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Trifluoperazine Reproduces in Rat Islets the Effects of Calcium Omission on Insulin Secretion and *de novo* Lipid Synthesis, without Affecting $^{45}Ca^{2+}$-Uptake

E. Vara, C. García and J. Tamarit-Rodríguez*

Departamento de Bioquímica
Facultad de Medicina
28040 Madrid (Spain)

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Calmodulin is thought to mediate at least some of the effects produced by the elevation of cytosolic calcium in response to a B-cell secretagogue. Trifluoperazine, an inhibitor of calcium-calmodulin interaction, has been used to test, comparatively with calcium-omission, whether the changes of lipid metabolism accompanying the stimulation of insulin release by glucose and palmitate are dependent on activation by the calcium binding protein. Low doses of trifluoperazine (1 and 5 µmol/l) reproduced quantitatively and qualitatively the effects of calcium omission on both insulin secretion and *de novo* lipid synthesis, without altering islet $^{45}Ca^{2+}$-uptake. The apparent dependence on calcium-calmodulin of the «de novo» synthesis of neutral lipids, but not of acidic phospholipids, might reflect a possible regulation of islet phosphatidate phosphohydrolase by calcium.

Key words: Islets, Insulin Secretion, Lipid Synthesis, $^{45}Ca^{2+}$-uptake, Trifluoperazine.

Palmitate-potentiation of glucose-induced insulin secretion in rat islets has been demonstrated to accompany an increased rate of *de novo* lipid synthesis and of $^{45}Ca^{2+}$-turnover (18). These three phenomena are almost suppressed in islets from 48 h starved rats and they are restored by specific inhibitors of fatty acid oxidation that divert palmitate towards esterification into islet lipids (14, 18, 19). This experimental evidence points to a cause-effect relationship between palmitate esterification into islet lipids (*de novo* synthesis) and the acceleration of $^{45}Ca^{2+}$-turnover and insulin release. The stimulus induced elevation of cytosolic calcium (4) resulting from the modification of islet calcium-fluxes might conceavably lead to an increased secretion of insulin through calmodulin-mediated activation of enzyme activities or granule-plasma mem-

* To whom all correspondence should be addressed.

brane interactions (17). Trifluoperazine, a known inhibitor of calcium-calmodulin interaction (21), is also able to inhibit glucose-stimulation of insulin secretion (2, 6, 9-11, 15, 16). It has been used in the present work to investigate, comparatively to the effect of calcium-omission, the calcium-dependency of the «de novo» synthesis of the different islet lipids in relation to that of insulin release and $^{45}Ca^{2+}$-uptake.

Materials and Methods

D-(U-^{14}C) glucose, $^{45}CaCl_2$, and Na^{125}I were from The Radiochemical Centre, Amersham. Activated charcoal, Hepes (4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid) and standard lipids were from Sigma. Fatty acid-poor bovine serum albumin was from Miles Laboratories, Kankakee, IL. Crystalline pig and rat insulin were a generous gift from Novo Industri, Copenhagen (Denmark). Sephadex G-25 and G-75 were from Pharmacia Fine Chemicals. Collagenase, calf thymus DNA and trifluoperazine were from Boehringer Mannheim. 3,5-Diaminobenzoate dihydrochloride and Tris (hydroxymethyl) aminomethane (TRIS) were from Aldrich-Chemie, Steinheim, F.R.G. Versilube F-50 was from Klöckner, Duisburg (F.R.G.). Palmitic acid was from Serva, Heidelberg (F.R.G.). All organic solvents and inorganic salts were of analytical grade, from Merck, Darmstadt (F.R.G.).

Islets were isolated by collagenase digestion (12) of the pancreas from adult Wistar Albino rats (males weighing 250 g) fed *ad libitum*. Insulin secretion was studied in batch-type incubations (3 islets/300 µl) in Krebs Ringer bicarbonate buffered with 10 mmol/l Hepes, equilibrated with O_2/CO_2 (19:1), pH 7.4, and containing 2 % (w/v) albumin (fatty-acid free). ^{125}I-Iodoinsulin was prepared from crystalline pig insulin (8) and purified by gel filtration in Sephadex G-25 and G-75. Crystalline rat insulin was used as standard of radioimmunoassay (7).

The *de novo* synthesis of lipids was measured by the incorporation of D-(U-^{14}C) glucose (17 or 56 Ci/mol at 3 or 20 mmol/l, respectively), as previously described (18). DNA was measured in duplicate according to VYTASEK (20). The uptake of $^{45}Ca^{2+}$ into isolated islets was measured with a lanthanum-wash procedure (5, 18) which allows a better discrimination between extracellular and intracellular calcium.

The values represent means ± SEM and the number of animals used in each experimental condition is given by n or indicated in brackets. Statistical comparisons between pairs of means were made with Student's t-tests for non-paired values. A two-tail p value lower than 0.05 between means was accepted as statistically significant.

Results

Increasing the glucose concentration from 3 to 20 mmol/l induced a nine-fold increase of insulin secretion in the presence of 1 mmol/l palmitate (fig. 1). Calcium omission did not affect insulin release at 3 mmol/l glucose but it severely decreased the secretory response to 20 mmol/l. In the presence of trifluoperazine and irrespective of its concentration, the basal secretory rate (at 3 mmol/l glucose) of insulin was reduced to half the value obtained in the absence of calcium. At 1 µmol/l, trifluoperazine induced a statistically similar reduction of the secretory response to glucose than calcium omission. Higher concentrations of the drug allowed significantly smaller responses than calcium omission.

In the presence of 1 mmol/l palmitate, a change of D-(U-^{14}C) glucose concentration from 3 to 20 mmol/l increased several fold (2 to 10) the incorporation of labelled

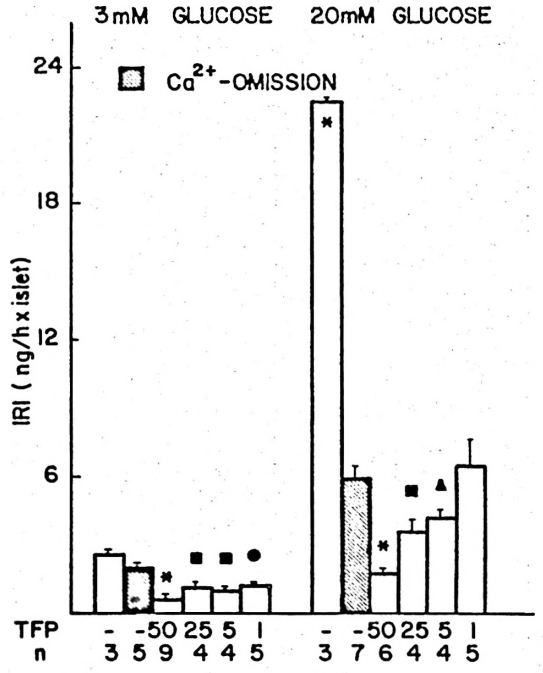

Fig. 1. *Effect of calcium omission and trifluoperazine (TFP, 1 to 50 µmol/l) on basal (3 mmol/l glucose) and stimulated (20 mmol/l glucose) insulin secretion of isolated rat islets.*
*p < 0.001, ■p < 0.02 and ▲p < 0.05 compared with the corresponding value in the absence of calcium.

sugar into the different lipid fractions investigated (fig. 2 and 3). Calcium omission reduced by approximately 50 % the incorporation of 20 mmol/l D-(U-^{14}C) glucose into triacylglycerols, phosphatidylcholine and phosphatidylethanolamine (fig. 2). However, it did not substantially modify labelled sugar incorporation into acidic phospholipids (fig. 3). Trifluoperazine exerted a dose-dependent inhibition of 20 mmol/l D-(U-^{14}C) glucose-incorporation into neutral lipids that was more clearly seen in phosphatidylcholine and triacylglicerols (fig. 2). At 1 and/or 5 µmol/l, the drug-induced decrease was statistically similar to that obtained by the absence of calcium in every neutral lipid investigated and the corresponding levels of labelled-sugar incorporated were significantly lower than those reached at normal calcium in the absence of trifluoperazine (fig. 2). At the lowest concentrations (1 and/or 5 µmol/l), trifluoperazine did not decrease 20 mmol/l D-(U-^{14}C) glucose incoporation into any of the acidic phospholipids (fig. 3).

In the presence of 1 mmol/l palmitate, both the short-term (15 min) and long-term (120 min) $^{45}Ca^{2+}$-uptake were stimulated (7- and 3.5 fold, respectively) by an increase of glucose concentration (from 3 to 20 mmol/l) (fig. 4). At 25 µmol/l, trifluoperazine completely blocked glucose-stimulation and it even drastically reduced long-term $^{45}Ca^{2+}$-uptake at 3 mmol/l glucose. Lower doses (1 and 5 µmol/l) did not exert any statistically significant effect although 5 µmol/l trifluoperazine produced a nonsignificant decrease of both short- and long-term stimulation of islet $^{45}Ca^{2+}$-uptake by 20 mmol/l glucose.

Discussion

Our results confirm the inhibitory action of trifluoperazine upon glucose stimulation of insulin secretion which had previously been reported by others (2, 6, 9-11, 17). They extend this observation showing that the glucose secretory response potentiated by palmitate is also decreased by trifluoperazine which at 1 µmol/l induces an equivalent inhibition to that caused by omission of calcium. Under this condition, the inhibition of secretion is not attributable to an impairment of calcium entry because trifluoperazine did not affect the stimulation of $^{45}Ca^{2+}$-uptake. In previous studies (6, 8, 17) trifluoperazine has been shown to decrease either short-and long-term $^{45}Ca^{2+}$-efflux in parallel to the inhibition of glucose-stimulated insulin secretion. Inhibition of $^{45}Ca^{2+}$-fluxes in these reports might be explained by high (10-50 µmol/l) doses of

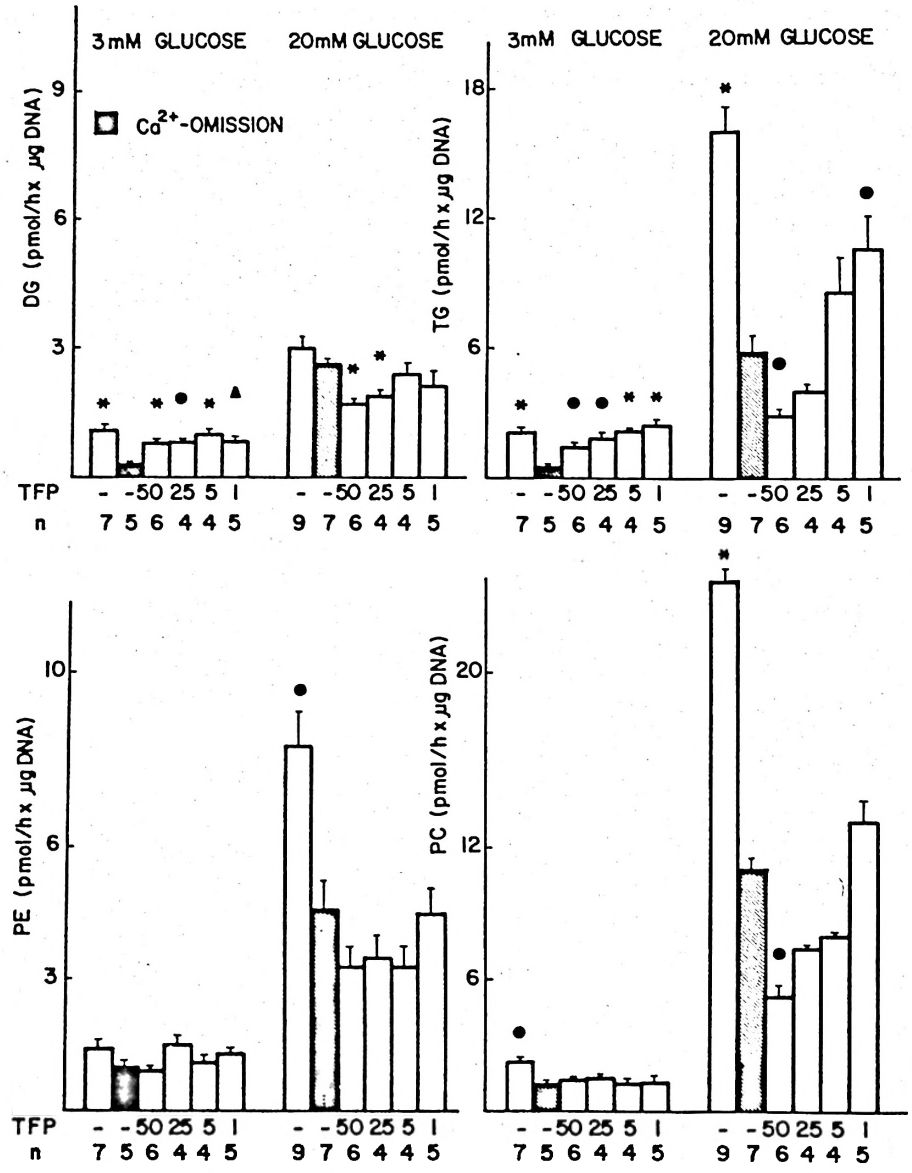

Fig. 2. *Effect of calcium omission and trifluoperazine (TFP 1 to 50 µmol/l) on 3 mmol/l (3 mM, set of columns to the left) and 20 mmol/l (20 mM, set of columns to the right in each panel) D-(U-^{14}C) glucose incorporation into islet diacylglycerols (DG), triacylglycerols (TG), phosphatidylethanolamine (PE) and phosphatidylcholine (PC).*

*p < 0.001, •p < 0.01 and ▲p < 0.05 compared with the corresponding value in the absence of calcium.

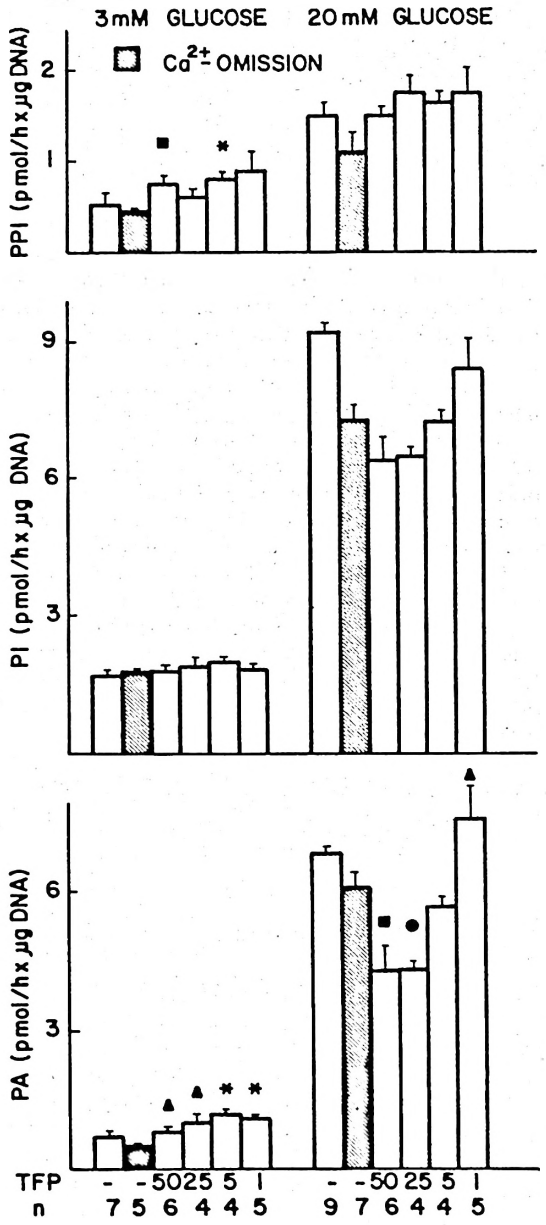

Fig. 3. *Effect of calcium omission and trifluoperazine (TFP, 1 to 50 µmol/l) on 3 mmol/l and 20 mmol/l D-(U-^{14}C) glucose incorporation into islet polyphosphoinositides (PPI), phosphatidylinositol (PI) and phosphatidic acid (PA).*
*p < 0.001, •p < 0.01, ■p < 0.02 and ▲p < 0.05 compared with the corresponding value in the absence of calcium.

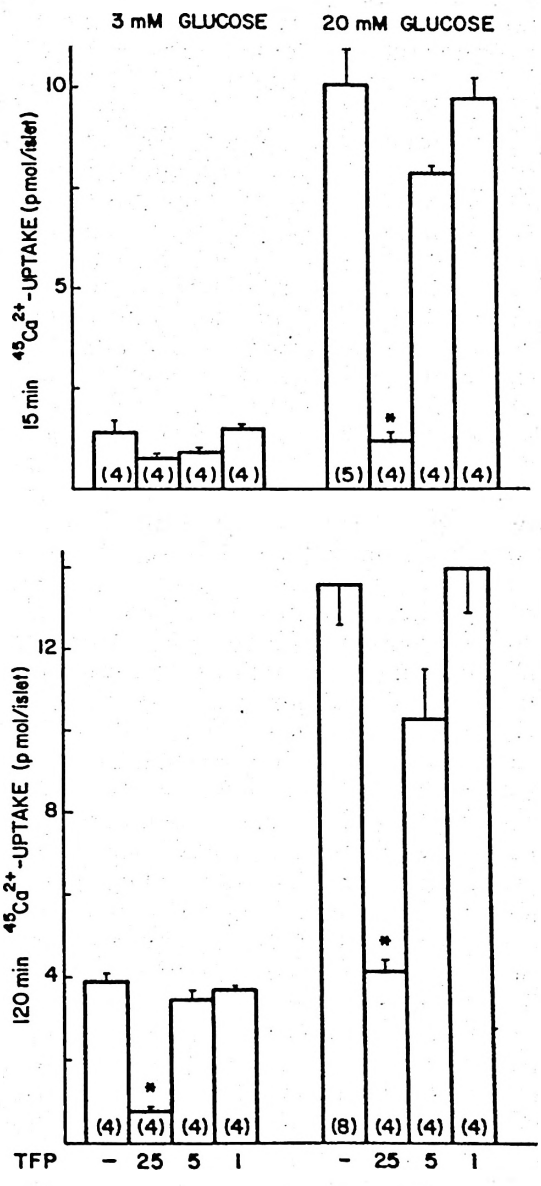

Fig. 4. *Effect of trifluoperazine (TFP, 1 to 25 µmol/l) on either short (15 min, upper panel) —and long (120 min, lower panel)— term $^{45}Ca^{2+}$-uptake at 3 mmol/l (3mM) and 20 mmol/l (20 mM) glucose.*
*p < 0.001 compared with the corresponding value in the absence of trifluoperazine.

trifluperazine or preexposure to the drug causing undesirable side effects (3, 15, 17) like a direct alteration of the membrane permeability to cations.

Trifluoperazine (1 µmol/l) did also mimick, both qualitatively and quantitatively, the effects of calcium omission on islet «de novo» lipid synthesis measured as the incorporation of D-(U-^{14}C) glucose after 2 hours of incubation. This experimental evidence reinforces our previous conclusion on the calcium-dependeny of the *de novo* synthesis of neutral lipids in contrast to the calcium-insensitivity of the *de novo* synthesis of acidic phospholipids (18). It also indirectly suggests that the enzyme phosphatidate phosphohydrolase which catalyzes phosphatidate conversion into diacylglycerol, the precursor of triacylglycerols and neutral phospholipids, may be stimulated by calcium-calmodulin. This enzyme activity is also present in islets and it is apparently translocated to plasma membranes when it is stimulated by glucose (1). This translocation might be promoted or enhanced by calcium-calmodulin. In support of these considerations, displacement of phosphatidate phosphohydrolase from hepatocyte membranes by chlorpromazine, which also binds to calmodulin and antagonizes some of its actions (21), results in the inhibition of triacylglycerol- and phosphatidylcholine-synthesis (13). It might be speculated that the translocation and activation of phosphatidate phosphohydrolase costitutes a possible mechanism of calcium-dependent generation of diacylglycerol at the plasma membrane of islet cells, thus providing one of the activators of protein kinase C.

The lack of effect of trifluoperazine (1 µmol/l) on the stimulation by glucose and palmitate of the *de novo* synthesis of acidic phospholipids strengthens its calcium independence. The calcium-independent stimulation of polyphosphoinositide synthesis might initiate or contribute to maintain an increased production of second messengers (inositol phosphates, diacylglycerol) which would secondarily accelerate calcium turnover and insulin secretion (18).

Resumen

La calmodulina puede mediar alguno de los efectos producidos por la elevación del calcio citoplásmico de las células B en respuesta a un secretagogo. La trifluoperazina, inhibidor de la interacción calcio-calmodulina, se ha utilizado para comprobar, comparativamente con la omisión de calcio, si los cambios del metabolismo lipídico que acompañan a la estimulación de la secreción de insulina por glucosa y palmítico son dependientes de su activación por la proteína combinadora de calcio. A bajas concentraciones (1 y 5 µmol/l), la trifluoperazina reproduce cuantitativamente los efectos de la omisión de calcio, tanto sobre la secreción de insulina como sobre la síntesis *de novo* de lípidos, sin alterar la captación de $^{45}Ca^{2+}$ por los islotes. La dependencia aparente de la síntesis *de novo* de lípidos neutros, pero no de fosfolípidos aniónicos, del complejo calcio-calmodulina podría reflejar una possible regulación de la actividad fosfatídico fosfohidrolasa por calcio.

Palabras clave: Islotes, Secreción de insulina, Síntesis de lípidos, Captación de $^{45}Ca^{2+}$, Trifluoperazina.

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