Dual Effects of Veratridine on Glucagon and Insulin Secretion

Dependence upon Extracellular and Intracellular Calcium

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SUMMARY

The stimulatory effect of the sodium ionophore, veratridine (10, 25, and 50 μM), on glucagon and insulin secretion was investigated using monolayer cultures of newborn rat pancreas. The results suggest that intracellular accumulation of sodium modulates hormone secretion from both alpha- and beta-cells. The action of veratridine is dependent, at least in part, on the extracellular calcium, as its effect was attenuated or lost when extracellular calcium was deleted. Its action was also dependent on intracellular calcium since preincubation of cells in low, normal, or high calcium to diminish, maintain, or increase intracellular calcium, followed by incubation with veratridine in the absence of calcium, altered the secretory responses of both glucagon and insulin. Ouabain (0.5 mM) stimulated glucagon and insulin secretion, although its effect was less than that of veratridine (50 μM). These results suggest that a common releasing mechanism, dependent on extra- and intracellular calcium, is involved in both endocrine cells. DIABETES *30*:446–450, May 1981.

Although the role of intracellular sodium in insulin secretion appears well documented,[1–3] its precise mechanism of action is still unclear, probably due to the technical difficulty of examining its fluxes across the plasma membrane.[4,5] In regard to glucagon secretion, which is stimulated oppositely with glucose, little is known about the role of sodium.[6] Therefore, we investigated the effect of veratridine on glucagon and insulin secretion. Veratridine, a sodium ionophore, has been reported to increase the permeability of β-cells to sodium,[3,5] inducing depolarization[7] and enhancing insulin secretion. Recently, it was suggested that the elevation of intracellular sodium by veratridine causes mobilization of calcium from intracellular storage sites[8] and facilitates glycolytic flux,[7,9] thereby stimulating insulin secretion.

In the present study, we used veratridine with different concentrations of calcium in the preincubation or incubation media to examine its effect upon both glucagon and insulin secretion. The postulated interaction between intracellular sodium and calcium and their concentrations outside the cell have been analyzed. Our results indicate that similar veratridine-sensitive sodium channels may be involved in both glucagon and insulin secretion from the islets of Langerhans.

MATERIALS AND METHODS

Monolayer cultures of the pancreas from neonatal rats were prepared as described previously,[10] according to the method of Lambert et al.[11] Cultures were maintained at 37°C in TCM 199 supplemented with Earle's salts, fetal calf serum (heat-inactivated, 10% v/v), and glucose (16.7 mM) in a humidified atmosphere of 95% O_2 and 5% CO_2. Experiments were performed on the 6–7th culture day. After preincubation for 60 min in Krebs-Ringer bicarbonate buffer containing dialyzed bovine serum albumin (0.5%, w/v) and glucose (0–5.6 mM), the cells were incubated for 30 or 90 min in the test medium, which consisted of the same buffer plus test substances. Calcium concentration of each medium was 0, 1.3, or 3.9 mM. EGTA [ethyleneglycol-bis(β-aminoethylenether)-N,N′-tetraacetic acid] was added in some experiments to chelate the calcium.

For those experiments in which the incubation period was 90 min, half of the medium was removed at 30 min and the remaining medium at 90 min. Otherwise, medium was removed at the end of the 30-min incubation period. In some experiments, cells were preincubated for 180 min in the presence of low (0.3 mM), normal (1.3 mM), or high calcium (3.9 mM), after which cells were incubated for 30 min without calcium in the presence or absence of veratridine. Samples were stored at −20°C until assay.

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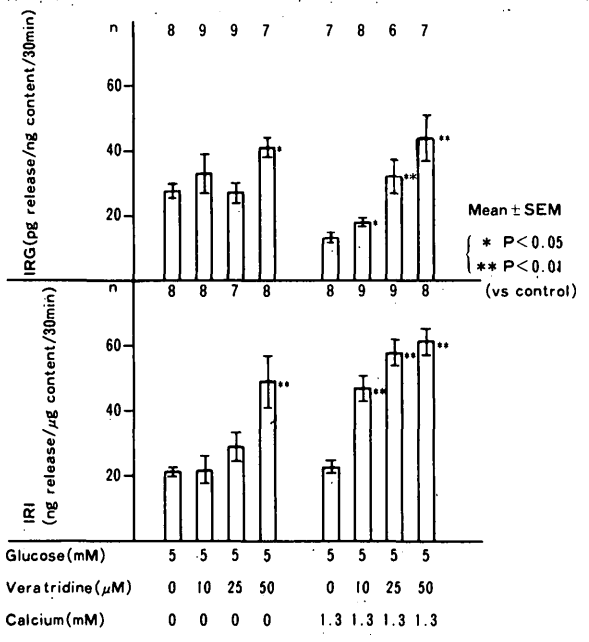

FIGURE 1. Release of immunoreactive glucagon (IRG) and insulin (IRI) from monolayer cultures of the endocrine pancreas of neonatal rats during a 30-min incubation period (mean ± SEM). Statistical significance for each column compared with control (absence of veratridin, calcium—0 or 1.3 mM). *P < 0.05, **P < 0.01.

Veratridine was donated by Serva Co. Ltd, and was a gift from Prof. W. J. Malaisse of Brussels, Belgium. It was first dissolved in dimethylsulfoxide (DMSO) and then diluted in the medium. This resulted in a final concentration of 0.5% DMSO, which by itself had no effect on either insulin or glucagon secretion from monolayer cultures of endocrine pancreas. EGTA, ouabain, and $LaCl_3$ were purchased from Sigma Chemical Company and TTX (Tetrodotoxin, Crystalline 3X) was purchased from Sankyo Co. Ltd., (Japan).

Insulin (IRI) was measured by double antibody radioimmunoassay,[12] and glucagon (IRG) by radioimmunoassay using a polyethylene-glycol (Mol. Wt. 6000) precipitation method. Monocomponent rat insulin and pork glucagon (Novo, Denmark) were used as standard. Veratridine did not affect these radioimmunoassay procedures.

Hormone secretion was expressed as the ratio of secretion in the medium (pg) per secretion in the medium plus content in the extracted tissue (ng). IRI and IRG contents of each culture dish were determined by extraction of monolayer cultures of endocrine pancreas with acid-ethanol.[13]

Statistical analysis was performed using Dunnett's test.

RESULTS

Effect of vertridine on glucagon and insulin secretion with and without calcium. In the presence of 5 mM glucose, which is a normal physiologic concentration in serum, the effects of various concentrations of veratridine on glucagon and insulin secretion were examined with and without calcium in the medium (Figure 1). Veratridine clearly stimulated glucagon as well as insulin secretion in dose-dependent manner in the presence of 1.3 mM calcium, a significant effect being seen with both hormones at a concentration of 10 μM. In the absence of calcium, 50 μM veratridine was required to demonstrate an effect.

The basal value of glucagon secretion was significantly augmented by the omission of calcium, although no change of insulin secretion was detected. By the observation under phase-contrast microscopy, the monolayer-cultured cells contract slightly and become rounded in the presence of veratridine; no changes were observed after 30 min of incubation in the absence of calcium.

Effect of EGTA on veratridine-induced glucagon and insulin secretion. To test the possible effect of a trace amount of calcium in the extracellular space, 0.5 mM EGTA was added in media without calcium or glucose. The cells were checked under the phase-contrast microscopy and it was confirmed that no detachment from the dish occurred after 30 min of static incubation. As shown in the upper part of Table 1, glucagon secretion was significantly increased without calcium in media and was further increased by the addition of EGTA. No significant changes of insulin secretion were produced by the omission of calcium, with or without EGTA.

In the presence of EGTA, the effect of 50 μM veratridine on the secretion of both hormones was significantly reduced when compared with that in the presence of 1.3 mM calcium in media.

Effects of TTX and $LaCl_3$ on veratridine-induced glucagon and insulin secretion. The effects of tetrodotoxin, a blocker of veratridine-sensitive sodium channels and $LaCl_3$

TABLE 1
Release of IRG and IRI from monolayer cultures of the endocrine pancreas of neonatal rats

no.	Glucose (mM)	Calcium (mM)	Veratridine (μM)	EGTA (mM)	TTX (μM)	$LaCl_3$ (mM)	IRG (pg release/ng content)	IRI (ng release/μg content)
1	0	1.3	0	0	0	0	12.0 ± 1.4 (12)	24.4 ± 3.2 (15)
2	0	0	0	0	0	0	21.7 ± 3.0 (14)*	24.6 ± 2.5 (15)
3	0	0	0	0.5	0	0	27.4 ± 3.4 (11)†	25.6 ± 2.0 (13)
4	20	0	0	0.5	0	0	29.9 ± 3.4 (7)†	30.5 ± 2.5 (7)
5	0	1.3	50	0	0	0	88.0 ± 3.6 (8)	70.5 ± 7.3 (8)
6	0	0	50	0	0	0	55.0 ± 8.5 (7)‡	64.5 ± 6.1 (9)
7	0	0	50	0.5	0	0	45.1 ± 8.8 (7)§	53.2 ± 4.7 (10)‡
8	0	0	50	0	0	0	39.2 ± 4.7 (6)	64.2 ± 3.7 (5)
9	0	0	50	0	3	0	19.8 ± 3.3 (4)‖	48.0 ± 3.4 (5)‖
10	0	0	50	0	0	0.5	15.1 ± 1.7 (4)¶	31.9 ± 3.9 (6)¶

After preincubation for 60 min in KRB buffer with 4–5 mM glucose, the cells were washed once with saline and incubated in various conditions for 30 min. Secretion of each hormone was expressed as secretion in the medium(pg)/secretion in the medium + content in the extracted tissue (ng); mean ± SEM. * $P < 0.05$ vs. no. 1; † $P < 0.01$ vs. no. 1; ‡ $P < 0.05$ vs. no. 5; § $P < 0.01$ vs. no. 5; ‖ $P < 0.05$ vs. no. 8; ¶ $P < 0.01$ vs. no. 8.

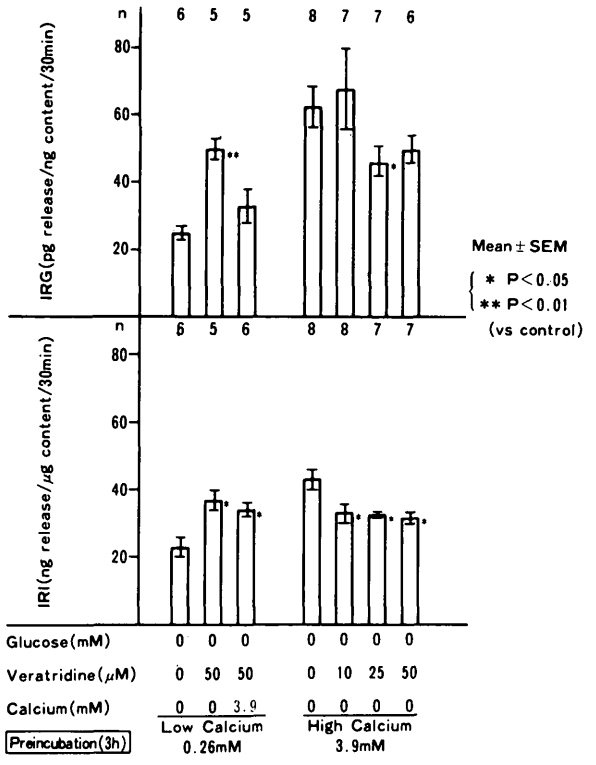

FIGURE 2. Release of IRG and IRI from monolayer cultures of the endocrine pancreas of neonatal rats in the absence of calcium and glucose during a 30-min incubation period (mean ± SEM). Statistical significance for each column compared with control (absence of veratridine, preincubation medium calcium concentration—0.26 or 3.9 mM). *P < 0.05, **P < 0.01.

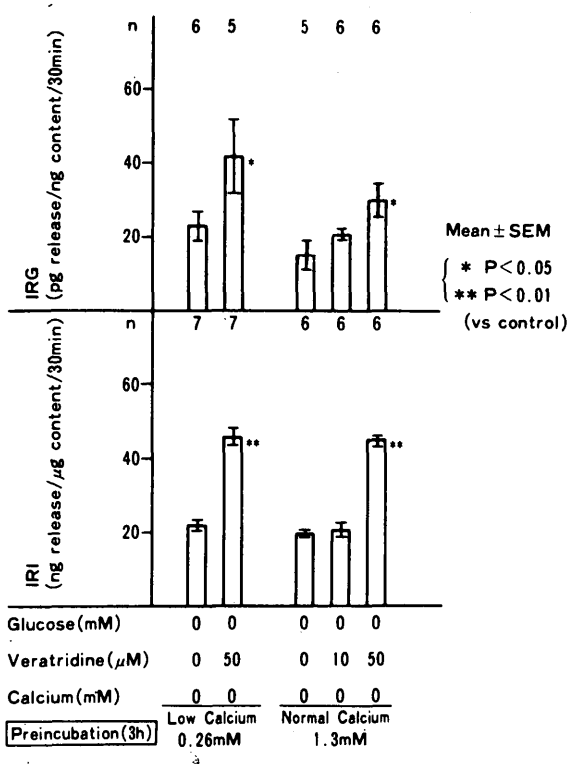

FIGURE 3. Release of IRG and IRI from monolayer cultures of the endocrine pancreas of neonatal rats in the absence of calcium and glucose during a 30-min incubation period (mean ± SEM). Statistical significance for each column compared with control (absence of veratridine, preincubation medium calcium concentration—0.26 or 1.3 mM). *P < 0.05, **P < 0.01.

that displaces superficial membrane calcium and blocks transmembrane calcium fluxes,[23] were examined in the absence of calcium in the medium. As shown in the lower part of Table 1, glucagon and insulin secretion induced by 50 μM veratridine was significantly inhibited by 3 μM TTX, or 0.5 mM $LaCl_3$. $LaCl_3$ seemed to be more potent than TTX.

Effect of preincubation in low (0.26 mM), normal (1.3 mM), or high (3.9 mM) concentration of calcium in the media. In an attempt to change the content of calcium in islet cells, monolayer cultures were preincubated for 3 h in low, normal, or high (0.26, 1.3, or 3.9 mM) calcium with 5.6 mM glucose in media. As shown in Figure 2, after the preincubation in high calcium, the effect of 50 μM veratridine in the absence of calcium was inhibitory on both glucagon and insulin secretion. However, the basal level of secretion for both hormones was significantly higher after preincubation in high calcium than it was in low or normal calcium media. When 3.9 mM calcium was added into the medium after the preincubation in low calcium, glucagon release was significantly inhibited in the presence of 50 μM veratridine.

As shown in Figure 3, the effect of 50 μM veratridine after preincubation in low calcium for 3 h was almost the same as that after preincubation with normal calcium concentration.

Effect of veratridine and ouabain with and without glucose. As shown in Figure 4, the effects of 50 μM veratridine and 0.5 mM ouabain were compared with and without glucose. In these studies, secretions of glucagon and insulin were measured during 0–30 min (early) and 30–90 min (late), respectively. The effect of 50 μM veratridine was predominantly on early secretion, except for the stimulation of both early and late insulin secretion when glucose was present. The rather small effect of 0.5 mM ouabain seemed to be on early hormone secretion, although no effect was seen on glucagon secretion in the absence of glucose.

DISCUSSION

The present study shows that both incubation and preincubation calcium concentrations affected glucagon and insulin secretion in the absence or presence of veratridine. Basal glucagon secretion was greatly enhanced when calcium was omitted from the incubation medium as compared with the situation with normal medium calcium. The same results were more clearly obtained in the presence of 0.5 mM EGTA. This observation is consonant with the report of Wollheim et al.[14,15] and Leclercq-Meyer et al.[16] Glucose seemed to have no effect on secretion of either hormone in the absence of extracellular calcium (Table 1), being different from the report by Leclercq-Meyer et al.[17]

Although in the presence of normal calcium veratridine caused a dose-dependent enhancement of glucagon secretion with an effect being seen at 10 μM veratridine, dose dependency was lost when calcium was omitted from the medium. Insulin secretion also showed a dose-dependent increase with veratridine in normal calcium, an effect being seen at 10 μM veratridine. This result is somewhat different from what was reported by Pace.[9] However, the effect was greatly attenuated or lost at lower concentrations of veratridine when calcium was absent in media. These results suggest that the action of veratridine is, at least in part, depen-

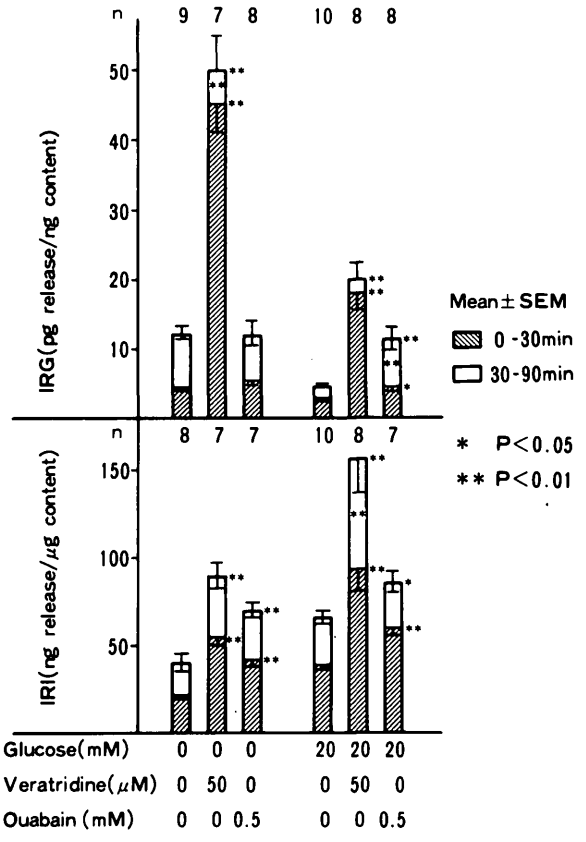

FIGURE 4. Release of IRG and IRI from monolayer cultures of the endocrine pancreas of neonatal rats. Release during the first 30 min (0–30 min: shaded column) and during the entire 90 min (shaded plus open column) of incubation are shown as mean ± SEM. Statistical significance is indicated at the right upper part of each column or in each column (30–60 min). *P < 0.05, **P < 0.01.

dent on extracellular calcium. It is possible that enhanced uptake of calcium by cells may occur when incubated with veratridine, due to a generalized increase in membrane permeability to ions or, alternatively, to Na^+-Ca^{2+} exchange.[18] The effect of 50 μM veratridine on glucagon and insulin secretion was clearly shown in the absence of calcium with or without EGTA. This means that 50 μM veratridine is potent enough to derive sufficient intracellular calcium from storage sites to induce glucagon and insulin secretion.[8]

The presence of 3 μM TTX significantly blocked the effect of veratridine on secretion of both hormones in the absence of calcium. Almost the same results was obtained by 3 μM TTX in the presence of 1.3 mM Ca (data are not shown), indicating the direct blocking effect of TTX to veratridine.[3,19] These results may suggest an important role for intracellular sodium in regulating concentration of intracellular calcium.[8]

We then evaluated glucagon and insulin secretion in the absence or presence of veratridine after omission of calcium from the incubation medium to investigate the effects of intracellular calcium. After cells had been preincubated for 3 h in either low (0.26 mM), normal (1.3 mM), or high (3.9 mM) calcium in medium, the actual incubations were performed for 30 min. We assume that the accumulation of cellular calcium is dependent on the extracellular calcium concentration.[20] Basal glucagon and insulin secretions were significantly increased after preincubation in high calcium when compared with low or normal calcium. The effect of veratridine in the absence of extracellular calcium, but with possible different content of intracellular calcium, was strikingly different among the various conditions of preincubation. The effect of veratridine on glucagon and insulin secretion when cells were preincubated in normal calcium was similar to that shown previously, i.e., absent at 10 μM but present at 50 μM veratridine. Preincubation in high calcium resulted in a diminution of glucagon and insulin secretion by 50 μM veratridine. Thus, in the absence of extracellular calcium, the results suggest that the content of calcium in cells may affect mobilization of intracellular calcium from the storage sites under various conditions.[8,21,22]

$LaCl_3$, as shown in Table 1, seemed to have a strong effect on veratridine-induced hormone secretion, resulting in almost complete inhibition. As La^{3+} is reported to be entirely restricted to the exterior of the cells,[23] this may suggest an important role of membrane-bound calcium in the regulation of hormone secretion. It remains to be clarified whether calcium bound in the membrane represents the pool from which the triggering level of calcium in the cell is desired or acts as a physicochemical factor required for the exocytosis of granules out of the membranes.

The effect of veratridine was compared with that of the Na^+-K^+ ATPase inhibitor, ouabain, in the absence or presence of 20 mM glucose (Figure 4). The effect of 50 μM veratridine was much more striking than that of 0.5 mM ouabain, especially with regard to glucagon secretion. In these studies, secretion was measured during the 0–90-min periods. This allows an estimate of the relative contribution of the early (0–30 min) versus the late (30–90 min) periods of secretion to the total hormone secretion. In monolayer cultures of neonatal rat pancreas, insulin secretion induced by high glucose has already been shown to be more prominent in early than in late periods.[10] The effect of veratridine was predominantly on early secretion, except for a stimulation of both early and late insulin secretion when glucose was present. The effect of ouabain seemed to be on early hormone secretion except for the absence of any effect upon glucagon secretion when glucose was omitted. These differences in effects are not surprising, as ouabain may alter intracellular K^+ and Na^+, and these changes may in turn affect calcium movements.[24]

The effect of glucose upon calcium movement is also relevant,[18,25] and further studies are required to distinguish more clearly this effect from those of calcium concentration per se or of cellular requirements for glucose as an energy source.[26,27]

In conclusion, these results indicate the existence of veratridine-sensitive sodium channels in both α- and β-cells, and also suggest that the effect of veratridine on glucagon as well as insulin secretion cannot be solely attributed to these sodium channels, but may have other actions as well.[7] Nevertheless, it is clear from our studies that veratridine and, to a lesser extent, ouabain, have significant effects upon α- as well as β-cell function, indicating that a common secretion mechanism is involved in both endocrine cells.

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