Tissue Distribution and Metabolism of Drugs VI: Effect of Second Drugs on Pancreatic Distribution and Insulin-Releasing Activity of Sulfonylureas in Perfused Rat Pancreas

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Abstract □ The distribution of sulfonylureas and their insulin-releasing potency as a pharmacological response were studied in isolated perfused rat pancreas. Sulfonylurea concentrations in the perfused pancreas in the presence or absence of a second drug were determined after perfusion for 15 min. Sulfonylureas could be distributed throughout the pancreas readily, and the tissue sulfonylurea concentration was reduced by the addition of sulfaphenazole, sulfadimethoxine, and salicylic acid. The insulin secretion rate stimulated by tolbutamide also was reduced by these three drugs; sulfanilamide, which could not displace the tolbutamide distribution, did not affect the tolbutamide-mediated secretion of insulin. These results document the importance of drug concentration in the tissue or receptor site with regard to insulin secretion and show that the sulfonylurea-mediated secretion of insulin can be modified easily by concomitant perfusion of a second drug that displaces sulfonylurea in the pancreas. These findings suggest that the drug interaction at the target organ or receptor site should be understood to provide adequate drug therapy.

Keyphrases □ Pancreas—distribution of sulfonylureas, displacement of sulfonylurea by second drug □ Distribution—sulfonylureas in the perfused pancreas, correlation with pharmacological response □ Sulfonylureas—distribution in pancreas, displacement by second drug □ Insulin secretion—stimulation by sulfonylurea, inhibition by presence of second drug

Hypoglycemic sulfonylureas are generally accepted to bind strongly to serum protein (1, 2). In 5% albumin solution, only 10% or less of tolbutamide was present in active unbound molecules at clinical levels (1, 2).

Even minute alteration of the protein binding capability changes the amount of the active unbound form of sulfonylureas. Clinically, ineffective therapy or toxic responses could result.

Displacement of such sulfonylurea–protein binding by other drugs has been demonstrated in plasma and in albumin solutions (3, 4). These phenomena may also occur in various organs or tissues (5, 6), but little is known about drug binding in tissues. The displacement at the receptor or binding site in the target organ may directly reflect therapeutic efficacy.

It has been generally accepted that the distribution dynamics of a drug are closely related to its efficacy or adverse reaction (6). However, technical difficulties in performing such investigations have limited available information. It is difficult to determine accurately the drug concentration in organs as compared to the blood, and it is almost impossible to measure the amount at isolated receptors. Moreover, it is particularly hard to obtain a simple and precise pharmacological marker.

In a previous paper (7), drug disposition dynamics in the exocrine pancreas were reported. Drugs are transported to the pancreas through the lipid barrier and excreted into the pancreatic juice through the rather tight lipoid and molecular sieve barriers.

In the present paper, the pancreatic distribution of sulfonylureas is correlated with insulin secretagogic capacity as the pharmacological marker using isolated perfused rat pancreas.

EXPERIMENTAL

Materials—Dextran (mol. wt. 60,000–90,000) was purchased in powder[1] or liquid[2] form (dextran 70 JP IX). Tolbutamide[3] and carbutamide[4] were the sulfonylureas studied. All other chemicals were obtained from commercially available sources and were analytical reagent grade.

Animals—Male Wistar rats, 200–300 g, were housed in a constant-temperature room with free access to water and food.

Procedure—The technique for pancreas isolation was practically the same as the reported pancreas–duodenum isolation method (8). The insertion of the cannulas was modified and directed upward just below the superior mesenteric artery. Furthermore, to simplify the procedure, the left renal, internal spermatic, and iliolumbar vessels were not occluded. The pancreas–duodenum was isolated from the rat and perfused on a constant-temperature table (37.4°).

The perfusion medium was Krebs–Henseleit bicarbonate solution supplemented with glucose (100 mg %). Colloidal osmotic pressure was maintained by the addition of dextran (4.5%) in accordance with the method of Toyota *et al.* (9). The perfusate was supplied by a peristaltic pump at the rate of 2 ml/min (net perfusion pressure was 30–60 mm Hg) and oxygenated with 95% O_2–5% CO_2 through a fiber-type oxygenator[5]. The perfusion time was 15 min for study of the pancreatic uptake of sulfonylureas. The perfusion experiment was carried out for 45 min to permit comparative study of insulin secretion stimulated by sulfonylureas with or without second drugs. The first 10 min was used as a stabilizing control period, and the next three 10-min periods and the last 5 min served as the experimental period to determine the effect of sulfonylurea and second drugs.

Four kinds of experiments were conducted in this comparative study. The first was a control experiment using basal Krebs–Henseleit bicarbonate solution, the second determined the direct effect of second drugs, the third was the tolbutamide control study, and the last was a combined experiment using tolbutamide and a second drug. Every 2.5 min, samples of effluent from the cannula in the portal vein were collected for insulin assay.

Analytical Methods—Tolbutamide and carbutamide were determined by the methods of Martin and Rowland (10) and of Bratton and Marshall (11), respectively, after Somozyi's deproteinization. Insulin secreted in each experimental period was assayed as immunoreactive insulin (IRI) by a single-antibody precipitation method using polyethylene glycol (12). Rat insulin was used as the reference standard. Aprotinin[6] was added to each borosilicated culture tube[7] (1.2 × 75 mm) in accordance with the method of Toyota *et al.* (13).

[1] Wako Chemicals Co., Osaka, Japan.
[2] Green Cross Co., Osaka, Japan.
[3] Provided by Dr. R. Konishi.
[4] Provided by Dr. H. Matsui.
[5] Bio-Fiber 5 minitube, Bio-Rad Laboratories, or microporous hollow fiber, Mitsubishi Rayon Co. & Hiromaito Manufacturing Co., Hiroshima, Japan.
[6] Trasylol (10,000 KIU/ml), Bayer Co. Ltd.
[7] Corning Co., Corning, N.Y.

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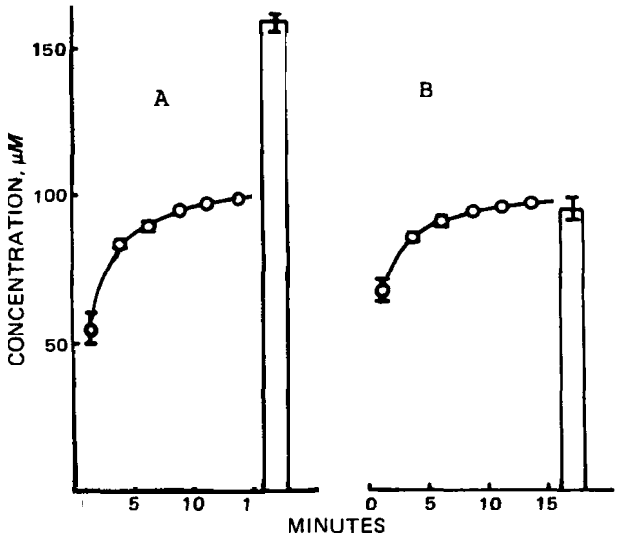

Figure 1—*Concentration profile of sulfonylureas in the portal effluent of the perfusate. Key: A, tolbutamide (100 μM) perfused; and B, carbutamide (100 μM) perfused. Open bars denote drug concentration in the pancreas.*

The effect of the second drug on the insulin secretion stimulated by tolbutamide was judged by the total amount of insulin secreted during the perfusion period of 15–45 min, because the insulin secretion stimulated by tolbutamide alone was almost stable during this period. The insulin secretion from 15 to 20 min and from 30 to 40 min was expressed as secretion in a combined experiment (microunits per minute), and the insulin secretion from 20 to 30 min and from 40 to 45 min was adopted as the control experiment in which only tolbutamide was perfused. The two values obtained from the two periods were compared. Statistical analysis was performed using the Student t test of paired samples.

RESULTS AND DISCUSSION

The concentration profile of tolbutamide and carbutamide in the effluent of the perfusate from the cannula in the portal vein when both drug solutions of 100 μM were perfused in the pancreas is shown in Fig. 1. After perfusion for 15 min, the sulfonylurea concentration in the whole pancreas was determined since equilibrium between the perfusate and the pancreas was nearly achieved within 15 min. The control data of pancreatic uptake of tolbutamide and carbutamide were 158 and 95 μM, respectively. These values were compared with the data based on concomitant use of second drugs.

Although serum albumin is used in almost every laboratory to maintain the colloidal osmotic pressure in the perfused pancreas, dextran was selected to differentiate between effects caused by competition for plasma protein binding sites as opposed to β-cell binding sites. The fact that dextran had no binding capacity with tolbutamide was confirmed by an ultrafiltration technique (14). Next, the effects of sulfaphenazole, sulfadimethoxine, sulfanilamide, and salicylic acid were examined to determine how they affect sulfonylurea distribution in the pancreas. Sulfaphenazole, sulfadimethoxine, and salicylic acid inhibited the pancreatic uptake of sulfonylureas by 30–40% (Table I), but sulfanilamide had little or no effect on the pancreatic uptake of tolbutamide.

These results correlated well with the displacement phenomenon of these drugs in the plasma or in an albumin solution (3). Christensen *et al.* (15) reported on the severe hypoglycemia provoked by the concomitant administration of tolbutamide and sulfaphenazole. Other investigators (16, 17) also reported that a similar phenomenon occurred with phenylbutazone or dicumarol. Hypoglycemia was attributable to a

Table I—Effect of the Second Drug on the Pancreatic Uptake of Sulfonylureas

Sulfonylurea	Second Drug	Pancreatic Uptake[a], μM	Uptake Ratio, %
Tolbutamide (100 μM)	None	158 ± 3[b] (6)[c]	100
	Sulfanilamide (2.5 mM)	150 ± 7 (3)	95
	Sulfaphenazole (2.5 mM)	109 ± 7 (5)	69[d]
	Salicylic acid (2.5 mM)	105 ± 6 (5)	67[d]
	Sulfadimethoxine (1 mM)	92 ± 9 (5)	58[d]
Carbutamide (100 μM)	None	95 ± 4 (8)	100
	Salicylic acid (2.5 mM)	70 ± 2 (9)	73[d]

[a] These values are of 15-min perfusion. [b] Values are mean ± *SEM*. [c] Values in parentheses are the number of experiments. [d] Significant differences were detected at $p < 0.001$.

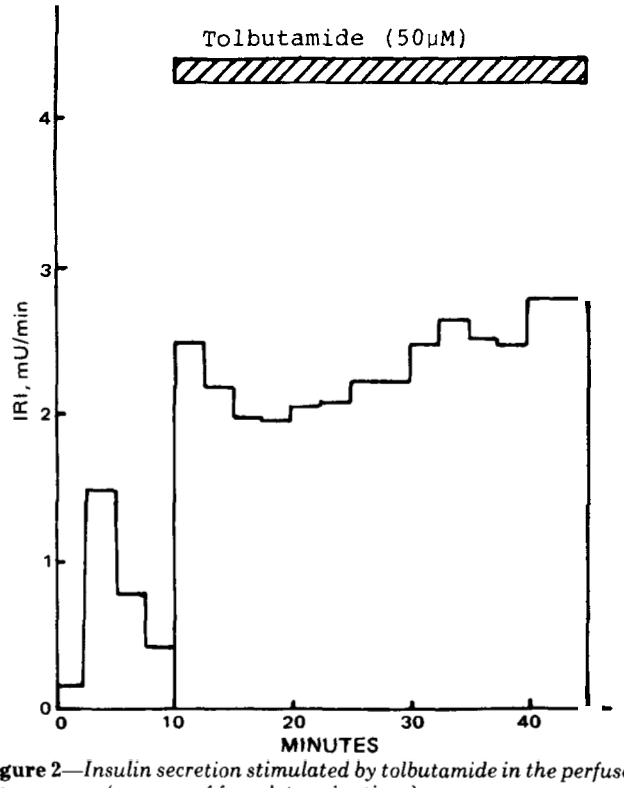

Figure 2—*Insulin secretion stimulated by tolbutamide in the perfused rat pancreas (average of four determinations).*

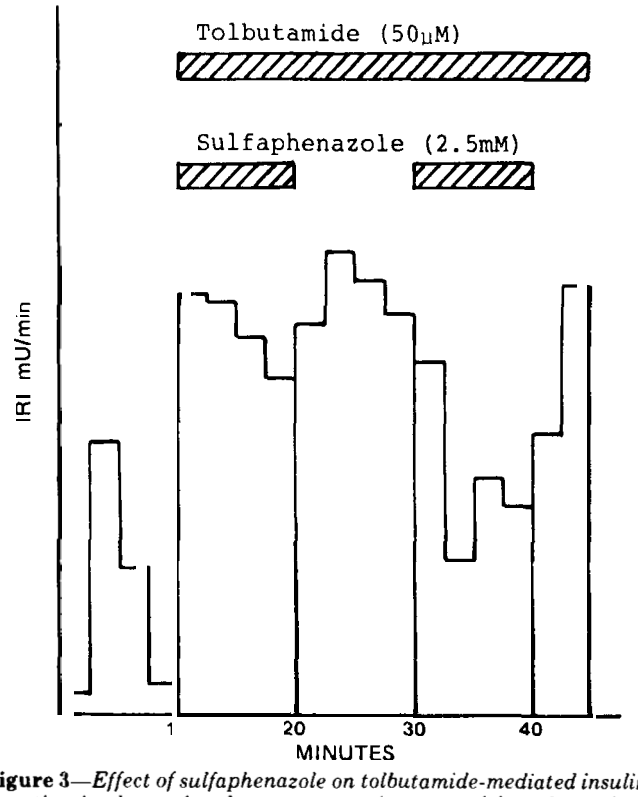

Figure 3—*Effect of sulfaphenazole on tolbutamide-mediated insulin secretion in the perfused rat pancreas (average of four determinations).*

Table II—Effect of the Second Drug on the Insulin Secretion in the Absence of Tolbutamide

Second Drug	Secreted Insulin, mU/min: Basal Medium[a]	Basal Medium plus Second Drug[b]	Statistics[c]
None	0.31	0.37	NS (3)[d]
Sulfanilamide (2.5 m*M*)	0.21	0.22	NS (3)
Sulfaphenazole (2.5 m*M*)	0.40	0.55	NS (4)
Salicylic acid (2.5 m*M*)	0.51	0.42	NS (5)
Sulfadimethoxine (1 m*M*)	0.23	0.36	NS (4)

[a] Insulin secretion during the 20–30- and 40–45-min periods. [b] Insulin secretion during the 15–20- and 30–40-min periods. [c] Paired *t* test. [d] Values in parentheses are the number of experiments.

Table III—Effect of the Second Drug on the Insulin Secretion Stimulated by Tolbutamide

Second Drug	Secreted Insulin, mU/min[a]: Tolbutamide Only[b]	Tolbutamide plus Second Drug[c]	Statistics[d]
None	2.35	2.34	NS (4)[e]
Sulfanilamide (2.5 m*M*)	1.81	1.99	NS (3)
Sulfaphenazole (2.5 m*M*)	2.68	1.89	$p < 0.01$ (4)
Salicylic acid (2.5 m*M*)	2.21	1.99	$p < 0.05$ (4)
Sulfadimethoxine (1 m*M*)	1.93	1.71	$p < 0.05$ (4)

[a] Concentration of tolbutamide was 50 μM. [b] Insulin secretion during the 20–30- and 40–50-min periods. [c] Insulin secretion during the 15–20- and 30–40-min periods. [d] Paired *t* test. [e] Values in parentheses are the number of experiments.

combination of causes, *i.e.*, inhibition of hepatic metabolism, increased protein-unbound sulfonylurea, and its displacement from tissue-binding sites in the liver (18). The displacement phenomenon in the blood is one main cause of hypoglycemia, but it is not totally responsible. The results of the present study showed that the pancreatic uptake of sulfonylureas decreased in accordance with their displacement phenomenon in plasma by the addition of second drugs with high hydrophobicity.

In considering the action site of sulfonylureas in the pancreas, the possibility that drug binding to the β-cell membrane may be related to secretagogic action (19–23) has received much attention. Drug displacement may occur at the receptor site as demonstrated in plasma or in the whole pancreas, and a converse pharmacological effect of what happened as a result of displacement in plasma may be seen. The possibility of displacement of tolbutamide at the receptor or binding site also was investigated in this study using the secreted insulin as a marker of the pharmacological effect. The basal pattern and tolbutamide-stimulated pattern are illustrated in Fig. 2.

Since it has been considered that sulfonylurea binding to the β-cell membranes is closely related to the secretagogic potency, it was expected that the drug interaction at the receptor level might be detected effectively by this experimental system. Sulfaphenazole, sulfadimethoxine, and salicylic acid, which inhibited the pancreatic uptake of tolbutamide, were investigated to determine their effect on the insulin secretion stimulated by tolbutamide. As shown in Fig. 3, the insulin secretory rate during the concomitant perfusion of tolbutamide and sulfaphenazole was significantly lower than that obtained during tolbutamide perfusion alone. When only sulfaphenazole was perfused, no significant effect was detected in the insulin secretory rate (Table II).

The effect of sulfadimethoxine and salicylic acid on insulin secretion stimulated by tolbutamide also was examined; smaller, but significant, inhibitory effects were observed. Insulin secretion rates when tolbutamide alone was perfused and when tolbutamide and a second drug were perfused are listed in Table III. These results are well explained by the hypothesis that expects competition at the binding or receptor site between tolbutamide and the second drug on the β-cell membrane.

Hellman *et al.* (22) reported on a similar phenomenon using 4-acetamide-4′-isothiocyanate-stilben-2,2′-disulfonic acid in incubated rat Langerhans islet. It depressed the islet uptake of glyburide (glibenclamide) and, when present solely with the islets, it activated the insulin secretion strongly. When it coexisted with glyburide, the two secretagogues acted less actively than the additive effect of glyburide and 4-acetamide-4′-isothiocyanate-stilben-2,2′-disulfonic acid. Hellman *et al.* (22) pointed out the possibility that 4-acetamide-4′-isothiocyanate-stilben-2,2′-disulfonic acid, a membrane probe, prevented the secretagogic recognition of glyburide by displacing this sulfonylurea from the β-cell membranes. Although their hypothesis may explain the present data, tolbutamide possibly could react directly with sulfaphenazole, sulfadimethoxine, or salicylic acid and form a less active complex compound. To exclude this possibility, the complex formation of these drugs was examined by the solubility method (24). The complex formation was no more than merely 1% of tolbutamide in the perfusate (stability constant = 5.3 M^{-1}). Thus, it could not explain the remarkably decreased insulin secretion.

On the other hand, the finding that tolbutamide-mediated secretion was inhibited by the addition of these three drugs could have been caused by their direct action if they had the capacity to inhibit insulin secretion. A perfusion study indicated that these drugs made a small, but insignificant, change in the insulin secretion (Table II). Consequently, direct inhibition of insulin secretion by these drugs apparently was not the reason for this phenomenon.

When sulfanilamide was perfused concomitantly with tolbutamide, the insulin secretion rate was slightly, but not significantly, stimulated (Table III). Since sulfanilamide was hardly able to displace the pancreatic uptake of tolbutamide, this result is consistent with the hypothesis that drug concentration at the tissue level might reflect the pharmacological response.

The importance of the drug protein binding in plasma has been stressed. It has been postulated widely that the unbound free fraction of a drug in plasma could serve as the marker of therapeutic efficacy. However, a correlation between the serum free fraction value and the tissue free fraction value is unlikely in the presence of some specific accumulation of drug in the tissue. From this point of view, drug–tissue binding or interaction in connection with pharmacological or toxicological response has received attention recently. The presented data show the important role of drug disposition dynamics in tissues, especially in target organs. Thus, drug disposition in target organs or receptor sites should always be considered when preparing a dosing regimen for optimal therapy.

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