9.2 ± 0.77 (5)	N.S.	6.0 ± 0.64 (6)	< 0.001

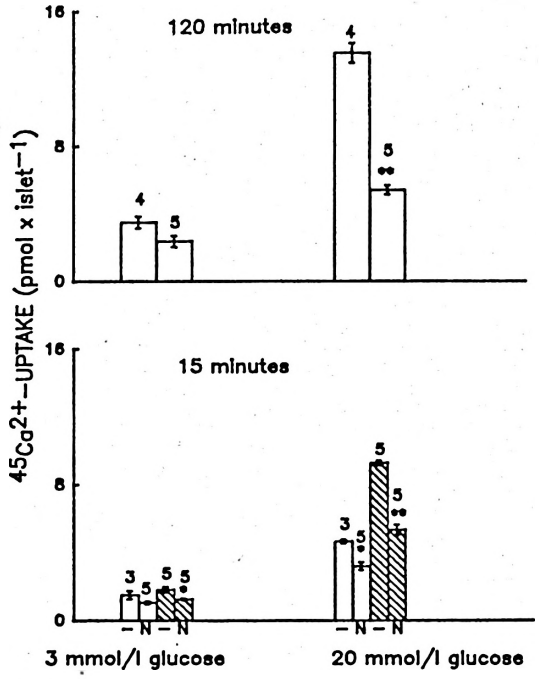

Fig. 3. *Short (15 min)- and long (120 min)-term $^{45}Ca^{2+}$-uptake induced by glucose, alone or with 1 mmol/l palmitate (hatched columns) are reduced by nifedipine (N).*
The number of experiments is given at the top of each column. * $p < 0.01$ and ** $p < 0.001$, compared with the corresponding control (first column to left within each glucose concentration).

has also been shown to fully antagonize the increase of both cytoplasmic calcium and hormone secretion evoked by K^+-depolarization in insulin producing cells. Therefore, it seems clear that K^+-stimulation of insulin secretion may be fully accounted for by the opening of voltage-gated calcium channels which are sensitive to the known types of channel-antagonists. In a recent report, the lack of ability of 10 μmol/l nifedipine to completely suppress K^+-depolarization-induced insulin secretion, in front of a full inhibition of glucose-stimulated release, was argued in favour of the existence of dihydropyridine-insensitive, voltage-dependent channels in rat islets (5). However, this indirect evidence should be confirmed by investigating nifedipine effects on calcium fluxes. Norepinephrine inhibits the secretory response to K^+-depolarization without altering $^{45}Ca^{2+}$-uptake whereas it reduces proportionately both parameters in glucose-stimulated islets (14). This confirms a previous report (8) supporting the idea of the amine acting at a site in the stimulus-secretion coupling which is distal to the generation of second messengers (12).

At variance with K^+-depolarization, glucose-stimulation of insulin secretion was only partially inhibited by either verapamil or nifedipine. However, nifedipine exerted a proportional reduction of both short-and long-term $^{45}Ca^{2+}$-uptake, whereas verapamil only inhibited the long-term uptake at the higher concentration. A decreased sensitivity to verapamil of nutrient as compared to K^+-stimulation has already been reported (2, 3, 6, 16) and our results demonstrate that the same applies qualitatively to nifedipine.

Therefore, glucose might be able to either change the properties of the voltage-dependent calcium channels and/or to open another type of calcium channels (6). There is no direct experimental support for any of these hypothetical explanations. However, a change of channel properties might explain the relatively higher resistance of glucose stimulated $^{45}Ca^{2+}$-influx and insulin release to verapamil as compared to nifedipine since these antagonists are supposed to act on different sites of the channel (11). Activation of a hypothetical voltage-non dependent calcium channel by glucose would not fully explain why the apparent size of the exchangeable pool of intracellular calcium (120 min $^{45}Ca^{2+}$-uptake) is almost two-fold greater than that recorded after K^+-depolarization. Therefore, it seems more plausible that glucose, besides activating (and modifying?) the voltage-dependent Ca^{2+}-channels, also enhances

(or initiates) the turnover of an intracellular pool of calcium not affected by K^+-depolarization. In support of this suggestion, $^{45}Ca^{2+}$-uptake induced by K^+-depolarization was affected neither by norepinephrine nor by starvation and fatty acid-oxidation inhibitors that are known to inhibit and restore, respectively, the glucose-dependent uptake (13).

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Resumen

Se utilizan dos antagonistas de canales de calcio, verapamil y nifedipina, para estudiar la dependencia de la secreción de insulina con respecto a la apertura de canales de calcio dependientes del voltaje. Ambos antagonistas suprimen la captación inicial de calcio y la secreción de insulina en respuesta a la depolarización por K^+. Sin embargo, sólo inhiben parcialmente la secreción de insulina y captación de $^{45}Ca^{2+}$ inducidas por glucosa, sola o en presencia de ácido palmítico. La norepinefrina inhibe la respuesta secretora a la depolarización por K^+ sin alterar la captación de $^{45}Ca^{2+}$, mientras que reduce ambos parámetros en islotes estimulados por glucosa. El ayuno o los inhibidores de la oxidación de ácidos grasos tampoco modifican la captación de $^{45}Ca^{2+}$ inducida por depolarización con K^+. Por tanto, se sugiere que la glucosa podría actuar modificando las propiedades de canales de calcio dependientes del voltaje y/o acelerando el recambio de un compartimiento intracelular de calcio.

Palabras clave: Islotes, Secreción de Insulina, Captación de calcio, Antagonistas de canales de calcio.

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