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CALCIUM ANTAGONISTS AND ISLET FUNCTION—III

THE POSSIBLE SITE OF ACTION OF VERAPAMIL

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Abstract Verapamil is known to abolish glucose- or sulfonylurea-induced insulin release by the isolated perfused rat pancreas. The mode of action of verapamil upon islet function is investigated. The drug apparently does not interfere with the process of glucose metabolism and recognition by the B-cell, since the utilization and oxidation of glucose, the total production of lactate, the synthesis of proinsulin, and the inhibitory effect of glucose on ^{45}calcium efflux in isolated islets were all unaffected by verapamil. The drug inhibited basal and glucose- or sulfonylurea-stimulated ^{45}calcium net uptake, without facilitating ^{45}calcium efflux from perifused islets. It failed to abolish the increase in both ^{45}calcium efflux and insulin output evoked by theophylline. These data suggest that a major effect of verapamil is to inhibit calcium entry in the B-cell, as if such an influx were to occur, in part at least, through calcium channels analogous to those described in myocardium and myometrium. The proper effect of glucose on calcium handling by the islets was not abolished by verapamil and may, therefore, not require the functional integrity of the system responsible for calcium inward transport in the B-cell.

Verapamil, often described as a selective Ca^{2+}-antagonist, is currently used to probe the significance of Ca^{2+} inward transport in cells exerting a Ca^{2+}-dependent function [1–5]. For instance, it was proposed that verapamil might help to elucidate the precise modality by which agents such as glucose or theophylline exert their insulinotropic action [6, 7]. In the present study, reported in abstract form elsewhere [8], it was investigated whether the influence of verapamil upon various parameters of islet function is indeed compatible with its postulated mode of action, namely the inhibition of Ca^{2+} influx in the B-cell.

MATERIALS AND METHODS

The methods used for the measurement of insulin release [9], proinsulin biosynthesis [10, 11], glucose utilization as judged by the production of ^3H_2O from [5-^{3}H]glucose [12, 13], glucose oxidation [14], lactate production [15], ^{45}calcium net uptake [16] and ^{45}calcium efflux [17] in isolated islets removed from fully fed female albino rats were previously described.

RESULTS

Effect of verapamil upon insulin release. Figure 1 illustrates the dose–action relationship for the inhibitory effect of verapamil upon insulin release evoked by glucose (16.7 mM) in isolated islets. Whereas verapamil at a 0.01 mM concentration suppressed glucose-induced insulin release, it did not abolish the enhancing action of theophylline (Table 1). The latter experiments were performed in the presence of glucose (16.7 mM) for, at the concentration here used (1.4 mM), theophylline does not exert any significant insulinotropic action in the absence of glucose [18]. Phentolamine, which is known to abolish the inhibitory effect of epinephrine upon glucose-induced insulin release [19] failed to protect the B-cell against the inhibitory action of verapamil (Table 1).

Effect of verapamil upon insulin biosynthesis. The effect of verapamil upon proinsulin synthesis was tested at two glucose concentrations (4.2 and 16.7 mM), the first close to the threshold value for the stimulant action of glucose upon proinsulin synthesis and the second inducing a near-to-maximal

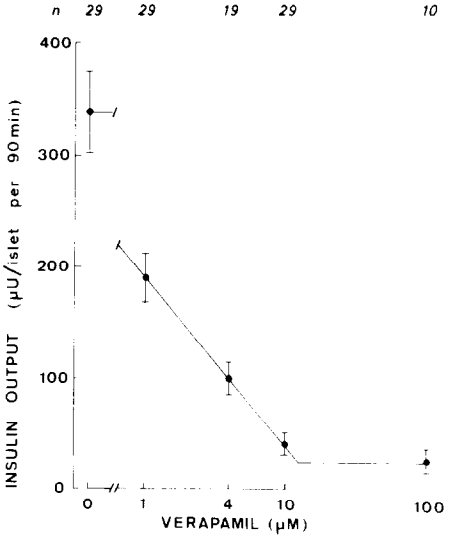

Fig. 1. Mean values (± SEM) for insulin release evoked by glucose (16.7 mM) at various verapamil concentrations (logarithmic scale) are shown together with the number of individual experiments (*n*).

Table 1. Effect of verapamil, theophylline and phentolamine upon insulin release evoked by glucose (16.7 mM) in isolated islets

Verapamil (mM)	Theophylline (mM)	Phentolamine (mM)	Insulin output* (%)
—	—	—	100.0 ± 5.1 (56)
0.01	—	—	15.6 ± 4.2 (55)
0.01	—	0.1	18.6 ± 3.0 (9)
—	1.4	—	255.1 ± 15.9 (18)
0.01	1.4	—	62.3 ± 6.3 (18)

* Mean values (± SEM) for insulin output are shown together with the number of individual experiments (in parentheses) and are expressed in percent of the mean control value found in the presence of glucose alone within the same experiment(s), such a control value averaging 292 ± 21 μU/islet per 90 min.

Table 2. Effect of glucose and verapamil upon proinsulin biosynthesis and conversion by isolated islets

Glucose (mM)	Verapamil (mM)	Proinsulin synthesis* (cpm. 10^{-3}/25 islets)	Proinsulin synthesis* (% of total synthesis)	Proinsulin conversion* (%)
4.2	—	45.3 ± 14.6	33.4 ± 8.5	43.7 ± 6.8
4.2	0.01	40.5 ± 2.6	33.2 ± 5.2	40.5 ± 7.2
16.7	—	136.8 ± 24.0	55.7 ± 4.1	34.8 ± 3.7
16.7	0.01	122.4 ± 21.8	54.7 ± 6.1	43.7 ± 6.7

* Mean values (± SEM) are each derived from 4 individual measurements.

Table 3. Effect of verapamil and theophylline upon glucose metabolism in isolated islets

Glucose (mM)	16.7	16.7	16.7	16.7
Verapamil (mM)	—	0.01	—	0.01
Theophylline (mM)	—	—	1.4	1.4
Glucose utilization*	105 ± 10 (20)	106 ± 9 (18)	98 ± 11 (16)	84 ± 7 (18)
Lactate output*	65 ± 5 (11)	44 ± 4[a] (11)	58 ± 5 (11)	57 ± 6 (11)
Glucose oxidation*	30 ± 4 (17)	29 ± 5 (16)	35 ± 4 (17)	26 ± 3 (17)

* Mean values (± SEM) for the utilization of glucose (as judged by the production of ^3H_2O from [5-^{3}H]glucose over 120 min incubation), the output of lactate in the incubation medium (measured over 90 min incubation) and the oxidation of glucose (as judged by the production of $^{14}CO_2$ from [U-^{14}C]glucose over 120 min incubation) in the islets are invariably expressed as pmol of glucose residues metabolized per islet and per hr, and are shown together with both the number of individual determinations (in parentheses) and the statistical significance (a: $P < 0.01$) of differences between experimental and control (glucose alone) data.

biosynthetic response [10]. Verapamil failed to significantly affect the various parameters of islets biosynthetic activity at these two glucose levels (Table 2).

Effect of verapamil upon glucose metabolism. At high glucose concentration (16.7 mM), verapamil (0.01 mM), theophylline (1.4 mM) and the combination of both agents failed to affect glucose utilization and oxidation by the islets (Table 3). Verapamil also failed to affect the total production of lactate by the islets but apparently retarded its exit from the islets (Fig. 2), so that the amount of lactate released by the islets in the medium was decreased. Such a reduction was not encountered when theophylline was also present in the incubation medium (Table 3).

Effect of verapamil upon ^{45}calcium uptake. The results of the measurements of ^{45}calcium net uptake are summarized in Table 4. Verapamil (0.01 mM) inhibited basal net uptake (Table 4, lines 1 and 2). Theophylline (1.4 mM) failed to protect against such an inhibitory effect (Table 4, line 3). Verapamil (0.01 mM) also reduced tolbutamide- and gliclazide-stimulated calcium net uptake (Table 4, lines 4 to 7). In the presence of glucose (16.7 mM), verapamil (0.002 to 0.1 mM) cause a dose related inhibition of calcium net uptake (Table 4, lines 8–10 and 12), a significant effect ($P < 0.005$) being already noted with the lowest concentration of verapamil here examined (0.002 mM). Theophylline (1.4 mM) again failed to protect against the inhibitory action of verapamil (Table 4, lines 10 and 11). The mean degree of inhibition in calcium net uptake attributable to verapamil (0.01 mM), relative to the appropriate control value found in its absence within the same experiment(s), was invariably and significantly ($P < 0.001$) less pronounced in the presence of glucose (16.7 mM; inhibitory effect of verapamil: $-21 \pm 6\%$; $n = 66$) than

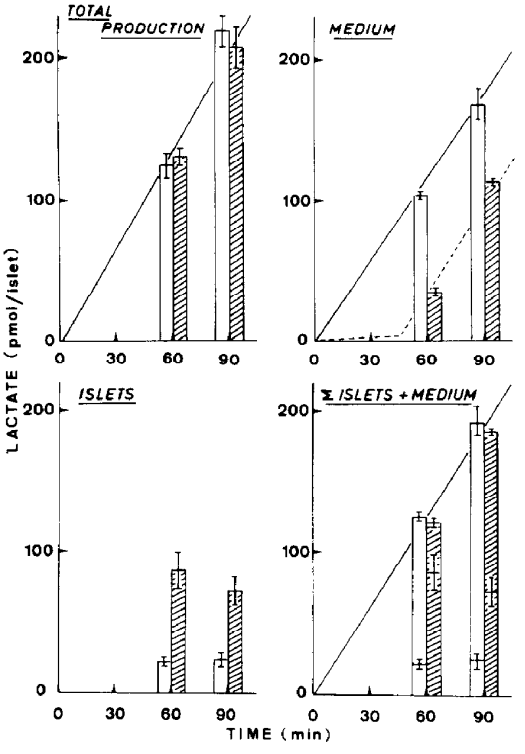

Fig. 2. Isolated islets were incubated for 60 or 90 min with glucose (16.7 mM) in the absence (open columns) or presence (shaded columns) of verapamil (0.01 mM). After incubation, lactate was measured either after sonification of the islets in their incubation medium (total production; upper left panel), or separately in the medium (upper right panel) and in the islets homogenates (lower left panel), the sum of these two latter measurements being shown in the lower left panel. Each value represents the mean (± SEM) of four individual experiments.

either in its absence (basal uptake; $-59 \pm 8\%$; $n = 43$) or when tolbutamide ($-72 \pm 6\%$; $n = 20$) and gliclazide ($-73 \pm 5\%$; $n = 19$) were used as the insulinotropic agents.

Effect of verapamil upon ^{45}calcium efflux. We have examined the effect of verapamil upon ^{45}calcium efflux from islets which were first exposed to glucose and ^{45}calcium for 60 min, extensively washed, and eventually placed in a perifusion chamber where they were exposed to media deprived of calcium and enriched with EGTA (ethyleneglycol-bis-(β-aminoethyl ether)*N*,*N*-tetraacetic acid, 1.0 mM). The latter media were used to prevent the release of ^{45}calcium often associated with the exocytosis of secretory granules [17].

Addition of verapamil (0.01 mM) to the perifusate, whether in the absence (Fig. 3) or presence (Fig. 4) of glucose, failed to cause any obvious accident in

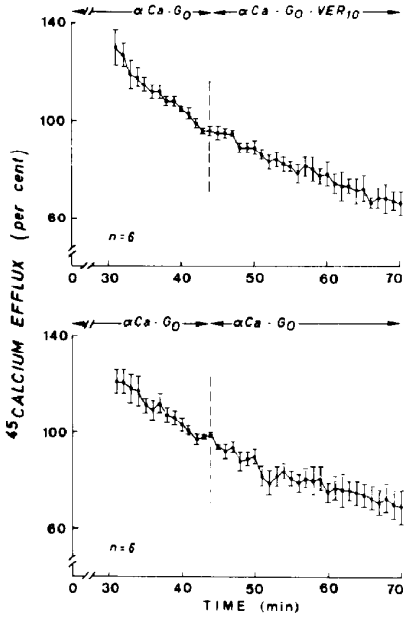

Fig. 3. Effect of verapamil upon ^{45}calcium efflux in the absence of glucose. The islets were perifused with calcium-deprived media enriched with EGTA (αCa) and containing no glucose (G_0). Verapamil (0.01 mM; VER_{10}) was added at the time shown by the dotted line (upper panel). Control experiments performed throughout in the absence of verapamil are shown in the lower panel. Individual values for ^{45}calcium efflux are expressed in percent of the mean control value found within the same experiment between 40–44 min. Mean values (± SEM) are shown together with the number of individual experiments in each group (*n*).

Table 4. Effect of verapamil and other insulinotropic agents upon ^{45}calcium net uptake by isolated islets

Line (Nr)	Glucose (mM)	Theophylline (mM)	Tolbutamide (mM)	Gliclazide (mM)	Verapamil (mM)	Calcium uptake* (pg/islet at 90th min)
1	—	—	—	—	—	67.0 ± 3.5 (93)
2	—	—	—	—	0.010	31.6 ± 3.3 (43)
3	—	1.4	—	—	0.010	32.0 ± 3.9 (24)
4	—	—	0.75	—	—	186.5 ± 7.5 (40)
5	—	—	0.75	—	0.010	56.3 ± 7.4 (20)
6	—	—	—	0.055	—	220.5 ± 8.3 (40)
7	—	—	—	0.055	0.010	67.3 ± 5.9 (19)
8	16.7	—	—	—	—	152.5 ± 5.9 (85)
9	16.7	—	—	—	0.002	124.3 ± 6.7 (59)
10	16.7	—	—	—	0.010	109.1 ± 4.8 (66)
11	16.7	1.4	—	—	0.010	100.2 ± 7.0 (29)
12	16.7	—	—	—	0.100	64.2 ± 6.2 (30)

* Mean values (± SEM) are shown together with the number of individual determinations (in parentheses).

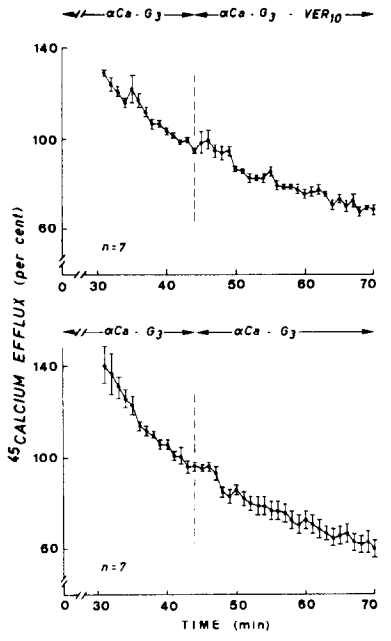

Fig. 4. Effect of verapamil upon ^{45}calcium efflux in the presence of glucose. Same presentation as in Fig. 3. Glucose (16.7 mM; G_3) was present throughout the perifusion.

the pattern of progressively decreasing effluent radioactivity normally observed in the present system. Verapamil did not prevent the immediate reduction in ^{45}calcium efflux normally seen on addition of glucose (16.7 mM) to the perifusate (Fig. 5). Thus, both in the absence and presence of verapamil (0.01 mM), the rate of fall in effluent radioactivity was significantly increased by glucose (Table 5, lines 5 and 6). Likewise, verapamil did not prevent the immediate increase in ^{45}calcium efflux normally seen on addition of theophylline (2.0 mM) to the perifusate (Fig. 6).

A closer examination of the experimental data suggested that verapamil may tend to minimize the effect of theophylline on ^{45}calcium efflux, both in the presence and absence of glucose (Table 5, lines 7–10). This behaviour contrasts with that seen in the absence of theophylline, where an opposite trend towards facilitation of ^{45}calcium efflux was noted upon addition of verapamil to the system (Table 5, lines 1–4). Although none of these changes achieved statistical significance, it should be noted that a clear-cut facilitation of ^{45}calcium efflux, characterized by an increase in the effluent radioactivity, was observed when verapamil was added to the perifusate in a much higher concentration (0.1 mM), the latter phenomenon being observed both in the absence and presence of glucose (data not shown).

DISCUSSION

The inhibitory effect of verapamil upon glucose-induced insulin release is apparently not due to any major defect in the process of glucose metabolism and/or recognition by the B-cell. The utilization and oxidation of glucose, its inhibitory effect on ^{45}calcium efflux and its stimulant action upon proinsulin biosynthesis were all unaffected by the calcium-antagonist.

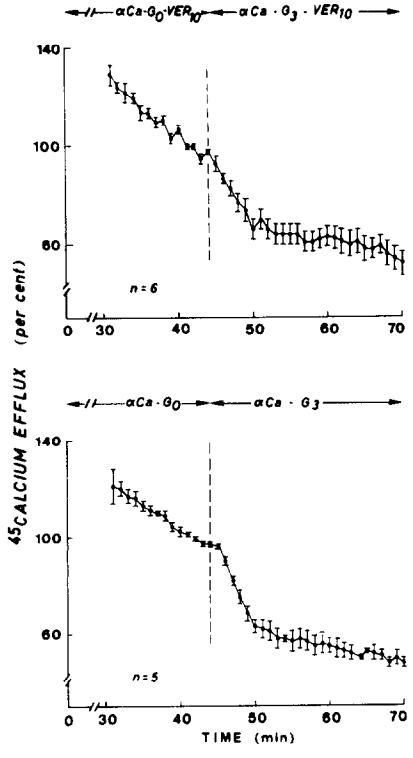

Fig. 5. Effect of glucose upon ^{45}calcium efflux in the absence (lower panel) or presence (upper panel) of verapamil. Same presentation as in Fig. 3. Glucose (16.7 mM; G_3) was added at the time shown by the dotted line.

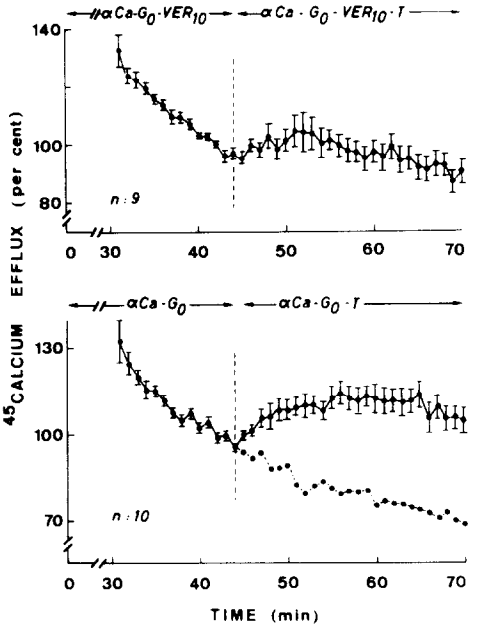

Fig. 6. Effect of theophylline upon ^{45}calcium efflux in the absence (lower panel) or presence (upper panel) of verapamil. Same presentation as in Fig. 3. Theophylline (2.0 mM; T) was added at the time shown by the dotted line. The broken line in the lower panel refer to mean control data obtained when no theophylline was added to the system (see Fig. 3).

Table 5. Effect of glucose, verapamil and theophylline upon ^{45}calcium efflux

Line (Nr)	Expts. (n)	Period: min 38–44 Conditions*	b (%.min^{-1})‡	Period: min 44–50 Conditions*	b (%.min^{-1})‡	Change in slope (Δb)‡	
1	(6)	G_0	−1.64 ± 0.34	G_0	−1.46 ± 0.43	+0.18 ± 0.55	NS
2	(6)	G_0	−2.36 ± 0.22	$G_0 + V_{10}$	−1.39 ± 0.33	+0.97 ± 0.40[a]	
3	(7)	G_3	−2.39 ± 0.26	G_3	−2.39 ± 0.56	0.00 ± 0.62	NS
4	(7)	G_3	−1.96 ± 0.40	$G_3 + V_{10}$	−1.36 ± 0.62	+0.60 ± 0.74	
5	(5)	G_0	−1.89 ± 0.27	G_3	−6.18 ± 0.40	−4.29 ± 0.48[b]	NS
6	(6)	$G_0 + V_{10}$	−2.21 ± 0.55	$G_3 + V_{10}$	−5.14 ± 0.22	−2.93 ± 0.59[b]	
7	(10)	G_0	−1.75 ± 0.42	$G_0 + T$	+2.25 ± 0.29	+4.00 ± 0.51[b]	NS
8	(9)	$G_0 + V_{10}$	−1.99 ± 0.44	$G_0 + V_{10} + T$	+0.78 ± 0.34	+2.77 ± 0.56[b]	
9	(8)	G_3	−2.01 ± 0.50	$G_3 + T$	+1.43 ± 0.41	+3.44 ± 0.65[b]	NS
10	(9)	$G_3 + V_{10}$	−1.76 ± 0.21	$G_3 + V_{10} + T$	+0.12 ± 0.69	+1.88 ± 0.72[a]	

* Verapamil (0.01 mM; V_{10}) and theophylline (2.0 mM; T) were used in the absence (G_0) or presence of glucose (16.7 mM; G_3).

‡ The slope (b) of the regression line characterizing the rate of change in mean effluent radioactivity is expressed as percent per min (%.min^{-1}) for two successive periods of 7 min each (min 38–44 and min 44–50). Whenever a change in composition of the perifusate was introduced, the change took place at min 44. Also shown are the number of individual experiments in each group (n) and the statistical significance for the change in slope over the two successive periods (a: $P < 0.05$; b: $P < 0.001$) and for the difference between changes observed in different sets of experiments (NS: not significant).

A more likely explanation for the effect of verapamil B-cell function would be an inhibition of calcium entry in the B-cell, since the drug, at concentration up to 0.01 mM, inhibited the net uptake of ^{45}calcium by the islets without causing any obvious facilitation of ^{45}calcium efflux. A primary effect of verapamil upon the channel for calcium influx in the B-cell would also explain why the drug inhibited basal as well as glucose-stimulated calcium net uptake and why it abolished sulfonylurea-induced calcium net uptake and subsequent insulin release. Last, because the insulinotropic action of theophylline is thought to result from an intracellular translocation rather than a change in the net uptake of calcium by the B-cell [20, 21], it is not surprising that the methylxanthine protected glucose-induced insulin release against the inhibitory action of verapamil [7] but failed to protect against the verapamil-induced reduction in calcium net uptake.

Within the framework of such a hypothesis, the following unexpected findings should be considered. First, verapamil (0.01 mM) caused lactate accumulation in the islets and retarded its appearance in the incubation medium. Second, the verapamil-induced reduction in glucose-stimulated ^{45}calcium net uptake was, in relative terms, less marked than that evoked by the calcium-antagonist in the absence of glucose or presence of sulfonylurea. Third, there might have been a tendency for verapamil to minimize the theophylline-induced increase in ^{45}calcium efflux from perifused islets. And last, from preliminary observations, it would appear that, in islets exposed to verapamil, there is no obvious reduction in the amount of calcium pyroantimonate precipitates detected in the secretory granules, whereas a significant depletion of this cationic pool can be evoked by incubating the islets in the presence of a calcium-chelating agent (M. Ravazzola and F. Malaisse-Lagae, personal communication). Further work is obviously required to explore these phenomena and their possible interrelationship.

Meanwhile, our experimental data are compatible with the view that a major effect of verapamil upon islet function is to inhibit calcium entry in the B-cell, as if such an influx were to occur, in part at least, through calcium channels analogous to those described in myocardium and myometrium [1, 2]. That, despite the impairment of calcium entry, glucose was still able both to stimulate calcium net uptake by the islets and to support the insulinotropic action of theophylline suggests that the proper effect of glucose on calcium metabolism is not dependent on the functional integrity of the system responsible for calcium inward transport in the B-cell. Such a conclusion is consistent with the idea that glucose mainly regulates some other step of calcium handling by the B-cell, such as the outward transport of the cation across the B-cell membrane [17].

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