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Effects of α-adrenoceptor antagonists on clonidine-induced inhibition of insulin secretion by isolated pancreatic islets

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1 The effects of clonidine, yohimbine, corynanthine and prazosin on glucose-induced insulin secretion by incubated or perifused mouse pancreatic islets were investigated.
2 Clonidine (0.1 μM) inhibited glucose-induced insulin secretion alone and in the presence of yohimbine (0.1 μM), corynanthine (10 μM) or prazosin (1 μM).
3 In higher concentrations, yohimbine (1–10 μM) antagonized the inhibitory effect of clonidine (0.1 μM) upon glucose-induced insulin secretion by incubated islets and by perifused islets.
4 The results support the view that adrenergic inhibition of insulin secretion is mediated by α_2-adrenoceptors on pancreatic β-cells.

Introduction

Adrenaline inhibits glucose-induced insulin secretion *in vivo* (Porte Jr., Graber, Kuzuya & Williams, 1966) and in isolated pancreatic islets (Coll-Garcia & Gill, 1969). The classification of α-adrenoceptors into α_1- and α_2-subtypes (Langer, 1974; Starke, 1977) led Nakaki, Nakadate & Kato (1980) and Nakaki, Nakadate, Ishii & Kato (1981) to examine the receptors involved in the inhibitory effect of adrenaline on insulin secretion from incubated pancreatic islets. At equimolar concentrations yohimbine, which preferentially blocks α_2-adrenoceptors, antagonized the inhibitory effect of adrenaline more effectively than prazosin which preferentially blocks α_1-adrenoceptors. The latter authors concluded that α_2-adrenoceptors on pancreatic β-cells mediate the adrenaline-induced inhibition of insulin release.

However in their studies involving incubation of rat pancreatic islets, the β-cells were exposed to high concentrations of the secretory products from the other types of islet cells e.g. glucagon and somatostatin which may modulate β-cell function (Unger & Orci, 1977; Pace & Tarvin, 1981) (not excluding the possibility of α-adrenoceptors being located on the other cell types). In order to explore this possibility we studied mouse islets, which contain a lower proportion of non-β-cells than rat islets (Dean, 1973; Hoftiezer & Carpenter, 1973), and which were perifused in order to minimize the effects of secretory products on β-cells. In addition, we used the more selective α_2-adrenoceptor agonist, clonidine instead of adrenaline in order to avoid stimulation of β-adrenoceptors which would lead to stimulation of insulin secretion (Porte Jr., 1967; Loubatières, Mariani, Sorel & Savi, 1971) and hence complicate the evaluation of the potency of α-adrenoceptor antagonists.

Methods

Isolation, incubation and perifusion of islets

Pancreatic islets were isolated from male albino mice (NMRI, 11–15 weeks old, 35–45 g, fed *ad libitum*) by collagenase digestion (Lernmark, 1974) in basal medium supplemented with 5 mM D-glucose. This basal medium was also used in the incubation and perifusion experiments and has been described previously (Panten, Biermann & Graen, 1981). Batches of 20 islets were preincubated and incubated at 37°C in 40 μl of medium. Batches of 50 islets were perifused at 0.9 ml/min at 37°C. Incubations and perifusions were performed as described by Panten, Ishida, Schauder, Frerichs & Hasselblatt (1977) and by Panten *et al.* (1981). From 48–120 min islets were perifused with 20 mM glucose.

Insulin determinations

Insulin was measured by radioimmunoassay (Zaharko & Beck, 1968) using ^{125}I-labelled porcine insulin (Behringwerke, Frankfurt, West Germany), with mouse insulin as the standard (Novo, Bagsvaerd, Denmark) and an antibody obtained from guinea-pigs in our laboratory. The bound fraction was precipitated by ammonium sulphate (Joost, 1979). Intra-assay variation was less than 5%, inter-assay variation less than 10%. It was possible to detect 0.1 ng/ml accurately.

Drugs and chemicals

The following drugs were used: clonidine HCl (Boehringer, Ingelheim, West Germany); yohimbine HCl and corynanthine HCl (Sigma, St. Louis, MO, U.S.A.); prazosin HCl (Pfizer, Karlsruhe, West Germany). Stock solutions of the test substances were prepared daily with distilled water. The sources of the other chemicals used have been stated previously (Panten *et al.*, 1981).

Presentation of results

Results are presented as mean values ± s.e.mean for independent experiments. In incubation experiments, the difference between the insulin release at

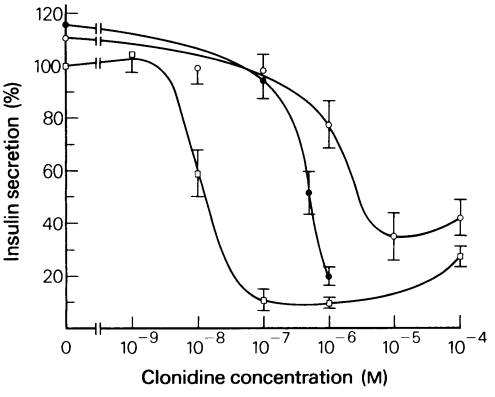

Figure 1 Effects of clonidine and yohimbine on glucose-induced insulin secretion from incubated pancreatic islets. Batches of 20 islets were preincubated for 45 min in basal medium containing 4 mM glucose and then incubated for 60 min in basal medium containing 20 mM glucose and the appropriate test substances. The difference between the insulin release in 4 mM glucose or 20 mM glucose was taken as 100%. Values shown are means of $n = 7-16$; ± s.e.mean indicated by vertical lines. Insulin secretion in the presence of 20 mM glucose (□) insulin secretion in the presence of 20 mM glucose, 1 μM yohimbine (●); insulin secretion in the presence of 20 mM glucose, 10 μM yohimbine (○).

Table 1 Effects of clonidine, yohimbine, prazosin and corynanthine on glucose-induced insulin secretion

Group	*Test substances* (μM)	*Mean insulin secretion rate from 92–120 min* (% of 76 min value)	*P*
A	None	136.0 ± 10.1	
B	Yohimbine (1)	154.3 ± 12.7	NS
C	Yohimbine (10)	138.3 ± 9.7	NS
D	Prazosin (1)	115.2 ± 14.6	NS
E	Corynanthine (10)	122.0 ± 7.3	NS
F	Clonidine (0.1)	21.9 ± 2.3*	<0.002
G	Clonidine (0.1) + yohimbine (0.1)	30.0 ± 3.1	NS
H	Clonidine (0.1) + yohimbine (1)	86.3 ± 11.6*	<0.002
I	Clonidine (0.1) + yohimbine (10)	120.5 ± 36.6*	<0.002
K	Clonidine (0.1) + prazosin (1)	31.6 ± 3.5	NS
L	Clonidine (0.1) + corynanthine (10)	32.8 ± 7.7	NS

During perifusion with 20 mM glucose, test substances were added at 80 min. In each single perifusion experiment the mean insulin secretion rate from 92–120 min was calculated (the rate at 76 min was taken as 100%). Values of groups A and C–L were derived from the experiments shown in Figures 2 and 3. Results are expressed as means ± s.e.mean ($n = 6-7$). Significance was calculated by the two-tailed nonparametric U-test of Wilcoxon and of Mann & Whitney. A *P* value of less than 5% was considered significant. The *P* values of groups B–F refer to comparison with group A and those of groups G–L refer to comparison with group F. The *P* values for comparison of C with I, H with K or H with L are NS, <0.01 or <0.02, respectively.

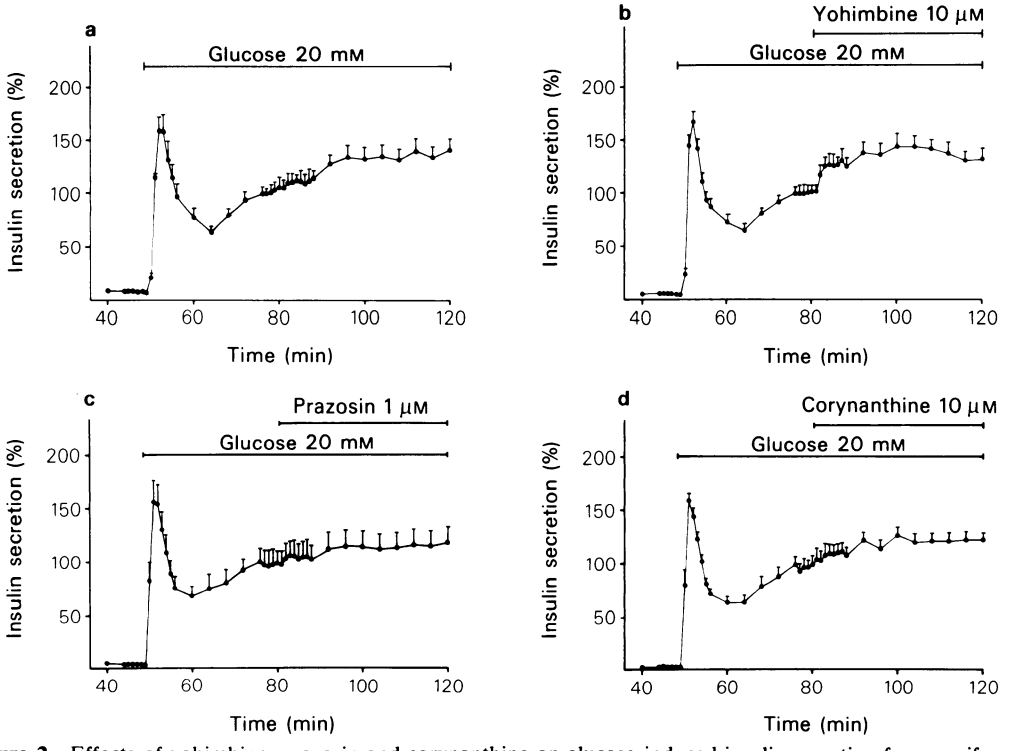

Figure 2 Effects of yohimbine, prazosin and corynanthine on glucose-induced insulin secretion from perifused pancreatic islets. Batches of 50 islets were perifused for 48 min with basal medium containing 5 mM glucose. At 48 min the fluid was changed to basal medium containing 20 mM glucose and at 80 min to basal medium containing 20 mM glucose and the following test substances: none (a); 10 μM yohimbine (b); 1 μM prazosin (c); 10 μM corynanthine (d). Insulin release was measured at 1 and 4 min intervals as described in the Methods section. In each single perifusion the insulin secretion rate at 76 min was taken as 100%. Values shown are means of $n = 6$ in (a), (b), (c) and (d); s.e.mean indicated by vertical lines.

4 mM glucose and 20 mM glucose was taken as 100%. In perifusion experiments, the effects of test substances were compared by calculating the mean insulin secretion rate from 92–120 min. In each single perifusion the insulin secretion rate at 76 min was taken as 100%. Significance was calculated by the two-tailed nonparametric U-test of Wilcoxon (1945) and of Mann & Whitney (1947). A P value of less than 5% was considered significant.

Results

The effects of clonidine and yohimbine on glucose-induced insulin secretion from incubated pancreatic islets are shown in Figure 1. Clonidine 0.01 μM inhibited glucose-induced insulin secretion by 40%. At 0.1 μM or 1 μM clonidine caused approximately 90% inhibition of insulin release. However, at concentrations above 1 μM the inhibitory effect of clonidine declined. Yohimbine 1 μM blocked the effect of 0.1 μM clonidine almost completely ($P < 0.002$) and reversed the effect of 0.5 μM clonidine by about 50%. No significant antagonistic effect of 1 μM yohimbine could be demonstrated in the presence of an equimolar clonidine concentration. Yohimbine 10 μM reversed the inhibition of insulin release induced by 0.01–1 μM clonidine by 100–75%. No clear antagonism between yohimbine and clonidine at high clonidine concentrations (10 μM or 100 μM) could be demonstrated. However, at high concentrations nonspecific membrane effects have been demonstrated both with clonidine (Starke, Wagner & Schümann, 1972) and yohimbine (Shaw, Holman & Mackenzie, 1955).

The results from the incubation experiments were compared with those from perifusion experiments (Figures 2 and 3, Table 1). The typical biphasic insulin release profile in the presence of 20 mM glucose is shown in Figure 2a. In this experiment the 100% value of insulin release from 50 islets at 76 min was 2.25 ± 0.17 ng/min. Control studies demon-

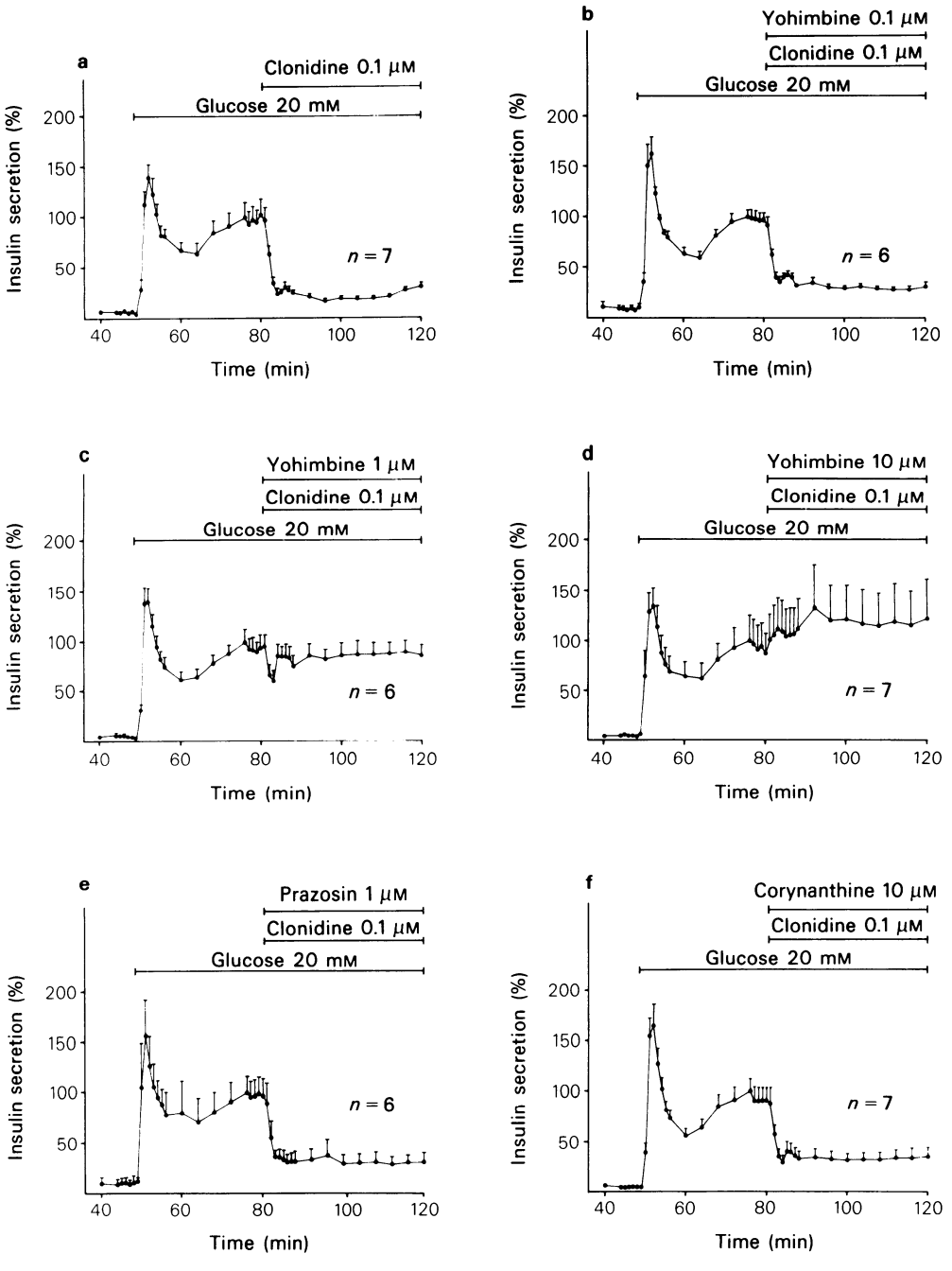

Figure 3 Effects of yohimbine, prazosin and corynanthine on clonidine-induced inhibition of insulin secretion from perifused pancreatic islets. Batches of 50 islets were perifused for 48 min with basal medium containing 5 mM glucose. At 48 min the fluid was changed to basal medium containing 20 mM glucose and at 80 min to basal medium containing 20 mM glucose, 0.1 μM clonidine and the following test substances: none (a); 0.1 μM yohimbine (b); 1 μM yohimbine (c); 10 μM yohimbine (d); 1 μM prazosin (e); 10 μM corynanthine (f). Insulin release was measured at 1 and 4 min intervals as described in the Methods section. In each single perifusion the insulin secretion rate at 76 min was taken as 100%. Values shown are means of $n = 6-7$; s.e.mean indicated by vertical lines.

strated that during perifusion in the presence of 5 mM glucose, insulin secretion remained constant: at 40, 60, 80, 100 or 120 min rates (per 50 islets) were 0.095 ± 0.009, 0.085 ± 0.013, 0.099 ± 0.014, 0.115 ± 0.017 or 0.125 ± 0.017 ng/min, respectively (n =8; secretion profile not shown). Yohimbine (1 or 10 μM), prazosin (1 μM) and corynanthine (10 μM) did not significantly alter glucose-induced insulin secretion (Figure 2b–d; Table 1). Clonidine (0.1 μM) inhibited glucose-induced insulin secretion by 76% within 4 min and by 84% from 92–120 min (Figure 3a, Table 1). The inhibitory effect of clonidine (0.1 μM) was 37% in the presence of 1 μM yohimbine and not detectable with 10 μM yohimbine (Figure 3, c and d; Table1). Yohimbine at a low concentration (0.1 μM), prazosin (1 μM) or corynanthine (10 μM) did not antagonize clonidine-induced inhibition of insulin secretion (Figure 3, b, e and f; Table 1).

Discussion

Our results confirm previous findings that clonidine, which preferentially activates α_2-adrenoceptors (Starke, 1981), strongly inhibits insulin release (Senft, Sitt, Losert, Schultz & Hoffmann, 1968; Metz, Halter & Robertson, 1978; Leclercq-Meyer, Herchuelz, Valverde, Couturier, Marchand & Malaisse, 1980; Nakaki *et al.*, 1981). At a concentration of 0.1 μM, clonidine inhibited glucose-induced insulin secretion by 84–90% (Table 1, Figures 1 and 3a) and the effect was largely reversed by 1 μM yohimbine (Figures 1 and 3c, Table 1). In other systems yohimbine (1 μM) has been shown to block α_2-adrenoceptors preferentially (Starke, Borowski & Endo, 1975; Borowski, Starke & Endo, 1977) while prazosin (1 μM) and the yohimbine diastereoisomer corynanthine (10 μM), which were only weak antagonists in the present study (Figure 3, e and f, Table 1), cause preferential α_1-adrenoceptor blockade in rabbit pulmonary arteries (Cambridge, Davey & Massingham, 1977; Weitzell, Tanaka & Starke, 1979). Since the chemical structures of corynanthine and yohimbine are closely related, it seems unlikely that responses to yohimbine found here are nonspecific. Thus our results support the conclusions of Nakaki *et al.* (1980, 1981) and Yamazaki, Katada & Ui (1982) that postsynaptic α_2-adrenoceptors modulate insulin secretion from pancreatic islets.

However, in the studies of Yamazaki *et al.* (1982), 3-isobutyl-1-methyl-xanthine (IBMX) was included in the incubation media at concentrations sufficient to initiate insulin release in the absence of other stimuli (Wiedenkeller & Sharp, 1981). Since it is generally believed that glucose and purine bases trigger insulin secretion by different mechanisms (Montague & Howell, 1975; Sharp, 1979) the results of Nakaki *et al.* (1980, 1981) are more relevant to glucose-induced insulin release. The static-incubation experiments of the latter did not explain the roles of the different cell types and their secretion products during yohimbine-induced blockade of α_2-adrenoceptors in pancreatic islets. In perifused islets the concentrations of the secretory products from islet cells are much lower than in islets incubated in small volumes of media. Therefore the demonstration of strong antagonizing properties of 1 and 10 μM yohimbine both in a micro-incubation and in a perifusion system (Figure 1 and 3; Table 1) supports the view that α_2-adrenoceptors on β-cells directly mediate the inhibitory effects of sympathomimetic compounds on insulin release from pancreatic islets.

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