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Research Article

The role of calmodulin in insulin secretion from rat pancreatic islets has been examined by the use of trifluoperazine, an inhibitor of calmodulin-Ca⁺⁺-directed functions. It was found that 30 microM trifluoperazine caused 50% inhibition, and 100 microM, up to 73% inhibition of 16.7 mM glucose-stimulated insulin release. 100 microM trifluoperazine caused a similar inhibition of 10 mM glyceraldehyde-stimulated release. Therefore, the site of action of trifluoperazine in glucose stimulus-secretion coupling appears to be after the trioses. As trifluoperazine had no effect upon insulin release stimulated by 1 mM 3-isobutyl-1-methylxanthine, the inhibitory effect of trifluoperazine appears to be rather specific. Further, the process of exocytosis per se is not affected. It was also found that although trifluoperazine inhibited the effect of glucose to stimulate insulin release, it did not affect the synergism between glucose and 3-isobutyl-1-methylxanthine to potentiate insulin release. It may be concluded that trifluoperazine selectively inhibits one part of the mechanism by which glucose stimulates insulin release. Calmodulin plays a role in the stimulation of insulin release by glucose at a site between metabolism of trioses and elevation of cytosol Ca⁺⁺, but is not involved in the final process of exocytosis.

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Possible Role for Calmodulin in Insulin Release

STUDIES WITH TRIFLUOPERAZINE IN RAT PANCREATIC ISLETS

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ABSTRACT The role of calmodulin in insulin secretion from rat pancreatic islets has been examined by the use of trifluoperazine, an inhibitor of calmodulin-Ca⁺⁺-directed functions. It was found that 30 μ M trifluoperazine caused 50% inhibition, and 100 μ M, up to 73% inhibition of 16.7 mM glucose-stimulated insulin release. 100 μ M trifluoperazine caused a similar inhibition of 10 mM glyceraldehyde-stimulated release. Therefore, the site of action of trifluoperazine in glucose stimulus-secretion coupling appears to be after the trioses. As trifluoperazine had no effect upon insulin release stimulated by 1 mM 3-isobutyl-1-methylxanthine, the inhibitory effect of trifluoperazine appears to be rather specific. Further, the process of exocytosis *per se* is not affected. It was also found that although trifluoperazine inhibited the effect of glucose to stimulate insulin release, it did not affect the synergism between glucose and 3-isobutyl-1-methylxanthine to potentiate insulin release. It may be concluded that trifluoperazine selectively inhibits one part of the mechanism by which glucose stimulates insulin release. Calmodulin plays a role in the stimulation of insulin release by glucose at a site between metabolism of trioses and elevation of cytosol Ca⁺⁺, but is not involved in the final process of exocytosis.

INTRODUCTION

Glucose exerts a major controlling influence over the rate of insulin secretion. A rise in the cytosolic concentration of ionized calcium is considered to be an important factor in stimulus-secretion coupling (1-4), but the mechanism by which Ca⁺⁺ induces exocytosis and insulin secretion is unknown. However, calcium-dependent modulator proteins, such as calmodulin, are

known to mediate some Ca⁺⁺-stimulated processes (5, 6). Calmodulin is present in islets and has been shown to activate adenylate cyclase from islets (7-9). It seems important, therefore, to evaluate the possible role of calmodulin in the regulation of insulin secretion. As a first step, the effects of trifluoperazine, a specific inhibitor of the calmodulin-Ca⁺⁺ complex (10), have been examined with respect to various stimulators of insulin secretion.

METHODS

Preparation and incubation of islets. Pancreatic islets were isolated from fed male Wistar rats by collagenase digestion (11). After a 30-min preincubation at 37°C in Krebs-Ringer bicarbonate buffer containing 10 mM Hepes buffer, 0.5% bovine serum albumin, and 2.8 mM glucose, groups of 20 islets were incubated in 500 μ l of the same buffer, but with 2.8 or 16.7 mM glucose and the test substances as appropriate. After 30 min, the supernates were removed for insulin radioimmunoassay (12), using rat insulin as standard. Each control and experimental situation was usually performed in triplicate and the results meaned.

Statistical methods. Each experiment performed in triplicate is expressed as $n = 1$ for statistical analysis. Significance was assessed by *t* test using paired analysis.

Trifluoperazine was a gift from Smith Kline & French Laboratories, Philadelphia. Guinea pig antipork insulin serum was generously supplied by Dr. P. Wright.

RESULTS

Effects of trifluoperazine (100 μ M) on insulin release in the presence of 2.8 and 16.7 mM glucose (Fig. 1). At a low glucose concentration (2.8 mM) trifluoperazine caused a small stimulation of insulin release. 16.7 mM glucose caused an increase in insulin release rising from 21 ± 2 to 116 ± 13 pg/islet per min ($P < 0.001$) over the 30-min incubation period. This stimulated rate of insulin release was inhibited by 73% by trifluoperazine ($P < 0.001$). The percent inhibition was determined

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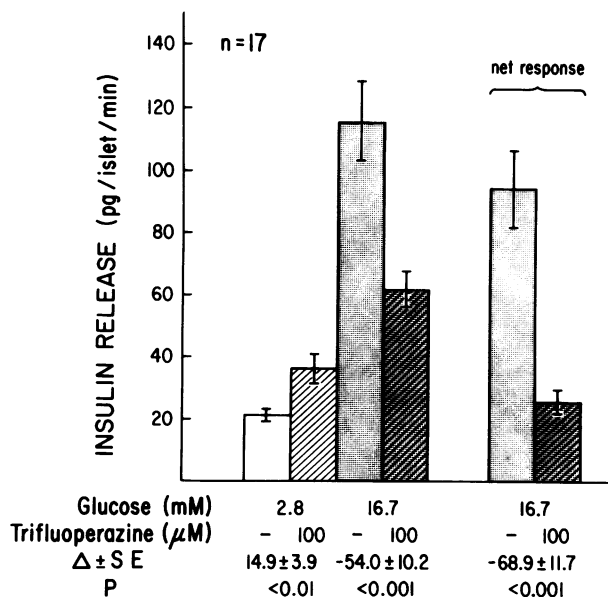


FIGURE 1 Effects of 100 μM trifluoperazine on insulin release in the presence of 2.8 and 16.7 mM glucose. The net response to high glucose with and without trifluoperazine is obtained by subtraction of the insulin response in the presence of 2.8 mM glucose.

from the increment in insulin release due to high glucose (95 ± 13 pg/islet per min) and the increment in insulin release remaining in the presence of trifluoperazine (26 ± 4 pg/islet per min) as shown in the two right-hand bars of Fig. 1.

Effects of different concentrations of trifluoperazine on insulin release in the presence of 2.8 and 16.7 mM glucose (Table I). 10 μM trifluoperazine had no effect upon insulin release either at low or high glucose.

30 μM trifluoperazine had no effect at low glucose, but caused a 50% inhibition of high glucose-stimulated insulin release (130 down to 64 pg). 100 μM trifluoperazine stimulated release at low glucose, and as expected had a marked inhibitory effect at high glucose.

Effect of trifluoperazine (100 μM) on insulin release stimulated by 10 mM D-glyceraldehyde (Fig. 2). Since the effects of glucose are mimicked by glyceraldehyde (13), to localize the site of action of trifluoperazine further, its effects on glyceraldehyde-induced insulin release were studied. 10 mM D-glyceraldehyde induced a 2.4-fold increase in the release rate, which was inhibited by 66% in the presence of trifluoperazine (P < 0.01). This is close to the 73% inhibition obtained for glucose-stimulated release.

Effect of trifluoperazine (100 μM) on insulin release stimulated by the phosphodiesterase inhibitor 3-isobutyl-1-methylxanthine (IBMX)¹ in the presence of 2.8 and 16.7 mM glucose (Fig. 3). In the presence of 2.8 mM glucose, 1 mM IBMX caused a 3.3-fold increase in the release rate from 16 ± 2 to 66 ± 5 pg/islet per min and the IBMX-stimulated release was unaffected by trifluoperazine. 16.7 mM glucose produced its anticipated stimulation of insulin release, which rose from 16 ± 2 to 82 ± 8 pg/islet per min. This was inhibited by trifluoperazine. The combination of 16.7 mM glucose and 1 mM IBMX caused a dramatic increase in the rate of insulin release. The potentiating effects of these two agents is shown by the fact that the increment in insulin release due to glucose and IBMX combined (171 ± 14 pg/islet per min) was significantly greater than the sum of the increments due to glucose (67 ± 8)

¹ Abbreviation used in this paper: IBMX, 3-isobutyl-1-methylxanthine.

TABLE I
Effects of Different Concentrations of Trifluoperazine on Insulin Release in the Presence of 2.8 and 16.7 mM Glucose

Glucose concentration	Trifluoperazine				Effect of trifluoperazine	
	0 μM	10 μM	30 μM	100 μM	Δ ± SEM	P
mM	pg/islet per min					
2.8	21 ± 4	24 ± 4	34 ± 9	51 ± 12	2 ± 2	>0.2
					13 ± 6	>0.05
					30 ± 9	<0.02
16.7	152 ± 27	130 ± 12	98 ± 14	76 ± 10	-22 ± 29	>0.4
					-53 ± 18	<0.02
					-76 ± 29	<0.05
Effect of high glucose	130 ± 29	106 ± 12	64 ± 15	25 ± 11	-24 ± 29	>0.4
Δ ± SEM					-66 ± 14	<0.01
					-105 ± 31	<0.05
P	<0.02	<0.001	<0.02	>0.05		

Insulin release is expressed as picograms immunoreactive insulin per islet per minute, n = 5.

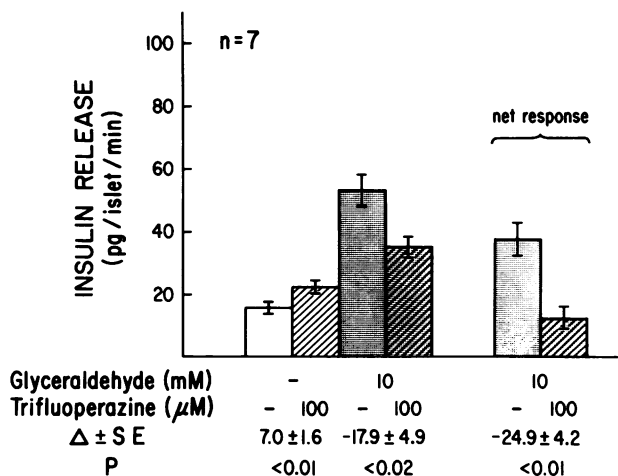


FIGURE 2 Effects of 100 μ M trifluoperazine on insulin release in the presence of 2.8 mM glucose and 2.8 mM glucose plus 10 mM D-glyceraldehyde.

and IBMX (51 ± 5) separately. The increase due to the potentiation was 54 ± 14 , $P < 0.01$, $n = 7$. Of interest was the fact that the absolute decrement due to trifluoperazine was identical in both 16.7 mM glucose and 16.7 mM glucose plus IBMX. Thus when the effect of IBMX and trifluoperazine is studied in the presence

of 16.7 mM glucose, the net response due to IBMX is unaffected by trifluoperazine (right-hand bars of Fig. 3). In the presence of trifluoperazine the potentiating effect due to the combination of glucose and IBMX was unaffected. Insulin release in the presence of glucose, IBMX, and trifluoperazine (128 ± 7 pg/islet per min) was significantly greater than the sum of the effects of glucose (23 ± 2) and IBMX (49 ± 7), both with trifluoperazine. The increase due to the potentiation was 56 ± 7 ($P < 0.001$, $n = 7$) which is similar to the potentiation seen in the absence of trifluoperazine. Strikingly, therefore, the synergism between glucose and IBMX is unaffected by trifluoperazine at a time when the stimulatory effects of glucose are inhibited.

DISCUSSION

Calmodulin has been found to mediate many of the effects of Ca^{++} in cellular functions (5, 6). To evaluate its role in the regulation of insulin secretion, the effects of trifluoperazine, a phenothiazine antipsychotic drug, have been examined in the presence of various stimulators of the secretion. This compound is thought to specifically bind to the Ca^{++} -calmodulin complex (10) and thereby inhibit the activity of various calmodulin-sensitive enzymes (14). The specificity of trifluoperazine has been shown by (a) the lack of

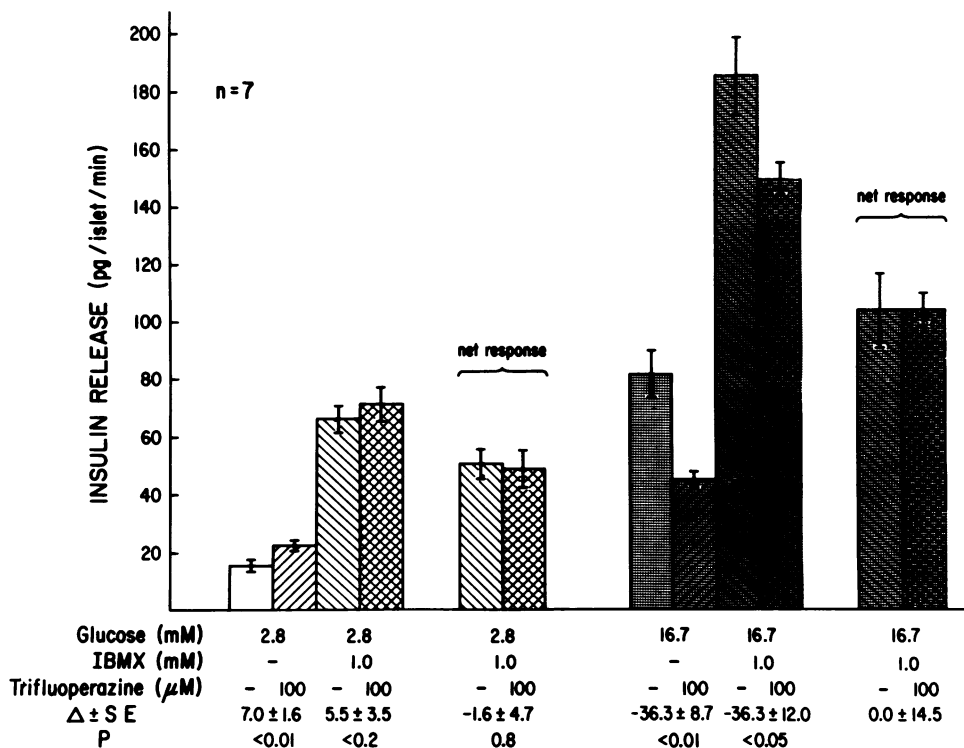


FIGURE 3 Effects of 100 μ M trifluoperazine on insulin release induced by 1 mM IBMX, in the presence of 2.8 mM glucose (left side) or 16.7 mM glucose (right side). \boxtimes , TFP; \boxplus , IBMX; \boxtimes , TFP + IBMX.

Ca⁺⁺-dependent binding of trifluoperazine to a variety of proteins (10); (b) the fact that the inhibitory effects of trifluoperazine are related to the presence of Ca⁺⁺ in concentrations that are required for activation of calmodulin-sensitive enzymes (10); and (c) the fact that the inhibitory effects of trifluoperazine and other phenothiazines are correlated with their binding affinities to calmodulin (15).

The results of the studies presented here, and of others, which demonstrate an inhibition of glucose-stimulated insulin release by trifluoperazine and the presence of calmodulin in islets (7), indicate the possible involvement of calmodulin in the mechanism of stimulus-secretion coupling. Trifluoperazine inhibited glyceraldehyde- and glucose-stimulated insulin release to the same extent, and this suggests that the calmodulin-sensitive step does not occur before trioses in glucose metabolism. It is important to note the complete absence of an inhibitory effect of trifluoperazine upon IBMX-induced release, because this strongly suggests that calmodulin is not involved in the process of exocytosis *per se*. In fact, as IBMX is thought to stimulate insulin release by raising cyclic AMP levels and mobilizing intracellular Ca⁺⁺ stores to raise cytosol Ca⁺⁺ (16), then all the steps between elevation of cytosol Ca⁺⁺ and exocytosis appear to be independent of calmodulin. Further, the mobilization of Ca⁺⁺ by IBMX is calmodulin independent. It was found that trifluoperazine had the same inhibitory effect on insulin release in the presence of IBMX at 16.7 mM glucose as it did with 16.7 mM glucose alone. Thus the action of IBMX was unaffected by trifluoperazine whether in the presence of low or high glucose concentrations. This leads to the striking conclusion that the potentiation of insulin release was the same in the presence and absence of trifluoperazine and that the synergism between glucose and IBMX is preserved at a time when the stimulation of insulin release by glucose is inhibited. This conclusion suggests that glucose potentiates the effect of IBMX, not that IBMX potentiates the effect of glucose, and that the mechanism of potentiation is unaffected by trifluoperazine and is therefore calmodulin insensitive.

The mechanisms underlying the observed effects of trifluoperazine are unknown. It is to be expected that inhibition of calmodulin-directed processes, because of their multiplicity, would lead to multiple effects. In islets this is manifested by (a) inhibition of glucose and glyceraldehyde-stimulated insulin release, and (b) a small stimulation of insulin release under basal conditions. Enzyme systems known to be sensitive to calmodulin, which if inhibited by trifluoperazine would lead to inhibition of insulin release, include adenylate cyclase and protein kinase. Activation of adenylate cyclase by Ca⁺⁺-calmodulin may underlie the Ca⁺⁺-dependent increase in cyclic AMP observed

in islets exposed to glucose (17–19), as suggested by the presence of Ca⁺⁺-calmodulin-dependent adenylate cyclase in islets (8, 9). Inhibition of the cyclic AMP response to glucose by trifluoperazine can be expected to be responsible to a small extent for inhibition of insulin release. Calmodulin-sensitive enzymes, which if inhibited could lead to stimulation of release, include cyclic nucleotide phosphodiesterase and Ca⁺⁺-ATPase. These and other potential sites for inhibition by trifluoperazine remain to be investigated.

The most important conclusions from these results are that (a) calmodulin appears to be involved in stimulus-secretion coupling for glucose and glyceraldehyde-induced insulin release; (b) the two effects of glucose to stimulate insulin release, and to potentiate release in the presence of IBMX, can be dissociated by trifluoperazine; stimulation of release is calmodulin dependent, whereas the potentiating effect is calmodulin independent; (c) calmodulin does not seem to be involved in stimulus-secretion coupling after the elevation of cytosol Ca⁺⁺, nor in the process of exocytosis itself.

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