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Calcium-Antagonists and Islet Function. IV. Effect of D600*

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Summary. D600 (2 to 20 μM; α-isopropyl-α [(N-methyl-N-homoveratril)-γ-aminopropyl]-3,4,5-trimethoxyphenyl-acetonitril) caused a dose-related, rapid and reversible inhibition of glucose-induced insulin release. It also suppressed the insulinotropic action of a sulphonylurea but failed to affect the enhancing action of theophylline upon glucose-induced release. The inhibitory effect of D600 was enhanced at low extracellular Ca^{2+} concentration. D600 reduced both basal and glucose-stimulated ^{45}calcium net uptake, whilst failing to affect the efflux of ^{45}calcium from perifused islets. The recognition of glucose by the B-cell was also unaffected by D600 as judged by the effect of the sugar upon both ^{45}calcium efflux and net uptake in the isolated islets. These findings are compatible with the hypothesis that the primary mode of action of D600 is to inhibit Ca^{2+} entry in the B-cell.

Key words: Insulin release, calcium, D600, glucose, sulphonylurea, theophylline.

If the Ca^{2+} dependency of insulin release is well documented, the precise influence of insulinotropic agents upon the movements of Ca^{2+} in the B-cell requires further investigation. In the search for specific tools to interfere with the handling of Ca^{2+} by islets tissue, we are presently investigating the insular effects of various potential Ca^{2+}-agonists and antagonists [1–3]. The present study, already partially reported in abstract form [4], deals with the effect of D600 (α-isopropyl-α[(N-methyl-N-homoveratril)-γ-aminopropyl]-3, 4, 5-trimethoxyphenyl-acetonitril) upon physiological events in the endocrine pancreas.

* (α – isopropyl- α [(N-methyl-N-homoveratril) – γ – amino-propyl] – 3, 4, 5-trimethoxyphenyl-acetonitril)

Materials and Methods

The method used for the measurement of insulin release by either isolated islets [5] or the isolated perfused pancreas [6] removed from fed rats, and for the uptake [7] and efflux of ^{45}calcium [8] in the isolated islets are described in detail elsewhere. D600 (Knoll A.G.; Ludwigshafen, FRG) was a gift of Dr. J.J. Dreifuss (Department of Physiology, Geneva University).

Results

Effect of D600 upon Insulin Release by Isolated Islets

D600 (2 to 20 μM) caused a dose-related inhibition of glucose-induced insulin release in isolated islets, rates of secretion close to the basal value normally found in the absence of glucose being observed at a D600 concentration of 20 μM (Fig. 1). A reduction in the Ca^{2+} concentration of the incubation medium sensitized the B-cell to the inhibitory effect of D600, so that suppression of insulin release to basal value was now observed with as little as 2 μM D600. Theophylline, on the contrary, apparently antagonized the inhibitory effect of the calcium-antagonist. Thus, at the usual Ca^{2+} concentration (2.0 mEq/1), the enhancing effect of theophylline upon glucose-induced insulin release displayed an invariable relative magnitude whether in the absence (enhancing action: × 2.57) or presence of D600 in concentrations of 2 μM (× 2.59) and 10 μM (× 2.76). Moreover, at a subnormal Ca^{2+} concentration (0.5 mEq/1) and in the presence of theophylline, the rates of glucose-induced insulin release, whether in the absence or presence of D600 (2 μM), were similar or somewhat higher than the corres-

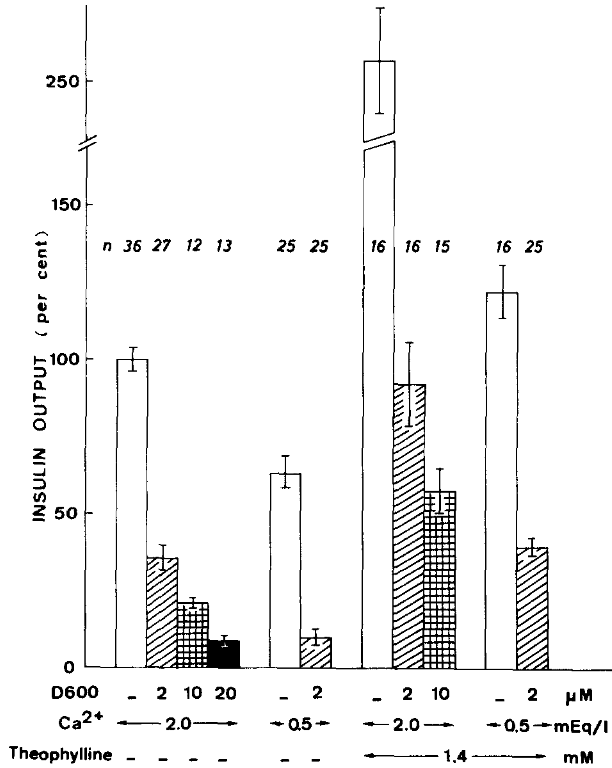

Fig. 1. Effect of D600, Ca^{2+} and theophylline (concentrations shown in the lower part of the figure) upon insulin release evoked by glucose (16.7 mM) in isolated islets. Mean values (± SEM) are shown relative to the mean control rate of secretion found within the same experiment(s) at a Ca^{2+} concentration of 2 mEq/1, in the absence of both D600 and theophylline. Such a control rate of insulin release averaged 182 ± 12 μU/islet per 90 min. Also shown is the number of individual observations (n) in each group. The basal release of insulin (no glucose present) amounts to 8.1 ± 2.6 per cent

ponding values otherwise found at normal Ca^{2+} concentration (2.0 mEq/1) in the absence of theophylline (Fig. 1).

Effect of D600 upon Insulin Release by the Isolated Perfused Pancreas

The method of the isolated perfused pancreas was used to investigate the effect of D600 upon the dynamics of insulin release. D600 inhibited in a dose-dependent fashion both the early peak and later build-up in secretion rate evoked by glucose (Fig. 2). The short-lived secretory peak evoked by gliclazide at a low glucose concentration was also abolished by D600 (Fig. 3). At two calcium levels (2 and 4 mEq/1), the inhibitory effect of the calcium-antagonist was almost immediate and at least partially reversible (Fig. 4).

Effect of D600 upon ^{45}Calcium Net Uptake

D600 (2 to 10 μM) significantly inhibited ($P < 0.005$ or less) both basal and glucose-induced ^{45}calcium net uptake at normal Ca^{2+} concentration (2.0 mEq/1). However, the stimulant action of glucose upon Ca^{2+} net uptake relative to basal value was not less marked in the presence of D600 (4 μM) than in its absence (Table 1). When the extracellular Ca^{2+} concentration was lowered to 0.5 mEq/1, the net uptake of ^{45}calcium found in the absence of D600 was less than at normal Ca^{2+} concentration ($P < 0.025$), and addition of the calcium-antagonist to the incubation medium provoked a further dose-related reduction ($P < 0.02$ or less). Comparison of the data for insulin release

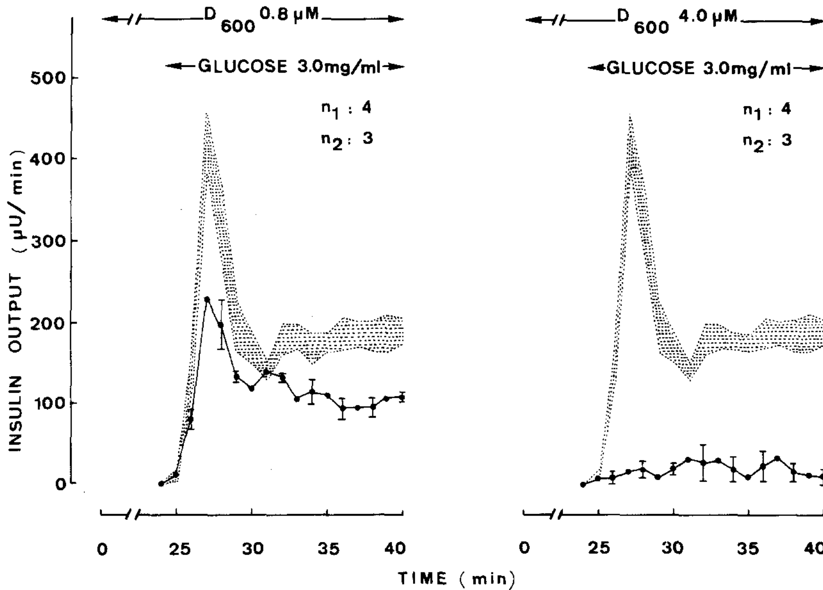

Fig. 2. Effect of D600 upon insulin release evoked by glucose (16.7 mM) in the isolated perfused rat pancreas. Mean values (± SEM) for insulin output in the absence (n_1, shaded area) and presence of D600 (n_2) are shown together with the number of individual experiments in each group (n). The experiments were performed at a Ca^{2+} concentration of 2 mEq/l

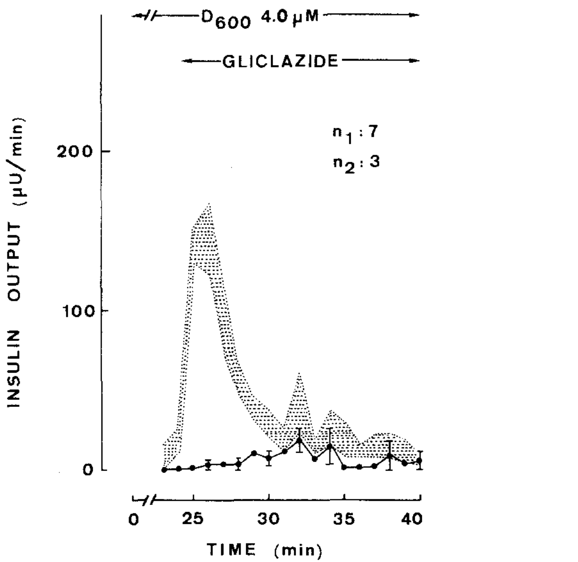

Fig. 3. Effect of D600 upon insulin release evoked by gliclazide (20 μg/ml) in the isolated perfused rat pancreas. Mean values (± SEM) for insulin output in the absence (n_1, shaded area) or presence of D600 (n_2) are shown together with the number of individual experiments in each group (n). Glucose (1.7 mM) was present throughout the experiments, which were performed at a Ca^{2+} concentration of 2 mEq/l

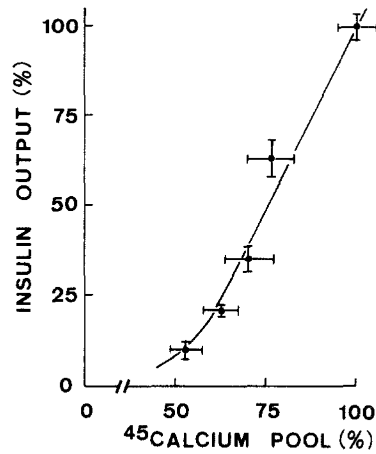

Fig. 5. Effect of D600 and Ca^{2+} on the relationship between glucose-induced insulin output and ^{45}calcium net uptake in isolated islets, both parameters (mean ± SEM) being shown as per cent of the mean control value found at normal Ca^{2+} concentration (2 mEq/l) and in the absence of D600. The experimental data are taken from Fig. 1 and Table 1

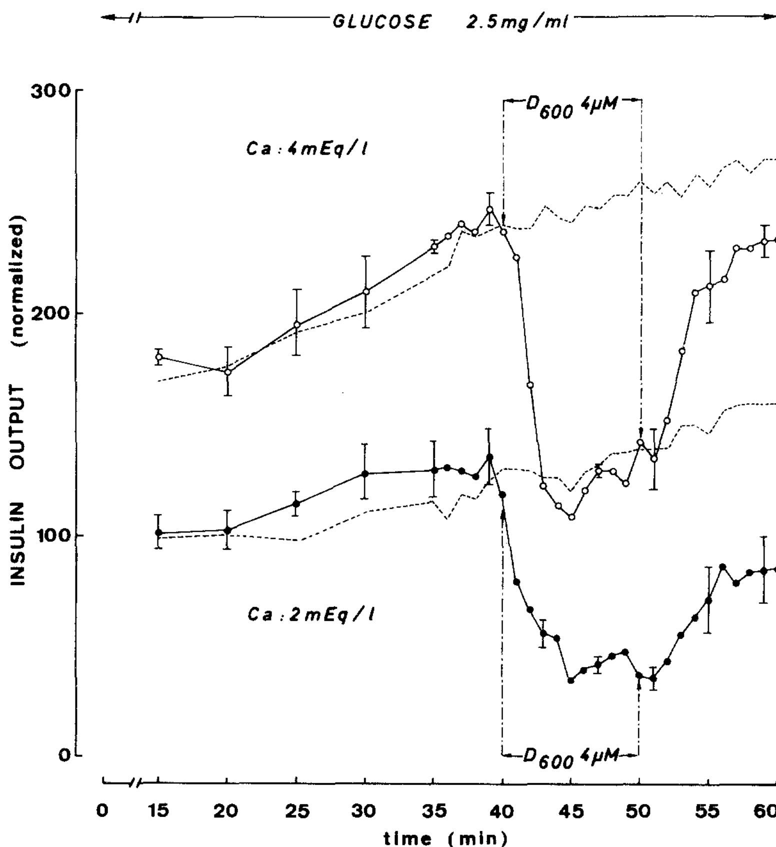

Fig. 4. Rapidity and reversibility of the inhibitory effect of D600 upon glucose-induced insulin release in the isolated perfused rat pancreas. The experiments were performed at two Ca^{2+} concentrations (2 and 4 mEq/l), in the absence (dotted lines) or presence of D600 (administered from the 40th to 50th min; solid lines), glucose (13.9 mM) being invariably present throughout the experiment. All rates of secretion are expressed relative to the mean control value found at a Ca^{2+} concentration of 2 mEq/l between the 15th and 20th min. Mean values (± SEM) refer to 2–3 individual experiments in each group

(Fig. 1) and calcium uptake (Table 1) indicated that the interaction of glucose, extracellular Ca^{2+} and D600 upon these two parameters of islet function did not alter their normal relationship as previously observed in the present system (7, 9). This relationship (Fig. 5) is characterized by the fact that insulin output increases above its basal value when the size of the Ca^{2+} exchangeable pool exceeds half of its normal value at high glucose concentration (16.7 mM).

Effect of D600 upon ^{45}Calcium Efflux

D600 failed to cause any obvious alteration in the pattern of ^{45}calcium efflux from perifused islets exposed to Ca^{2+}-depleted media, whether in the presence or absence of glucose (Fig. 6). The Ca^{2+}-antagonist did not suppress the normal fall in effluent radioactivity caused in the present system by the addition of glucose to the perfusate (Fig. 7).

Discussion

The present data, which complement our study with verapamil (1–3), suggest that D600 does not affect the process by which the B-cell recognizes glucose as an insulinotropic agent, since both the inhibition of Ca^{2+} efflux (Fig. 7) and subsequent relative increase in Ca^{2+} net uptake (Table 1) normally evoked by glucose were not altered in the presence of the Ca^{2+}-antagonist, although the drug was used at a concentration sufficient to cause marked inhibition of insulin release (Figs. 1 and 2). These findings and the fact that D600 also suppressed gliclazide-induced insulin release (Fig. 3) could indicate that the drug acts upon the secretory sequence at a level distal to the site of recognition of the secretagogues.

Our results are compatible with the idea that D600 inhibits Ca^{2+} inward transport in the B-cell, since it inhibited the net uptake without facilitating the efflux of ^{45}calcium in the isolated islets. The postulated primary action of D600 on Ca^{2+} inward transport would also explain why the Ca^{2+}-antagonist inhibited basal net uptake of ^{45}calcium, accentuated the depressing effect of a reduced extracellular Ca^{2+} concentration upon both Ca^{2+} net uptake and insulin release, and failed to affect the insulinotropic action of theophylline (Fig. 1 and Table 1), which is thought to augment glucose-induced insulin release by causing an intracellular translocation rather than a net accumulation of Ca^{2+} in the B-cell [10, 11].

If our assumption is correct that D600 inhibits calcium entry in the B-cell like in other tissues [12–14], the rapidity of its inhibitory effect upon insulin release (Fig. 4) would suggest that the physiologi-

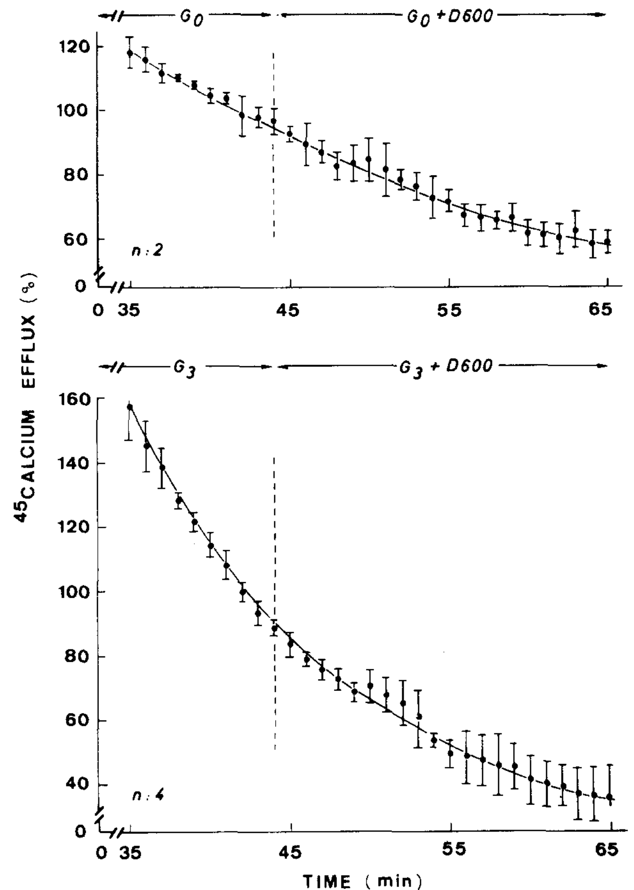

Fig. 6. Effect of D600 (2 μM; added at the time shown by the vertical dotted line) upon ^{45}calcium efflux from islets perifused in the absence (GO) or presence of glucose (G3; glucose 16.7 mM), the perifusion media being deprived of Ca^{2+} and enriched with EGTA (1 mM). Mean values (± SEM) for ^{45}calcium efflux are expressed in per cent of the mean value found within each experiment during the last 5 min prior to addition of D600. Also shown is the number of experiments (n) in each group

Table 1. Effect of glucose, D600 and extracellular Ca^{2+} upon ^{45}calcium net uptake in isolated rat islets. All results are expressed relative to the mean control value found within the same experiment(s) in the presence of glucose (16.7 mM), at normal Ca^{2+} concentration (2.0 mEq/l) and in the absence of D600. Such a control value averaged 199 ± 14 pg/islet at the 90th min of incubation. Mean values (± SEM) are shown together with the number of individual determinations in each group (in parentheses)

Glucose (mM)	D600 (μM)	Ca^{2+} (mEq/l)	^{45}Calcium net uptake (per cent)
–	–	2.0	49.7 ± 7.6 (10)
–	4	2.0	22.1 ± 2.8 (10)
16.7	–	2.0	100.0 ± 4.7 (51)
16.7	2	2.0	70.4 ± 6.8 (20)
16.7	4	2.0	66.7 ± 4.2 (10)
16.7	10	2.0	62.4 ± 5.0 (20)
16.7	–	0.5	76.2 ± 6.5 (16)
16.7	2	0.5	53.0 ± 4.7 (10)
16.7	10	0.5	36.4 ± 5.2 (9)

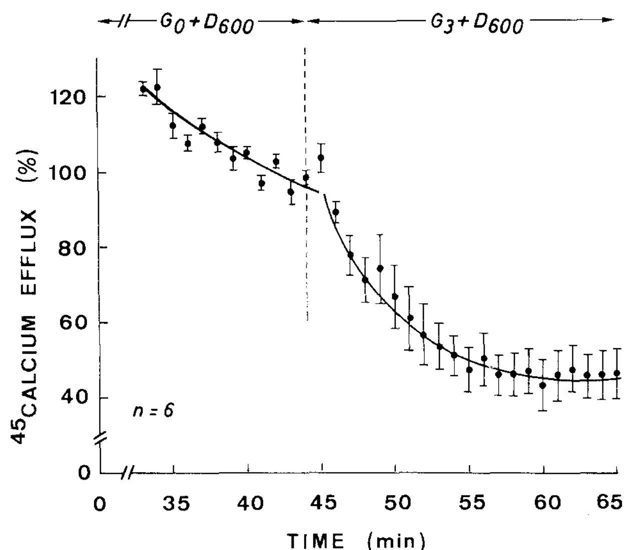

Fig. 7. Effect of glucose (16.7 mM) added at the time shown by the vertical dotted line) upon ^{45}calcium efflux from islets perifused in the presence of D600 (2 μM), the perifusion media being deprived of Ca^{2+} and enriched with EGTA (1 mM). Same presentation as in Fig. 6

cally active pool of Ca^{2+} in the B-cell is characterized by a rapid turn-over rate so that, under many if not all experimental conditions, a sufficient inward transport of Ca^{2+} would represent, to say the least, a permissive factor for the maintenance of the calcium-dependent insulin secretory process.

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