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EFFECTS OF DRUGS ON GLUCOSE AND TOLBUTAMIDE-STIMULATED INSULIN RELEASE FROM ISOLATED RAT ISLETS OF LANGERHANS

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Abstract—The effects of acetylsalicylic acid, sulphadimidine, phenylbutazone and chlorpromazine on glucose- and tolbutamide-stimulated insulin release by isolated rat islets of Langerhans have been investigated. Acetylsalicylic acid did not influence glucose-stimulated insulin secretion; although at 2.5 mM it potentiated the effect of tolbutamide. Sulphadimidine potentiated the effects of glucose and tolbutamide on insulin secretion, whereas phenylbutazone was found to augment glucose-stimulated insulin release and to antagonize the tolbutamide effect. The effects of glucose and tolbutamide on insulin secretion were inhibited by low concentrations of chlorpromazine (0.005 mM–0.01 mM) and were stimulated by high concentrations (0.5 mM–1 mM). These results are discussed in relation to the possibilities that certain drugs may affect tolbutamide-stimulated insulin release either by altering the binding of tolbutamide to the B-cell or to other proteins, or by affecting the cyclic AMP system of the B-cell.

A number of drugs, such as salicylates, sulphonamides and phenothiazines, have been shown to induce hypoglycaemia and/or hyperglycaemia in experimental animals and human subjects. Salicylates have in general been reported to cause hyperglycaemia [1–4] although hypoglycaemia has been observed [5, 6]. Hypoglycaemia has also been encountered with the use of sulphonamides [7]. Studies on the effects of chlorpromazine, a phenothiazine derivative, on carbohydrate metabolism have yielded conflicting results. Some reports have shown hyperglycaemia and decreased glucose tolerance [8–11], while other studies have shown hypoglycaemia [12].

Studies have also indicated that these drugs may potentiate or inhibit the hypoglycaemic action of sulphonylureas. Thus, salicylates [13–15], sulphonamides [16–18] and phenylbutazone [19–21] have been reported to potentiate the hypoglycaemic effect of sulphonylureas, whereas chlopromazine has been found either to inhibit [10, 22] or to potentiate [12] the hypoglycaemia.

Since it was possible that part of the effect of these drugs on plasma glucose concentrations might be related to changes in rates of insulin secretion, we have attempted to assess the effect of aspirin, sulphadimidine, phenylbutazone or chlorpromazine on insulin release stimulated by glucose and tolbutamide from islets of Langerhans. We have used islets of Langerhans isolated from rat pancreas since they provide an *in vitro* system for the study of insulin release that is highly sensitive to the effect of glucose and sulphonylureas [23, 24].

MATERIALS AND METHODS

Reagents. Collagenase was obtained from Sigma Chemical Company, Kingston, Surrey; bovine albumin from Armour Pharmaceutical Company Ltd., Eastbourne, Sussex; Tolbutamide from Hoechst Pharmaceuticals; acetylsalicyclic acid (Aspirin) from Evans Company, Liverpool; sulphadimidine (Sulphamezathine) from Imperial Chemical Industries Ltd., Pharmaceutical Division, Macclesfield, Cheshire; phenylbutazone (Butazolidin) from Geigy Pharmaceuticals, Macclesfield, Cheshire, and chlorpromazine (Largactil) from May and Baker, Dagenham, Essex.

Incubation medium. In all experiments using isolated islets of Langerhans, the incubation medium was that described by Gey and Gey [25]. In experiments in which insulin secretion from isolated islets was being studied, this medium was supplemented with bovine serum albumin Fraction V (1 mg/ml). Glucose and drugs were added to this medium as required.

Isolation of islets of Langerhans. Islets of Langerhans were prepared by the collagenase digestion of pancreatic tissue taken from female Sprague–Dawley rats, as described in detail by Howell and Taylor [26] and Montague and Taylor [27].

Insulin secretion from isolated rat islets. Islets were pre-incubated for 30 min at 37° in buffer containing 2 mM glucose. The incubation medium for insulin secretion studies contained either 8 mM glucose alone or 8 mM glucose plus the drug under study, and the gas phase was $O_2 + CO_2$ (95:5). Each drug was added to the medium alone or in combination with tolbutamide. In all the experiments the effects of glucose and tolbutamide were compared in the presence and absence of the drug under study. The islets were incubated in 0.6 ml of incubation buffer in groups of three at 37° for 60 min with gentle shaking. At the end of this period, a sample of the medium was removed and diluted for the determination of insulin content.

Insulin assay. Insulin was assayed in duplicate, using a double antibody technique based on that of Hales and Randle [28] with the reagents supplied by the Radiochemical Centre, Amersham, Bucks. These

reagents, which are basically used for the immunoassay of human insulin, react with equal sensitivity to rat insulin. These reagents can be used therefore to determine rat insulin [27].

RESULTS AND DISCUSSION

Studies on the effect of salicylates on blood sugar have indicated that their hyperglycaemic effect might be related to epinephrine-stimulated glycogenolysis [3]. Salicylate-induced hypoglycaemia, on the other hand, has been attributed to increased uptake and utilization of glucose by muscles and a decrease in the synthesis of glucose from non-carbohydrate precursors [29]. Since insulin might participate in the alterations of blood sugar caused by salicylates, we have investigated the effect of aspirin on insulin secretion by islets of Langerhans. Aspirin did not affect the release of insulin in response to glucose from isolated islets (Table 1). This is in agreement with the findings of a number of authors that the effect of salicylates on blood sugar is not mediated by alterations in the rate of insulin release [30–32]. At a small concentration (0.025 mM) aspirin inhibits tolbutamide-stimulated insulin secretion. However, there was a remarkable potentiation of the effect of tolbutamide by aspirin at the high concentration of 2.5 mM (Table 1). This potentiation might result from a displacement of tolbutamide from the albumin present in the incubation medium used in the present study [33], since the addition of salicylate to tolbutamide-containing serum has been shown to increase the proportion of the unbound sulphonylurea [34].

Sulphadimidine was found to potentiate the effects of glucose and tolbutamide on insulin release by islets (Table 2). An increased rate of insulin release might account for the potentiation by sulphonamides of the hypoglycaemic effect of sulphonylureas [8, 35]. Some authors have attributed the potentiating effect of sulphonamides to their capacity to increase the serum tolbutamide level by displacing it from serum protein [17, 35, 36]. Hellman [33] has shown that sulphonamides directly increase the binding of sulphonylureas to islets isolated from mice, an effect which might be related to its displacement from albumin present in the incubation medium.

Phenylbutazone was found to increase the effect of glucose on insulin secretion by islets, although it appeared to inhibit the effect of tolbutamide on insulin secretion (Table 3). The potentiation of sulphonyl-

Table 1. Effect of aspirin alone or in combination with tolbutamide on glucose-stimulated insulin release by islets of Langerhans *in vitro*. Isolated islets were incubated for 1 hr at 37° in Gey and Gey buffer containing albumin (1 mg/ml)

Treatment	Mean insulin secretion (ng/islet/hr)
Glucose (8 mM) control	5.82 ± 0.43 (20)*
Glucose (8 mM) + tolbutamide (1 mM)	19.06 ± 1.56† (12)
Glucose (8 mM) + aspirin (0.025 mM)	5.82 ± 0.43 (7)
Glucose (8 mM) + aspirin (0.5 mM)	4.70 ± 0.56 (4)
Glucose (8 mM) + aspirin (5 mM)	4.80 ± 0.67 (9)
Glucose (8 mM) + aspirin (0.025 mM) + tolbutamide (0.5 mM)	8.2 ± 1.9 (9)
Glucose (8 mM) + aspirin (2.5 mM) + tolbutamide (0.5 mM)	26.23 ± 5.05† (8)

* Values are means ± S.E.M.
† Significant difference from control at P = 0.001.
Figures in parenthesis are number of observations.

Table 2. Effect of sulphadimidine alone or in combination with tolbutamide on glucose-stimulated insulin release by islets of Langerhans *in vitro*. Isolated rat islets were incubated for 1 hr at 37° in Gey and Gey buffer containing albumin (1 mg/ml)

Treatment	Mean insulin secretion (ng/islet/hr)
Glucose (8 mM) control	5.5 ± 0.22 (12)*
Glucose (8 mM) + tolbutamide (1 mM)	18.60 ± 1.42† (12)
Glucose (8 mM) + sulphadimidine (0.025 mM)	13.83 ± 3.68‡ (5)
Glucose (8 mM) + sulphadimidine (0.25 mM)	7.80 ± 0.95‡ (8)
Glucose (8 mM) + sulphadimidine (2.5 mM)	9.68 ± 2.26 (5)
Glucose (8 mM) + sulphadimidine (0.125 mM) + tolbutamide (0.5 mM)	26.41 ± 3.6† (8)

* Values are means ± S.E.M.
† Significant differences from control at P = 0.001.
‡ Significant difference from control at P = 0.05.
Figures in parenthesis are number of observations.

Table 3. Effect of phenylbutazone alone or in combination with tolbutamide on glucose-stimulated insulin release by islets of Langerhans *in vitro*. Isolated rat islets were incubated for 1 hr at 37° in Gey and Gey buffer containing albumin (1 mg/ml)

Treatment	Mean insulin secretion (ng/islet/hr)
Glucose (8 mM) Control	5.4 ± 0.46 (9)*
Glucose (8 mM) + tolbutamide (1 mM)	18.15 ± 1.61† (12)
Glucose (8 mM) + phenylbutazone (0.025 mM)	8.33 ± 0.73‡ (5)
Glucose (8 mM) + phenylbutazone (0.25 mM)	8.00 ± 0.89§ (8)
Glucose (8 mM) + phenylbutazone (2.5 mM)	10.49 ± 1.21§ (5)
Glucose (8 mM) + phenylbutazone (0.125 mM) + tolbutamide (0.5 mM)	7.40 ± 0.71§ (10)

* Values are means ± S.E.M.
† Significant difference from control at P = 0.001.
‡ Significant difference from control at P = 0.01.
§ Significant difference from control at P = 0.05.
Figures in parenthesis are number of observations.

Table 4. Effect of chlorpromazine alone or in combination with tolbutamide on glucose-stimulated insulin release by islets of Langerhans *in vitro*. Isolated rat islets were incubated for 1 hr at 37° in Gey and Gey buffer containing albumin (1 mg/ml)

Treatment	Mean insulin secretion (ng/islet/hr)*
Glucose (8 mM) control	4.66 ± 0.35 (21)*
Glucose (8 mM) + tolbutamide (1 mM)	17.90 ± 0.85† (12)
Glucose (8 mM) + chlorpromazine (0.01 mM)	2.81 ± 0.39† (8)
Glucose (8 mM) + chlorpromazine (0.1 mM)	15.35 ± 2.45† (10)
Glucose (8 mM) + chlorpromazine (1 mM)	20.60 ± 0.93† (10)
Glucose (8 mM) + chlorpromazine (0.005 mM) + tolbutamide (0.5 mM)	2.37 ± 0.12† (7)
Glucose (8 mM) + chlorpromazine (0.05 mM) + tolbutamide (0.5 mM)	4.90 ± 0.91 (8)
Glucose (8 mM) + chlorpromazine (0.5 mM) + tolbutamide (0.5 mM)	38.6 ± 1.4† (10)

* Values are means ± S.E.M.
† Significant difference from control at P = 0.001.
Figures in parenthesis are number of observations.

urea-induced hypoglycaemia by phenylbutazone as previously reported [37, 38], does not therefore appear to be a result of drug effect on insulin secretion.

Chlorpromazine at a concentration of 0.01 mM was found to inhibit glucose-stimulated insulin secretion (Table 4). This result is in agreement with the observation of Ammon and Steinke [42] that chlorpromazine inhibits insulin secretion by isolated rat islets and with the suggestion of Sustin *et al.* [10] that chlorpromazine reduces the ability of the pancreatic B-cells to secrete insulin in response to glucose. Such an effect of chlorpromazine could contribute to the incidence of hyperglycaemia observed in human subjects [39–41] treated with the drug. Chlorpromazine at 0.005 mM was found to abolish completely tolbutamide-stimulated insulin release (Table 4), results which agree with the observations that chlorpromazine antagonizes tolbutamide-induced hypoglycaemia and decrease tolbutamide-induced elevation in serum insulin [10, 22]. Conversely, at higher concentrations, i.e. 0.1 mM and 1 mM, chlorpromazine produced very marked increases in insulin secretion (Table 4). A stimulatory effect of high concentrations of chlorpromazine has also been reported by Ammon and Steinke [42]. When chlorpromazine at a concentration of 0.5 mM was given in combination with tolbutamide (0.5 mM) potentiation was observed (Table 4) suggesting that the two drugs act by different mechanisms on the islet cells. Chloropromazine has been shown to increase fluoride-stimulated adenyl cyclase activity in a number of tissues [44] and it might therefore be expected to stimulate islet cell adenyl cyclase and elevate c-AMP level, thereby potentiating the secretory response to tolbutamide.

The results of this study indicate that the effects of a wide variety of drugs on blood glucose levels may result in part from changes in the rate of insulin release from the pancreas. Studies are currently in progress to define in detail the mode of action on insulin release of the drugs used in this study.

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