# Calcium Antagonists and Islet Function

## I. Inhibition of Insulin Release by Verapamil

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SUMMARY

**Verapamil is a potent calcium antagonist known to inhibit excitation-contraction coupling in both myocardium and myometrium. Its effect upon glucose- and sulfonylurea-induced insulin release was investigated in the isolated perfused rat pancreas.**

**After twenty-five minutes' pretreatment and at concentrations ranging between 0.8 and 8.1 μM, verapamil caused a dose-related inhibition of glucose-induced insulin release during both the early and late phase of the secretory process. At a concentration of 0.8 μM, the degree of inhibition was more marked when the exposure time to verapamil prior to stimulation with glucose was increased to sixty minutes. Verapamil also inhibited gliclazide-induced insulin release. Infusion of verapamil during the late phase of the secretory response to glucose demonstrated that the inhibition of insulin release was an immediate and reversible phenomenon. The inhibitory effect of verapamil was enhanced at a subnormal calcium concentration and reduced at a high calcium concentration.**

**These findings are consistent with the well-known calcium dependency of both glucose- and sulfonylurea-induced insulin release and suggest that verapamil might be a promising tool for further studies on the interactions between cations and secretagogues in the β-cell secretory process. DIABETES *24*:547-51, June, 1975.**

Calcium is known to play a critical role in insulin release, the secretory process being apparently triggered by a cytosolic accumulation of this cation.[1-3] Other cations also influence insulin secretion either through a direct effect on insulin calcium handling or by altering the recognition of secretagogues in the β-cell.[4-10] The present series of reports aims at a better understanding of such interrelationships between cations and insulinotropic agents in islet tissue. For this purpose, we have first examined the influence of verapamil on insulin release by the isolated perfused rat pancreas. A preliminary account of this work was reported in abstract form.[11]

Verapamil, α-isopropyl-α [(N-methyl-N-homoveratril) -γ-aminopropyl] -3, 4-dimethoxy-phenyl-acetonitril, is currently used as a coronary vasodilator and antiarrhythmic agent. Its inhibitory effect on the electromechanical coupling in such tissue as myocardium or myometrium is thought to be due to blockade of the channels responsible for calcium influx in myocardial and smooth-muscle cells,[12,13] rather than to any direct effect on calcium handling by microsomes or sarcoplasmic reticulum.[14,15] The metabolic effects of the drug, e.g. accumulation of high-energy phosphates and reduced oxygen consumption, are thought to be secondary to the changes in cation transport.

To our knowledge, only fragmentary information is currently available concerning the effect of verapamil or related drugs on calcium-dependent secretory processes. Malaisse et al.[16] first reported on the inhibitory effect of D600 (2 to 10 μM) upon glucose-induced $^{45}$calcium uptake and subsequent insulin release by isolated islets, while Dreifuss et al.[17] observed concomitant inhibition of oxytocine release and $^{45}$calcium uptake in neurohypophyses exposed to D600 (5 to 50 μM).

### MATERIAL AND METHODS

*Perfusion technic.* A detailed description of the perfusion technic was reported in an earlier publication.[18] Briefly, the pancreas was dissected according to the technic of Sussman et al.[19] Fully fed male albino rats (200-300 gm. body weight) were used as pancreas and blood donors. The perfusate was a bicarbonate-buffered solution containing albumin (40 mg./ml.; bovine albumin, fraction V; Sigma Chemical Co., St. Louis, Mo.) and heparinized rat blood (10 per cent, v/v). The perfusate was equilibrated against a mixture of $O_2$ (95 per cent) and $CO_2$ (5 per cent), and circu-

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lated through the perfusion unit for 10-20 minutes at 37°C. before the isolated pancreas was introduced (time zero). Afterwards, and for the rest of the experiment, no recirculation occurred, the venous effluent being either discarded or collected over individual periods of one minute each. The flow rate through the pancreas was maintained constant in all experiments at 2.0 ml./min. Glucose, gliclazide (S852; Laboratoires Servier, Paris, France) and verapamil (Isoptine; Knoll A.G., Ludwigshafen, Germany) were either immediately added to the perfusate or dissolved in a bicarbonate-buffered solution, which was administered into the arterial cannula at a flow rate of 0.05 ml./min. by means of a Braun pump (Braun, Melsungen, Germany). The control experiments and those performed with verapamil were carried out on different rats, but usually on the same day, six to eight individual experiments being performed each day. The arterial pressure, the arterial and venous $O_2$, and the pH of the perfusate were all maintained at physiological values,[18] no obvious influence of verapamil upon these parameters being noticed.

*Chemical determinations.* The samples of venous blood were processed as described in detail elsewhere.[18] Glucose was measured by a photoelectric method,[20] adapted to the Technicon AutoAnalyzer. The insulin concentration of the venous effluent was determined by a modification of the method of Wright et al.[21]

*Presentation of results.* In figures 1, 4, and 5, the mean value (± S.E.M.) for the rate of insulin secretion at each time interval and under any given experimental condition, is expressed in absolute terms (μU./min. per pancreas). In figures 3 and 6 the results are given in per cent of the mean total amount of insulin released over the entire period of stimulation in the appropriate control experiments. In order to quantify the effect of various concentrations of verapamil on the early and late phases of insulin secretion, respectively, the total mean amount of insulin released in the control experiments over the first five minutes of stimulation (phase I) and the ensuing nine minutes (phase II) were each considered as the 100 per cent reference value (figure 2). The statistical analysis is always based on a group comparison between control and experimental data, using Student's *t* test.

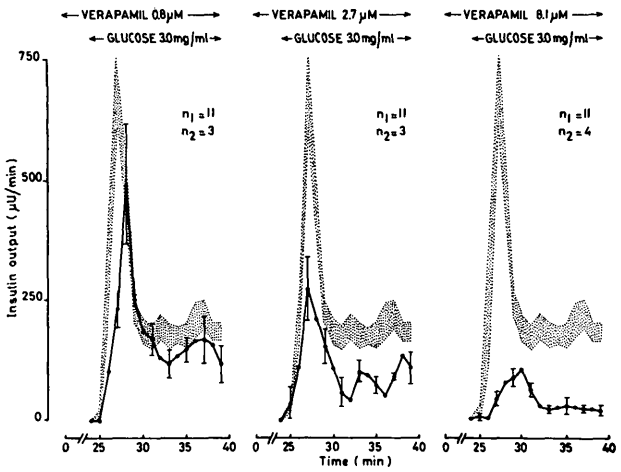


FIG. 1. Effect of verapamil on glucose-induced insulin release. Mean values (± S.E.M.) are expressed as μU./min. per pancreas and are shown together with the number of individual experiments in each group ($n_2$). Control experiments ($n_1$) are represented by the shaded area.

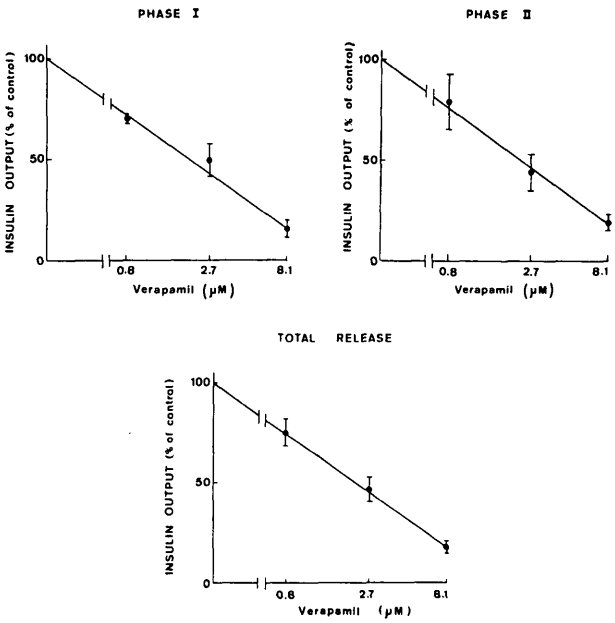


FIG. 2. Dose-dependent inhibitory effect of verapamil on both phases of insulin release. Mean values (± S.E.M.) for the integrated release during phase I (25th to 30th minute), phase II (30th to 39th minute), and total release (phase I + phase II) in the presence of verapamil are expressed in per cent of the corresponding control mean value found in the absence of the drug.

## RESULTS

### *Dose-dependent Effect of Verapamil on Glucose-induced Insulin Release*

As shown in figures 1 and 2, after twenty-five minutes of pretreatment with verapamil at the lowest concentration used (0.8 μM), glucose-induced insulin release during both phases I and II was slightly but not significantly reduced. The initial phase, estimated over a period of five minutes after the addition of glucose, corresponded to an integrated insulin release of 70 ± 2 per cent ($N$ = 3) in the presence of ver-

apamil, as distinct from 100 ± 10 per cent ($n$ = 11) in its absence. During the late phase (30th to 39th minute) insulin secretion in the presence of verapamil averaged 79 ± 14 per cent ($n$ = 3) of its appropriate control value (100 ± 11 per cent, $n$ = 11). When the verapamil concentration was enhanced to 2.7 μM, the insulin release fell to 50 ± 8 per cent ($n$ = 3, $p < 0.05$) of its control value during the early phase, and to 44 ± 9 per cent ($n$ = 3, $p < 0.05$) during the late phase. The highest verapamil concentration used (8.1 μM) caused a nearly complete inhibition of insulin secretion during both phases of insulin release (phase I: 16 ± 4 per cent, $n$ = 4, $p < 0.001$; and phase II: 19 ± 4 per cent, $n$ = 4, $p < 0.005$).

*Time-dependent Effect of Verapamil on Glucose-induced Insulin Release*

When the pretreatment period with the low concentration of verapamil (0.8 μM) was prolonged from twenty-five to sixty minutes, the degree of inhibition was more marked (figure 3). The insulin output averaged respectively 46 ± 10 per cent ($n$ = 3) of its control value (100 ± 5 per cent, $n$ = 4) during the first phase of insulin secretion ($p < 0.001$) and 47 ± 16 per cent ($n$ = 3) of the control value (100 ± 12 per cent, $n$ = 4, $p < 0.05$) during the late phase.

*Effect of Verapamil on Sulfonylurea-induced Insulin Release*

As shown in figure 4, at a concentration of 8.1 μM, verapamil caused an almost complete inhibition of

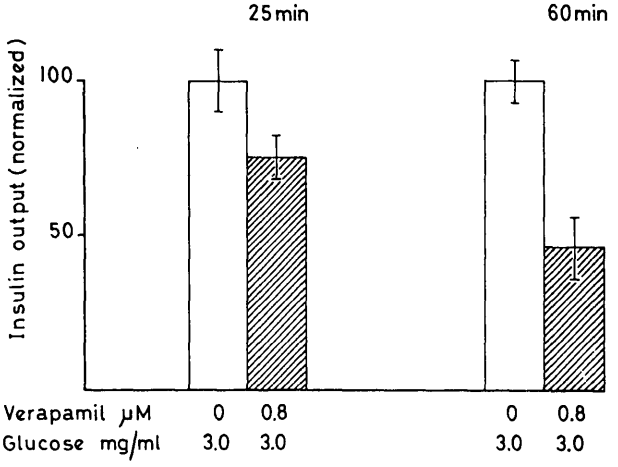


FIG. 3. Time-dependent effect of verapamil on glucose-induced insulin release. The integrated release of insulin over fifteen-minute exposure to glucose (3.0 mg./ml.) in the presence of verapamil (shaded columns) is expressed in per cent of the corresponding mean control output found in the absence of the drug (open columns). Mean values (± S.E.M.) are shown after twenty-five or sixty minutes' pretreatment with verapamil.

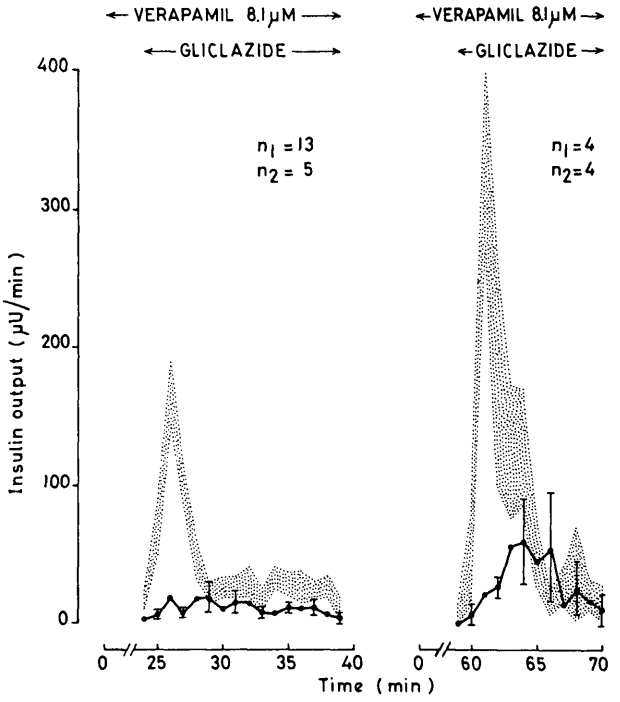


FIG. 4. Effect of verapamil on sulfonylurea-induced insulin release after different pretreatment periods (twenty-five and sixty minutes). Same presentation as in figure 1.

gliclazide-induced insulin secretion, whether after twenty-five or sixty minutes' pretreatment with the drug. Incidentally, and as already reported elsewhere,[22] the secretory response to gliclazide in the absence of verapamil (control experiments) was more marked at the 60th than at the 25th minute of the perfusion.

*Effect of Verapamil During the Late Phase of Glucose-induced Insulin Release*

When verapamil (2.7 μM) was administered during the late phase of glucose-induced insulin release, an immediate decrease in insulin output was observed (figure 5). The inhibitory action of verapamil also appeared rapidly reversible.

*Effect of Verapamil on Glucose-induced Insulin Release at Various Calcium Concentrations*

The effect of verapamil on glucose-induced insulin release was examined at three calcium concentrations. As shown in figure 6, the influence of the different calcium concentrations on glucose-induced insulin secretion in the absence of verapamil (control experiments) followed a pattern similar to that disclosed in previous studies.[6,23]

Relative to the mean control value found at the

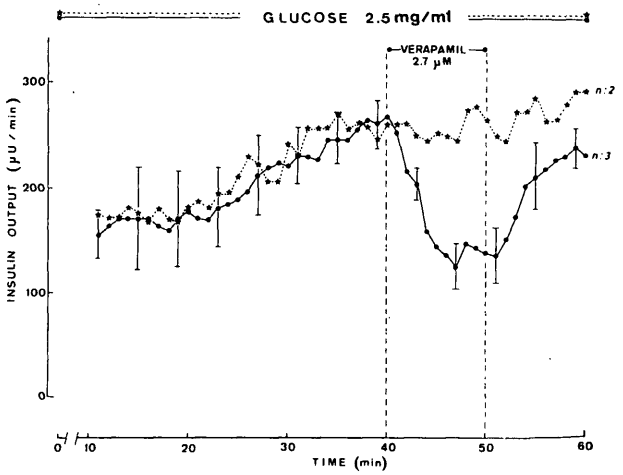


FIG. 5. Rapidity and reversibility of the verapamil-induced changes in insulin release. Control experiments are shown by the dotted line. Solid line shows the effect of verapamil (2.7 μM) administered from the 40th to the 50th minute. In both series of experiments, glucose (2.5 mg./ml.) was present from time zero until the end of the experiment. Mean values (± S.E.M.) are expressed as μU./min. per pancreas and shown with the number of individual experiments in each group (*n*).

same calcium concentration, insulin secretion was more sensitive to inhibition by verapamil at a low (0.6 mEq./L.) than at the usual (2.0 mEq./L.) calcium concentration. Thus, after twenty-five minutes of pretreatment with the low concentration of verapamil (0.8 μM), the integrated release of insulin evoked by glucose between the 25th and 39th minute amounted to 23 ± 8 per cent ($n = 5$) at a calcium concentration

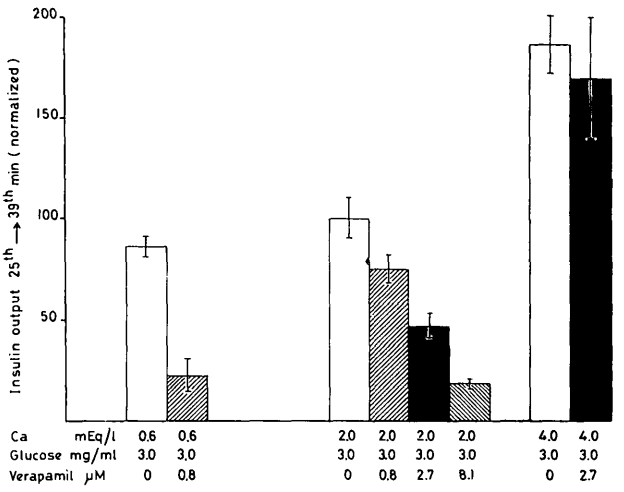


FIG. 6. Effect of verapamil on glucose-induced insulin release at various calcium concentrations. Total insulin secretion (± S.E.M.) over fifteen minutes (25th to 39th minute) stimulation by glucose (3.0 mg./ml.) is expressed in per cent of the mean control value found at a calcium concentration of 2 mEq./L. in the absence of verapamil.

of 0.6 mEq./L., as against 75 ± 7 per cent ($n = 3$) at a calcium concentration of 2.0 mEq./L. ($p < 0.005$). In other terms, insulin secretion evoked by glucose was depressed as much at the low calcium level (0.6 mEq./L.) and the lowest concentration of verapamil (0.8 μM) than at the usual calcium level (2.0 mEq./L.) and the highest concentration of verapamil (8.1 μM), residual insulin release relative to the appropriate control value found in the absence of verapamil averaging 23 ± 8 and 18 ± 3 per cent, respectively, under these two experimental conditions. These data indicate that the inhibitory effect of verapamil is enhanced at a subnormal calcium concentration.

Inversely, when the calcium concentration was raised to 4.0 mEq./L., the inhibitory effect of verapamil was minimized. Thus, at an intermediate concentration of verapamil (2.7 μM), the residual output of insulin relative to its appropriate control value averaged 47 ± 8 per cent ($n = 3$) and 91 ± 6 per cent ($n = 5$), respectively, at the usual (2.0 mEq./L.) and a higher (4.0 mEq./L.) calcium concentration. At the high calcium level, a higher concentration of verapamil (8.1 μM) was required to cause significant inhibition of insulin release (data not shown).

## DISCUSSION

The data presented in figures 1 to 3 demonstrate that verapamil provokes a time- and dose-related inhibition of glucose-induced insulin release. The degree of inhibition is the same for the first and second phases of the secretory process. The inhibitory effect of verapamil on glucose-induced insulin release is an immediate and reversible phenomenon (figure 5). The data illustrated in figure 6 are compatible with the view that verapamil inhibits insulin release through its calcium-antagonistic properties. Thus, the inhibitory action of verapamil was enhanced at a low extracellular calcium concentration while diminished in the presence of a high extracellular calcium concentration. The postulated calcium-antagonistic effect of verapamil could also account for the inhibition of sulfonylurea-induced insulin release (figure 4).

Although the present findings suggest that verapamil might interfere with the process of stimulus-secretion coupling in the β-cell in a manner comparable to that characterizing its effect on excitation-contraction coupling in muscle, namely by interfering with calcium transport across the plasma membrane, alternative explanations, such as a primary effect of the drug upon either the intracellular stores of cal-

cium, the metabolism of glucose in islet tissue, or the transport across the cell membrane of other cations,[24] can, at present, not be ruled out.

With this reservation in mind, verapamil appears as a promising tool for further studies on the interactions between cations and secretagogues in the process of insulin release by the pancreatic β-cell.

## ACKNOWLEDGMENT

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