Br. J. Pharmac. (1979), **67**, 265–267

INHIBITION OF INSULIN SECRETION FROM THE PERFUSED PANCREAS OF THE RAT BY PIZOTIFEN

H.G. JOOST

Institute of Pharmacology and Toxicology, University of Göttingen, Robert-Koch-Str. 40, D-3400 Göttingen, Federal Republic of Germany

1 In the perfused rat pancreas the effects of pizotifen on insulin release induced by 20 mM glucose were studied.

2 Pizotifen (10 and 100 μM) significantly reduced the insulin release during a 25 min perfusion period to 49% and 7% of the controls.

3 The same concentrations of the structurally related agents cyproheptadine, doxepin, and chlorpromazine produced a comparable inhibition.

Introduction

The tricyclic agent, pizotifen, is used in the prophylactic treatment of migraine attacks (Sicutery, Fanchi & Del Bianco, 1967), and has also been reported to have an antidepressant effect (Krumholz, Yaryara-Tobias & White, 1968; Dixon, Hill, Roemer & Scholtysik, 1977). A closely related agent, cyproheptadine, inhibited insulin release in the rat *in vivo* and *in vitro* (Joost, Poser & Panten, 1974; Rickert & Fischer, 1975; Richardson, McDaniel & Lacy, 1975). Similar effects were produced by two structurally related antidepressants, doxepin and amitriptyline (Joost *et al.*, 1974), and a neuroleptic agent, chlorpromazine (Ammon, Orci & Steinke, 1973). In the experiments to be described in this paper the effects of pizotifen on insulin release from the perfused pancreas of the rat were investigated and compared to the effects of the agents that are known to inhibit insulin release.

Methods

Male albino Wistar rats (200 to 250 g) which had been deprived of food overnight were used throughout. The animals were anaesthetized by an intraperitoneal injection of pentobarbitone (45 mg/kg body wt.). Rectum, colon, ileum, and jejunum were removed. As described previously (Grodsky, Batts, Bennett, Vcella, McWilliams & Smith, 1963) the pancreas, spleen, stomach, and proximal part of the duodenum were perfused through the cannulated abdominal aorta. The venous effluent from a cannula inserted into the portal vein was collected at 1 and 5 min intervals. The perfusion media consisted of a Krebs-Henseleit-bicarbonate buffer, containing (mM): sodium 143, potassium 5.9, calcium 2.5, magnesium 1.18, and phosphate 1.2. The bicarbonate concentration was 25 mM, yielding a pH of 7.4 when gassed with a mixture of 95% O_2 and 5% CO_2. The buffers contained no albumin. Glucose and the agents under investigation were added as indicated. The flow rate was 4 ml/min and provided a constant perfusion pressure of 60 to 80 mmHg. The medium was changed during the perfusion experiments by means of a valve. Immediately after collection, the samples were diluted with Tris-buffer (pH 7.4) containing albumin (0.1%). Insulin was determined radioimmunologically using antibodies obtained from guinea-pigs. The bound and free fractions were separated with cellulose according to Zaharko & Beck (1968). The results presented in Table 1 were obtained by adding the amounts of insulin released during the perfusion periods under investigation. The means ± s.e. mean of the perfusion experiments were calculated, and differences were tested for statistical significance with the U-test of Wilcoxon, Mann & Whitney.

Results

When agents were perfused 5 min before the high glucose stimulus (20 mM), neither a stimulatory nor an inhibitory effect on basal insulin secretion was observed (data not shown). Glucose (20 mM) produced a large release of insulin that was significantly inhibited by pizotifen as well as by cyproheptadine, doxepin, and chlorpromazine (Table 1). The inhibition by 10 and 100 μM pizotifen, cyproheptadine, and doxepin

0007-1188/79/100265-03$01.00

Table 1 Inhibition of glucose-induced (20 mM) insulin release from the perfused pancreas of the rat by pizotifen, cyproheptadine, doxepin, and chlorpromazine

	Insulin (ng/25 min)	*% of controls*
Control	615 ± 73	49
Pizotifen (10 μM)	304 ± 62*	
Control	591 ± 82	7
Pizotifen (100 μM)	42 ± 5*	
Control	863 ± 120	35
Cyproheptadine (10 μM)	252 ± 36*	
Control	531 ± 37	13
Cyproheptadine (100 μM)	67 ± 25*	
Control	372 ± 21	50
Doxepin (10 μM)	186 ± 30*	
Control	408 ± 76	9
Doxepin (100 μM)	35 ± 12*	
Control	488 ± 41	72
Chlorpromazine (10 μM)	354 ± 67	
Control	763 ± 85	33
Chlorpromazine (100 μM)	248 ± 34*	

Means ± s.e. mean of 6 experiments. (* $P < 0.001$).

was comparable but was less in the case of chlorpromazine.

Discussion

The results show that pizotifen inhibits insulin release in the rat *in vitro*. The concentrations of pizotifen needed to produce the inhibition are similar to those of cyproheptadine, doxepin, and chlorpromazine. Although the chemical structures of the agents under investigation exhibit certain similarities, the tricyclic part of the molecules varies: pizotifen is a thiophene derivative, chlorpromazine is a phenothiazine, and doxepin bears an oxygen atom in the middle ring. In contrast, the side chains of the agents are related: an ionogenic tertiary nitrogen at the same distance (three carbon atoms) from the tricyclic system. Thus the similarity of the side chains rather than of the tricyclic systems correlates the structure to the observed effects.

The similar chemical structure of the agents points to a common mode of action on the pancreatic β-cell. Usually the anti-5-hydroxytryptamine activity of cyproheptadine and pizotifen is emphasized when their mode of action, particularly in preventing migraine attacks, is discussed (Lance, Anthony & Somerville, 1970). However, the drugs also share antihistaminic and anticholinergic activity (Speight & Avery, 1972; Stone, Wenger, Ludden, Stavorsky & Ross, 1961). Thus their specificity as antitransmitter substances is relatively low. Accordingly it cannot be concluded that the effect of a certain transmitter substance on the pancreatic β-cell is blocked by the agents.

It has been proposed that biogenic amines modulate insulin secretion (Feldman & Lebovitz, 1971). However, if they control the insulin secretion at all, they control it in an inhibitory rather than in a stimulatory way (Feldman & Lebovitz, 1971; Lernmark, 1971; Ericson, Hakanson & Lundquist, 1977). Blockade of the receptors of biogenic amines should thus be expected to stimulate the release of insulin.

Cyproheptadine inhibited the calcium accumulation of isolated islets of the mouse (Joost, Beckmann, Lenzen & Hasselblatt, 1976), and altered the glucose-induced calcium distribution pattern as was shown ultracytochemically (Bommer, Joost & Klöppel, 1978). The inhibition of insulin release by cyproheptadine in the perfused pancreas of the rat was counteracted in part by theophylline or calcium (Joost, Beckmann, Holze, Lenzen, Poser & Hasselblatt, 1976). Further, chlorpromazine has been reported to displace membrane calcium (Kwant & Seeman, 1969). An interference with the calcium action on the β-cell might therefore be the common mode of action of the agents investigated in the present study.

The significance of the inhibitory effects for therapy, either as side effects or in the therapy of insulinoma patients, is unclear. No disturbance of carbohydrate metabolism due to pizotifen (Fraipont-Guyot, Luyckx & Lefebvre, 1970; Cerdan, Acosta & Jolin, 1975), doxepin, or cyproheptadine (Drash, Elliott, Langs, Lavenstein & Cooke, 1966) was observed. Only chlorpromazine produces a mild but significant impairment of carbohydrate tolerance in man (Erle, Basso, Federspil, Sicolo & Scandellari, 1977).

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(Received January 8, 1979. Revised February 26, 1979.)