Br. J. Pharmac. (1980), **70**, 355–362

MECHANISM BY WHICH CYPROHEPTADINE INHIBITS INSULIN SECRETION

P. DONATSCH, D.A. LOWE, B.P. RICHARDSON & PAMELA TAYLOR

Biological and Medical Research Division, Sandoz Ltd., 4002 Basle, Switzerland

1 Isolated islets of Langerhans from the rat have been used in studies designed to elucidate the mechanism by which cyproheptadine inhibits insulin secretion.

2 D-Glucose and tolbutamide, both of which require extracellular Ca^{2+} to produce insulin release, failed to evoke a secretory response from islets pretreated with cyproheptadine. Conversely veratridine, the calcium ionophore A23187 and theophylline, all of which are capable of mobilizing sufficient intracellular Ca^{2+} to evoke insulin secretion in the absence of extracellular Ca^{2+}, produced similar responses from cyproheptadine pretreated and control islets.

3 Cyproheptadine completely inhibited Ca^{2+} uptake induced by D-glucose and high K_o^+, two agents which depolarize the islet β-cell membrane, whilst Ca^{2+} uptake elicited by removal of extracellular Na^+ (i.e. Na^+-Ca^{2+} counter transport) was only slightly reduced.

4 A significant increase in Na^+ uptake produced by veratridine was sensitive to tetrodoxin but only partially reduced by cyproheptadine.

5 These results suggest that cyproheptadine inhibits depolarization-dependent calcium entry into pancreatic β-cells.

Introduction

The histamine and 5-hydroxytryptamine (5-HT) antagonist, cyproheptadine (CPH), inhibits glucose-mediated insulin release both *in vivo* and *in vitro* (Joost, Poser & Panten, 1974; Feldman, Chapman & Plonk, 1974; Rickert & Fischer, 1974). Recent studies on isolated islets of Langerhans from the rat have shown that this results from an immediate and direct action of CPH on the insulin secreting pancreatic β-cell (Richardson, McDaniel & Lacy, 1975). The present studies were conducted to elucidate the mechanism by which CPH produces this effect. A preliminary account of some aspects of this work has been presented to the British Pharmacological Society (Richardson, 1976).

Methods

Insulin release studies

Islets of Langerhans were isolated from the pancreata of two 8-week-old male albino rats (200 to 250 g) by collagenase digestion (Lacy & Kostianovsky, 1967). An exact number (80 to 100) of islets were placed in each of two small chambers which were then perfused at a rate of 1 ml/min as previously described (Lacy, Walker & Fink, 1972) with Krebs-Ringer bicarbonate solution (KRB) of the following composition (mM): NaCl 115.0, KCl 5.0, $NaHCO_3$ 24.0, $MgCl_2$ 1.0, $CaCl_2$ 2.5. The KRB was supplemented with 5 mg/ml fraction V bovine serum albumin (Fluka Ltd.) and 5.6 mM D-glucose except in studies where the ionophore A 23187 was used in which case 2.8 mM D-glucose was used. This procedure was adopted because A23187 is such a weak secretogogue that its stimulant effects on insulin release could only be clearly demonstrated when basal secretion had been suppressed in this way. Experiments were conducted at 37°C and the pH of all solutions maintained at 7.40 by constant bubbling with 95% O_2 and 5% CO_2.

The two sets of islets were left 45 min before exposure of one set to 10^{-4} M CPH for 5 min and subsequently stimulation of both with an insulin secretogogue. Fractions of the perifusate were collected at 5 min intervals during the settling period and then at 1 min intervals for 5 min immediately before CPH exposure and for the initial 20 min period in secretogogue. Thereafter fractions were again collected at 5 min intervals. The insulin content of all fractions was measured by radioimmunoassay (Wright Makulu, Vichick & Sussman, 1971) and expressed as μu secreted $islet^{-1}$ min^{-1}. Five experiments were conducted for each condition studied and the mean rates of secretion between the various phases and treatments compared statistically by Student's *t* tests.

0007-1188/80/110355-08 $01.00

$^{45}Ca^{2+}$ and $^{22}Na^{+}$ uptake by isolated islets

The method we employed for measuring $^{45}Ca^{2+}$ uptake by isolated islets in static incubation has been described in detail by Donatsch, Lowe, Richardson & Taylor (1977). In studies where the effect of isotonic KCl on $^{45}Ca^{2+}$ uptake was investigated, varying amounts of NaCl in the KRB were replaced by equivalent amounts of KCl. Where zero extracellular sodium ($0Na_o^+$) was used, all the NaCl in the KRB was replaced by an equivalent amount of choline chloride and all the $NaHCO_3$ by an equivalent amount of choline bicarbonate. In such experiments atropine 10 μM was added to all media in order to eliminate possible effects of the choline. At this concentration, atropine does not significantly ($P > 0.05$) alter $^{45}Ca^{2+}$ uptake by isolated islets (compare values for 'normal KRB' with those of 'normal KRB plus atropine', i.e. line 3 column 1 and line 6 column 1, Table 1).

The uptake of $^{22}Na^{+}$ by isolated pancreatic islets of the rat was determined in a similar manner, 5 μCi of the isotope (specific activity 288 μCi/mg, Radiochemical Centre, Amersham) was added to 0.5 ml of the KRB containing the isolated islets and the various compounds under investigation. Incubations lasted 30 min, after which the islets were washed free of extracellular $^{22}Na^{+}$ as in the $^{45}Ca^{2+}$ uptake studies, and the tissue $^{22}Na^{+}$ estimated by γ-counting. The isotope dilutions for both $^{45}Ca^{2+}$ and $^{22}Na^{+}$ were calculated and the uptake expressed in pmol islet^{-1} time^{-1}. Mean values for control and CPH-treated islets were compared statistically by Student's *t* tests.

Drugs

D-Glucose (Merck), tolbutamide (Upjohn), theophylline (Sigma), atropine and veratridine (Sandoz) were all dissolved directly in the KRB. The divalent cation ionophore A23187 (Eli Lilly) was dissolved in ethanol immediately before use and 100 μl of this stock was then added to 100 ml of KRB. This concentration of ethanol does not itself influence insulin secretion (Karl, Zawalich, Ferrendelli & Matschinsky, 1975). Tetrodotoxin (Sigma) was stored frozen as a stock solution in citrate buffer. Aliquots were thawed and added to the KRB immediately before use.

Results

Insulin release studies

Pre-exposure of isolated pancreatic islets to 100 μM CPH for 5 min completely inhibited glucose-mediated insulin release and the islets showed no signs of recovery during the subsequent 40 min stimulation period. Such pretreatment also abolished the responsiveness of islets to tolbutamide. The results are shown in Figures 1 and 2.

In contrast to the effects on D-glucose and tolbutamide-induced insulin secretion, the actions of theophylline (Figure 3) and the divalent cation ionophore A23187 (Figure 4) were unaffected by CPH pretreatment. Veratridine, which is an equally effective insulin secretogogue in the presence or absence of extracellular Ca^{2+} (Lowe, Richardson, Taylor & Donatsch, 1976) caused a three to four fold increase in insulin secretion in the absence of extracellular Ca^{2+}. Pretreatment of the islets with 100 μM CPH did not significantly ($P > 0.05$) affect this response (Figure 5).

$^{45}Ca^{2+}$ uptake studies

Raising the D-glucose concentration from 5.6 mM to 16.7 mM resulted in a significant ($P < 0.01$) increase in

Table 1 Effects of cyproheptadine (CPH) on Ca^{2+} uptake by isolated islets of Langerhans of the rat

Modification to KRB	*Ca^{2+} uptake* (pmol/islet ± s.e.) Control	100 μM CPH	P (*control versus CPH*)
Normal KRB	2.8 ± 1.1 (5)*	3.8 ± 1.7 (5)*	NS
D-Glucose 16.7 mM	15.9 ± 4.0 (5)*	4.4 ± 2.6 (5)*	<0.05
Normal KRB	2.1 ± 0.1 (7)	1.7 ± 0.1 (6)	NS
K^+ 50 mM	7.7 ± 0.7 (9)	2.6 ± 0.2 (10)	<0.001
K^+ 120 mM	14.3 ± 1.9 (10)	2.3 ± 0.2 (8)	<0.001
Normal KRB plus atropine 10 μM	1.6 ± 0.2 (8)	1.7 ± 0.1 (6)	NS
Na^+ 0 mM plus atropine 10 μM	4.3 ± 0.5 (8)	3.0 ± 0.3 (5)	NS

Figures in parentheses indicate number of experiments in each case. NS = not significant.
* Uptake per 60 min, all other values per 20 min.

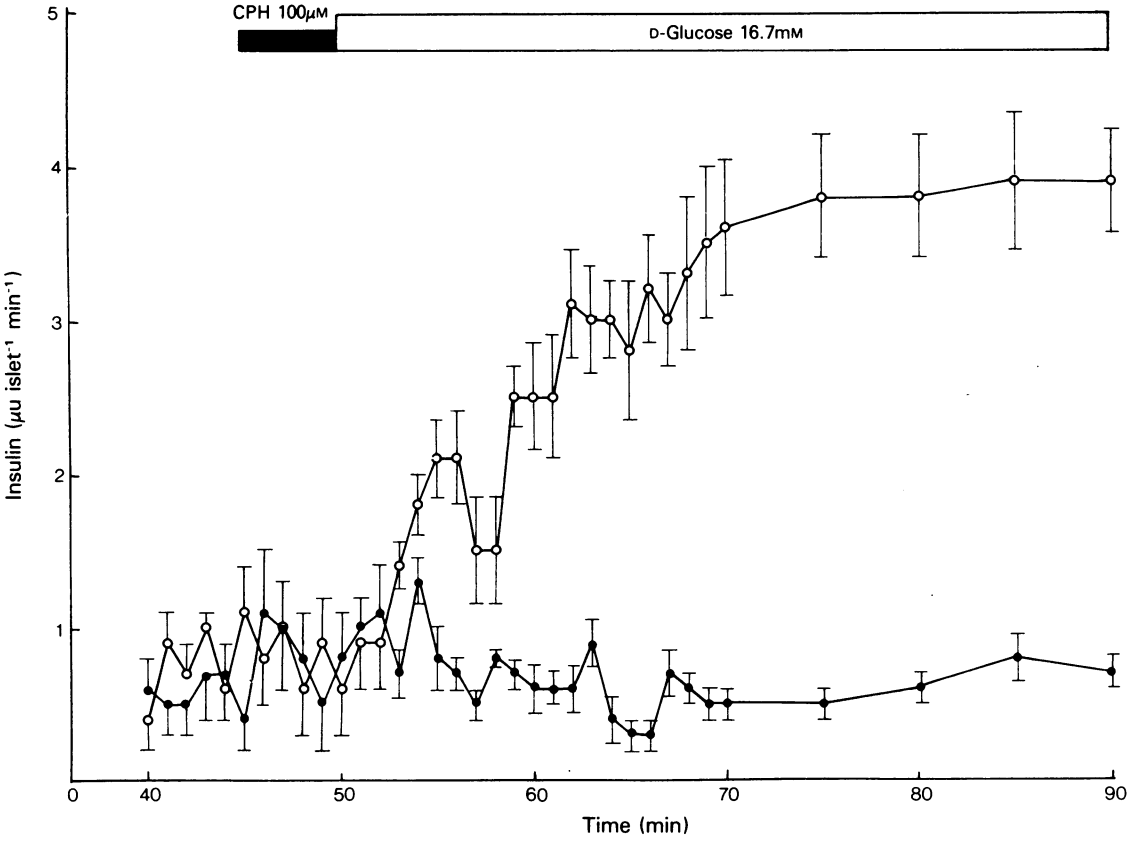

Figure 1 Effects of a 5 min pretreatment with cyproheptadine (CPH) 100 μM (solid bar) on D-glucose stimulated (open bar) insulin secretion from rat isolated pancreatic islets *in vitro*. Control (○); treated (●). Each point is the mean of five experiments and is shown with its standard error.

Ca^{2+} uptake by isolated pancreatic islands which was completely inhibited by CPH 100 μM. The basal Ca^{2+} uptake that occurred at the lower of the two glucose concentrations was not affected by the CPH treatment (Table 1).

Table 1 also shows that all but a very small portion of the Ca^{2+} uptake induced by 50 mM or 120 mM K^+ was inhibited by 100 μM CPH. Nevertheless the residual Ca^{2+} uptake into these islets was significantly ($P < 0.01$) greater than that into unstimulated controls. In contrast to its inhibitory effects on K^+-induced Ca^{2+} uptake, CPH did not significantly influence the uptake which occurred on totally replacing Na^+, with choline (Table 1).

Table 2 Effects of cyproheptadine (CPH) on veratridine-mediated Na^+ uptake by isolated islets of Langerhans of rat

Addition to KRB	n	*Na^+ uptake* (pmol islet^{-1} 30 min^{-1} ± s.e.)	P (*versus control*)
Control	9	63.3 ± 4.9	—
Veratridine 100 μM	12	213.8 ± 25.0*	<0.001
Veratridine 100 μM plus CPH 100 μM	12	130.9 ± 22.2*	<0.02
Veratridine 100 μM plus tetrodotoxin 3 μM	6	46.1 ± 9.3	NS

* Statistically different from one another, $P < 0.05$.

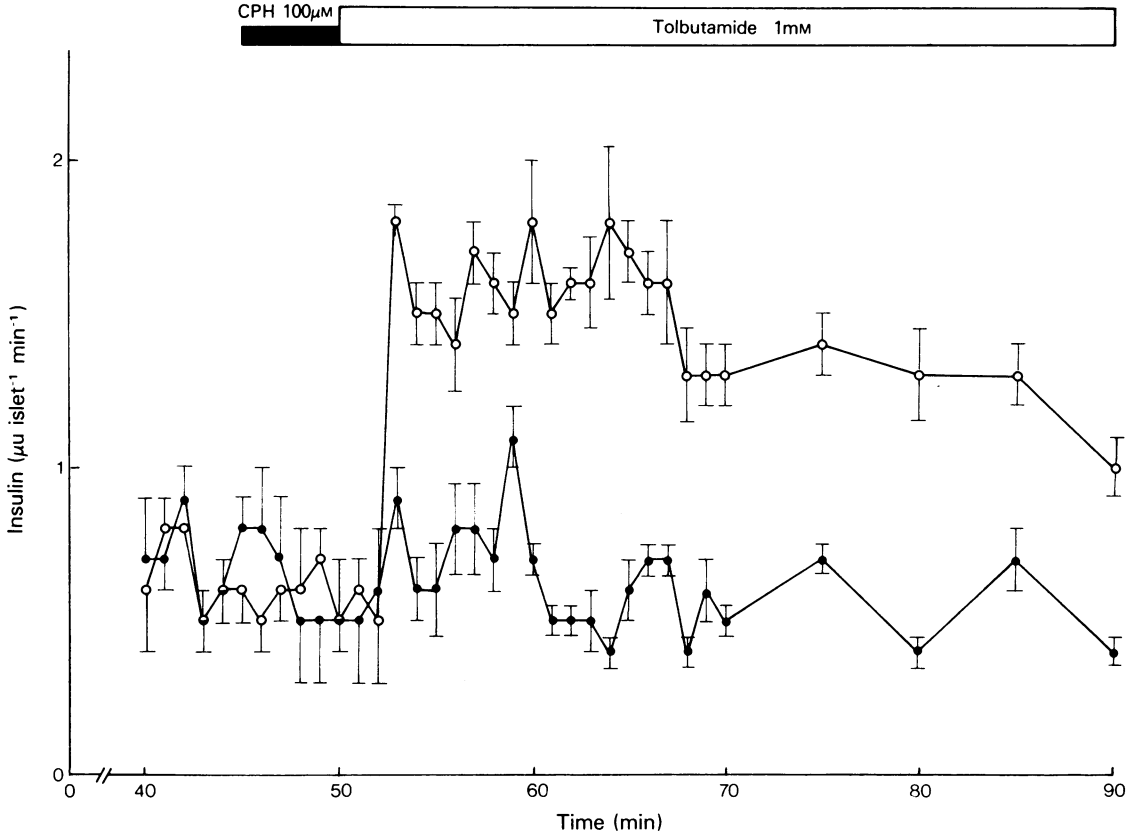

Figure 2 Effects of a 5 min pretreatment with cyproheptadine (CPH) 100 μM (solid bar) on tolbutamide-stimulated (open bar) insulin secretion from rat isolated pancreatic islets *in vitro*. Control (○); treated (●). Each point is the mean of five experiments and is shown with its standard error.

$^{22}Na^+$ *uptake studies*

As shown in Table 2, veratridine evoked a significant ($P < 0.001$) increase in Na_i^+ accumulation by isolated islets. This did not occur in the presence of tetrodotoxin 3 μM. CPH 100 μM significantly reduced the Na_i^+ accumulation induced by veratridine but did not completely inhibit it.

Discussion

D-Glucose probably has to be transported into the pancreatic β-cell and metabolized there before it can stimulate insulin secretion (Dean, Matthews & Sakomoto, 1975; Matthews, 1977). It seems very unlikely that CPH affects either of these steps since the production of $^{14}CO_2$ from [^{14}C]-glucose by isolated islets is unaltered by the presence of the drug in a concentration sufficient to inhibit completely glucose-mediated insulin release (Joost, Beckmann, Holze, Lenzen, Poser & Hasselblatt, 1976). Furthermore, the CPH-induced inhibition of insulin secretion is not restricted to the use of D-glucose as secretogogue since tolbutamide also failed to stimulate its production after exposure of the islets to the drug. Both D-glucose and tolbutamide depolarize the β-cell membrane, thereby opening voltage-dependent channels through which calcium ions subsequently pass down their electrochemical gradient into the cytosol from the extracellular space (Matthews & Sakamoto, 1975). Thus the possibility exists that CPH exerts its actions by preventing Ca^{2+} entry through the outer membrane. On the other hand, the movement of Ca^{2+} between intracellular compartments is apparently unaffected by the drug since veratridine, the ionophore and theophylline, which share the property of being able to mobilize such stores sufficiently to evoke insulin release in the absence of extracellular Ca^{2+} (Brisson, Malaisse-Legae & Malaisse, 1972; Ashby &

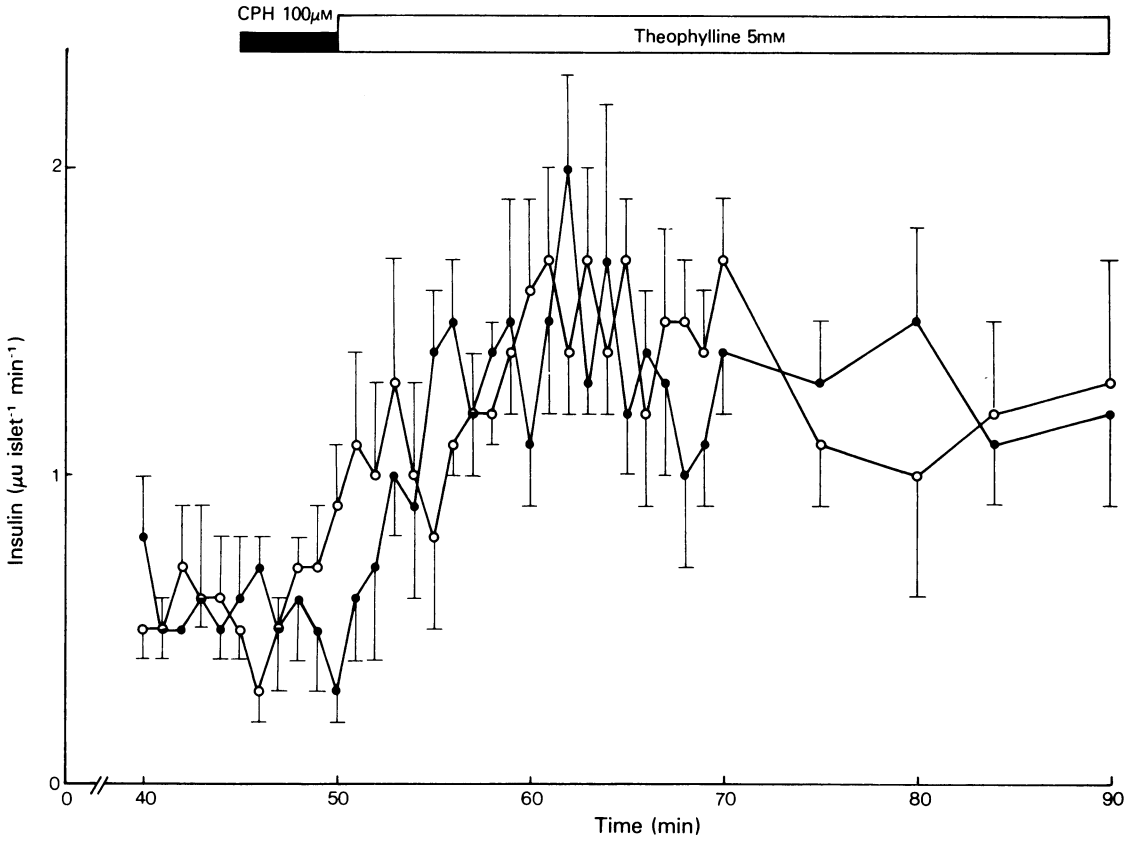

Figure 3 Effects of a 5 min pretreatment with cyproheptadine (CPH) 100 μM (solid bar) on theophylline-stimulated (open bar) insulin secretion from rat isolated pancreatic islets *in vitro*. Control (○); treated (●). Each point is the mean of five experiments and is shown with its standard error.

Speake, 1975; Charles, Lawecki, Pictet & Grodsky, 1975; Karl *et al.*, 1975; Lowe *et al.*, 1976), all elicited hormone secretion from CPH pretreated islets.

Direct evidence to support our hypothesis that CPH blocks depolarization-dependent Ca^{2+} entry was obtained from the isotope studies. The results confirm the findings of others (Dean & Matthews, 1970; Malaisse-Legae & Malaisse, 1971; Malaisse, 1973; Matthews & Sakamoto, 1975) that raising either the extracellular concentration of D-glucose or K_o^+ depolarizes β-cells and consequently causes uptake of Ca^{2+} from the extracellular space, and also show that this is almost totally abolished by CPH. The small residual increment in Ca^{2+} uptake that occurred in islets stimulated with either 50 mM or 120 mM K^+ and which was insensitive to the action of CPH very probably represents Ca^{2+} uptake resulting from the reduction in $[Na^+]_o$ rather than from the increased $[K^+]_o$ since we have observed that replacing 50 mM or 120 mM Na_o^+ by choline also increases Ca^{2+} uptake significantly. This interpretation is strongly supported by the observation that the Ca^{2+} uptake induced by the total replacement of Na_o^+ with choline was not significantly affected by CPH. Unlike elevating the concentration of D-glucose or K_o^+, Na_o^+ replacement does not alter the membrane potential of pancreatic β-cells (Dean & Matthews, 1970). Instead it reverses the normal inwardly directed Na^+ gradient, thus producing Na^+ efflux which is linked to Ca^{2+} uptake by the counter transport mechanism we have already recently described (Donatsch *et al.*, 1977). The lack of a marked inhibitory effect of CPH on this mechanism is therefore suggestive of a selective action of the drug on depolarization-dependent Ca^{2+} entry. In addition, the increase in P_{Na^+} caused by veratridine was only slightly reduced by CPH, this small effect probably being related to a slight degree of more general membrane stabilization that might be

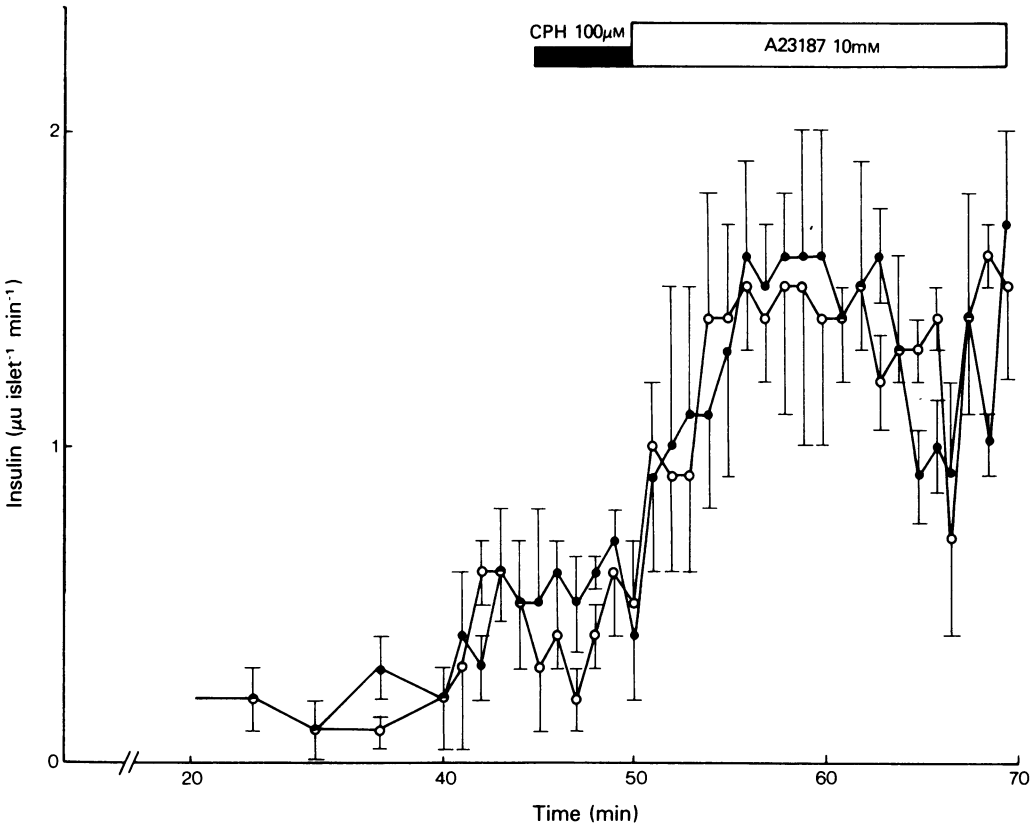

Figure 4 Effects of a 5 min pretreatment with cyproheptadine (CPH) 100 μM (solid bar) on insulin secretion from rat isolated pancreatic islets stimulated with the ionophore A23187 (open bar) *in vitro*. Control (○); treated (●). Each point is the mean of five experiments and is shown with its standard error.

expected for a tertiary amine such as CPH at higher concentrations (Seeman, 1972). On the other hand, tetrodotoxin totally inhibited the increase in P_{Na^+} that veratridine caused, confirming that the action of the alkaloid relates to a Na^+ channel effect.

Many of the peripheral actions of 5-HT and histamine are mediated by depolarization-dependent Ca^{2+} entry (Douglas, 1975). Our results may therefore explain why CPH antagonizes the actions of these two substances so effectively (Stone, Wenger, Ludden, Stavorski & Ross, 1961) and suggest a note of caution in interpreting earlier work with CPH because, although it is commonly claimed to be a 5-HT and histamine receptor antagonist, there is, in fact, no published data which permits the distinction between an inhibitory effect of the drug on histamine or 5-HT receptors and on channels which normally open subsequent to receptor activation.

One possibility to explain the action of CPH on channelled Ca^{2+} entry is that it increases the resting membrane potential such that the channel can no longer open. However, this seems unlikely since CPH would have to cause truly massive hyperpolarization for it to overcome the depolarization-evoked Ca^{2+} uptake caused by 120 mM K_o^+. In addition CPH did not markedly influence Ca^{2+}-Na^+ counter transport, a mechanism known to be strongly inhibited by hyperpolarization (Baker, 1976). In view of this we are currently investigating a further possibility to explain the action of CPH, namely that drug molecules bind to the Ca^{2+} channel, thereby hindering the passage of calcium ions through the channel complex.

We wish to thank Miss Erika Stöcklin and Miss Sharon Lowry for skilled technical assistance, and Mr W. Gehrig for preparation of the figures.

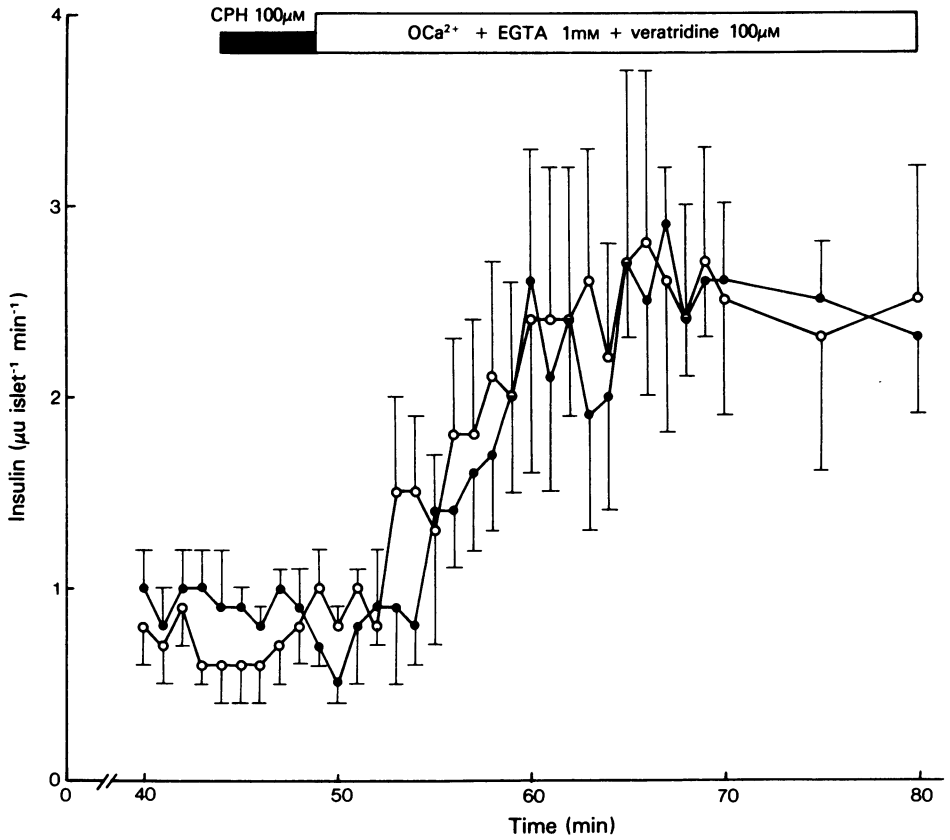

Figure 5 Effects of a 5 min pretreatment with cyproheptadine (CPH) 100 μM (solid bar) on insulin secretion from rat isolated pancreatic islets stimulated with veratridine in Ca^{2+}-free medium containing EGTA 1 mM (open bar) *in vitro*. Control (○); treated (●). Each point is the mean of five experiments and is shown with its standard error.

References

ASHBY, J.P. & SPEAKE, R.N. (1975). Insulin and glucogon secretion from isolated islets of Langerhans. The effects of calcium ionophores. *Biochem. J.*, **150**, 89–96.

BAKER, P.F. (1976). Regulation of intracellular Ca and Mg in squid axons. *Fedn Proc.*, **35**, 2589–2595.

BRISSON, G.R., MALAISSE-LEGAE, F. & MALAISSE, W.J. (1972). The stimulus–secretion coupling of glucose-induced insulin release—VIII. A proposed site of action for adenosine 3′:5′-cyclic monophosphate. *J. clin. Invest.*, **51**, 232–241.

CHARLES, M.A., LAWECKI, J., PICTET, R. & GRODSKY, G.M. (1975). Insulin secretion: interrelationships of glucose, cyclic adenosine 3′:5′-monophosphate and calcium. *J. biol. Chem.*, **250**, 6134–6140.

DEAN, P.M. & MATTHEWS, E.K. (1970). Electrical activity in pancreatic islet cells: effect of ions. *J. Physiol.*, **210**, 265–275.

DEAN, P.M., MATTHEWS, E.K. & SAKAMOTO, Y. (1975). Pancreatic islet cells: effects of monosaccharides, glycolytic intermediates and metabolic inhibitors on membrane potential and electrical activity. *J. Physiol.*, **246**, 459–478.

DONATSCH, P., LOWE, D., RICHARDSON, B.P. & TAYLOR, P. (1977). The functional significance of sodium channels in pancreatic islet cells: effect of ions. *J. Physiol.*, **210**, 265–275.

DOUGLAS, W.W. (1975). Autacoids. In *The Pharmacological Basis of Therapeutics*. 5th Edition ed. Goodman, L.S. & Gilman, A. pp. 589–629. New York, Toronto and London: Macmillan Publishing Co.

FELDMAN, J.M., CHAPMAN, B.A. & PLONK, J.M. (1974). Monoamine oxidase inhibition: possible mechanism for cyproheptadine induced alterations in insulin secretion. *Life Sci.*, **15**, 1121–1134.

JOOST, H.G., BECKMANN, J., HOLZE, S., LENZEN, S., POSER, W. & HASSELBLATT, A. (1976). Inhibition of insulin and glucagon release from the perfused rat pancreas by cyproheptadine. *Diabetologia*, **12**, 201–206.

JOOST, H.G., POSER, W. & PANTEN, U. (1974). Inhibition of insulin release from the rat pancreas by cyproheptadine

and tricyclic antidepressants. *Naunyn-Schmiedebergs Arch. Pharmac.*, **285**, 99–102.

KARL, R.C., ZAWALICH, W.S., FERRENDELLI, J.A. & MATSCHINSKY, F.M. (1975) The role of Ca^{2+} and cyclic adenosine 3′:5′-monophosphate in insulin release induced *in vitro* by the cation ionophore A23187. *J. biol. Chem.*, **250**, 4575–4579.

LACY, P.E. & KOSTIANOVSKY, M. (1967). Method for the isolation of intact islets of Langerhans from the rat pancreas. *Diabetes*, **16**, 35–39.

LACY, P.E., WALKER, M.M. & FINK, C.J. (1972). Perifusion of isolated rat islets *in vitro*. *Diabetes*, **21**, 987–998.

LOWE, D., RICHARDSON, B.P., TAYLOR, P. & DONATSCH, P. (1976). Increasing intracellular sodium triggers calcium release from bound pools. *Nature, Lond.*, **260**, 337–338.

MALAISSE-LEGAE, F. & MALAISSE, W. J. (1971). Stimulus–secretion coupling of glucose-induced insulin release III. Uptake of ^{45}calcium by isolated islets of Langerhans. *Endocrinology*, **88**, 72–80.

MALAISSE, W. J. (1973). Insulin secretion: multifactorial regulation for a single process of release. *Diabetologia*, **9**, 167–173.

MATTHEWS, E.K. (1977). Insulin Secretion. In *Hormones and Cell Regulation.* Inserm European Symposium, Vol. I. ed. Dumont, J. & Nunez, J. pp. 57–76. Amsterdam, New York and Oxford: North Holland Publishing Company.

MATTHEWS, E.K. & SAKAMOTO, Y. (1975). Electrical characteristics of pancreatic islet cells. *J. Physiol.*, **246**, 421–437.

RICHARDSON, B. P. (1976). The effects of cyproheptadine pretreatment on insulin release from isolated pancreatic islets. *Br. J. Pharmac.*, **56**, 356–357P.

RICHARDSON, B.P., MCDANIEL, M.L. & LACY, P.E. (1975). Effects of cyproheptadine on insulin secretion by isolated perfused rat islets. *Diabetes*, **24**, 836–841.

RICKERT, D.E. & FISCHER, L.J. (1975). Cyproheptadine and β-cell function in the rat: insulin secretion from pancreas segments *in vitro*. *Proc. Soc. exp. Biol. Med.*, **150**, 1–6.

SEEMAN, P. (1972). The membrane actions of anaesthetics and tranquilizers. *Pharmac. Rev.*, **24**, 583–655.

STONE, C.A., WENGER, H.C., LUDDEN, C.T., STAVORSKI, J.M. & ROSS, C.A. (1961). Antiserotonin–antihistaminic properties of cyproheptadine. *J. Pharmac. exp. Ther.*, **131**, 73–84.

WRIGHT, P.H., MAKULU, D.R., VICHICK, D. & SUSSMANN, K.E. (1971). Insulin immunoassay by back titration; some characteristics of the technic and the insulin precipitant action of alcohol. *Diabetes*, **20**, 33–45.

(Received January 18, 1979.
Revised January 4, 1980.)