PHARMACOLOGY AND MODE OF ACTION OF THE HYPOGLYCAEMIC SULPHONYLUREAS AND DIGUANIDES[1,2]

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The sulphonylureas and diguanides are the two types of compounds presently used as orally effective hypoglycaemic agents in the treatment of diabetes mellitus in man.

THE SULPHONYLUREAS

The hypoglycaemic effect of some sulphonamide-like compounds was first reported by Ruiz, Silva & Libenson (1) in 1930. This property was not further studied until Janbon et al. (2), in 1942, observed the occurrence of hypoglycaemia in some typhoid patients who had been treated with an antibacterial sulphathiazole, IPTD (*p*-amino-benzene sulphonyl-isopropylthiodiazole). In the next few years, Loubatières (3) and his associates systematically studied the hypoglycaemic action of such compounds in animals and suggested their possible therapeutic use in diabetes. Nevertheless, it was not until 1955 that Franke & Fuchs (4) reported the successful treatment of some insulin-independent diabetics with an antibacterial sulphonamide, carbutamide [N-(*p*-amino-benzenesulphonyl) = N-(*n*-butyl)urea]. Since then, numerous other hypoglycaemic sulphonylureas have been discovered and are now used for the treatment of 30 to 50 percent of the diabetic population in all countries. Those most commonly employed are carbutamide (Nadisan); tolbutamide [(N-(*p*-toluenesulphonyl) = N-(*n*-butyl)urea, Rastinon, Artosin, Orinase]; and chlorpropamide [N-(*p*-chlorobenzensulphonyl) = N-(*n*-propyl) urea, Diabinese]. These seem to have the same mode of hypoglycaemic action, but differ a little in their pharmacological properties.

Pharmacology

Many sulphonyl compounds having the general formula R_1-SO_2-NH-CO-NH-R_2 have been examined for hypoglycaemic properties (5–9). Linkage of the sulphone radicle to a ureide or similar group seems essential for hypoglycaemic activity, the extent and duration of the response depending on the nature of R_1 and R_2.

Sulphonylureas are rapidly absorbed from the gastrointestinal tract; maximum blood concentrations occur, on the average, 4 hr after oral administration (10, 11). Although excretion in the bile occurs, reabsorption is com-

[1] The survey of literature pertaining to this review was completed in May, 1964.
[2] The following abbreviations will be used: ILA (insulin-like activity); and NHGR (net hepatic glucose released).

plete (11). The volume of distribution of tolbutamide and chlorpropamide approximates that of the extra-cellular fluid (12, 13, 14), whereas carbutamide is distributed in a volume about twice as large (13, 15). The concentration of carbutamide in liver is ten times that in other organs (16), but there is no hepatic concentration of the other two sulphonylureas. Small amounts of sulphonylureas pass into the cerebrospinal fluid (15), but considerable quantities cross the placental barrier (17). About 50 percent of carbutamide and tolbutamide in the plasma is protein-bound (15, 18). In the body, carbutamide is acetylated and tolbutamide is carboxylated in the para position, forming inactive derivatives (18, 19). In the dog, however, tolbutamide is degraded by removal of the *n*-butyl group (18). Chlorpropamide does not undergo any comparable or significant metabolic changes in man (14). The plasma half life of an administered dose of tolbutamide varies from 4 to 8 hr, and that of carbutamide and chlorpropamide from 30 to 60 hr (10). The sulphonylureas are entirely eliminated from the body by the kidney, the proportions of the unchanged and metabolised forms in the urine varying with the drug administered, its dose, and on the individual animal and species. Its excretion is by glomerular filtration and renal tubular transport (12, 14, 20).

In acute toxicity studies, reviewed elsewhere (10, 11), very large doses caused neurological disturbances, leading to death of the experimental animal in the euglycaemic or hyperglycaemic state. These are probably attributable to a direct toxic effect on the nervous system; the hyperglyacemic response results from adrenal stimulation. Adrenalectomised and hypophysectomised animals are extremely sensitive to the hypoglycaemic action of the sulphonylureas (10, 11).

The results of the numerous long-term toxicity studies (10, 11) have varied according to the drug given, its dose and duration of administration, and the species and endocrine status of the animal employed. Long-term administration of effective hypoglycaemic doses did not retard growth of intact animals, although very large doses occasionally did so. Only minor histological and functional changes of the liver, kidney, and bone marrow have been reported. However, in partially depancreatised or alloxanised puppies, given a sulphonylurea for prolonged periods, these organs did show considerable damage. Carbutamide has a goitrogenic effect; but otherwise, the sulphonylureas do not influence endocrine glands other than the pancreas. Sexual and reproductive function remains unimpaired and only occasional teratogenic effects are found. There are many reports of the effect of long-term sulphonylurea administration on glucose tolerance, insulin sensitivity, islet cell volume and regeneration in intact, sub-diabetic, and hypophysectomised animals (10, 11, 21); the results have differed considerably and cannot be accurately summarised.

Mechanism of Hypoglycaemic Action

In the past twenty years, a plethora of relevant studies has been published of which there are several previous reviews (3, 10, 11, 22, 23, 24). In 1946,

Loubatières considered that the sulphonylureas lowered the blood glucose concentration by stimulating insulin secretion by the pancreatic β cells, and this view, with modifications, still best accords with the known facts. Before considering this hypothesis, other possibilities will be discussed and some at once dismissed.

Thus, the hypoglycaemic effect of the sulphonylureas is not attributable to their influence on the alimentary absorption or renal excretion of glucose, to acceleration of glycolysis in the blood, to decrease in the activity of other endocrine glands such as the pituitary, adrenal or thyroid, or to decrease of the secretion of glucagon by the pancreatic α cells or reduction of its effect on hepatic glycogenolysis (10, 11).

Direct action on peripheral tissues.—It is unequivocally proven and unanimously agreed that sulphonylureas have no hypoglycaemic effect when administered in single or repeated doses to "totally" diabetic depancreatised or alloxanised experimental animals not recently given exogenous insulin (10). Moreover, the exaggerated hypoglycaemic response of hypophysectomised or adrenalectomised animals is consistently abolished by pancreatectomy (25, 26). Although Mirsky & Gitelson (27) showed that tolbutamide elicited a fall in blood glucose when given to depancreatised fowls, it is emphasized that pancreatectomy does not induce diabetes in these animals (28). Thus, in the absence of endogenous or exogenous insulin, the sulphonylureas do not *in vivo*, have any demonstrable effect on the glucose uptake of peripheral tissues or output of glucose by the liver.

Studies in which a sulphonylurea was given to "totally" diabetic dogs, recently given or infused with insulin, have, however, usually shown the sulphonylurea to effect a small but consistent reduction in blood glucose (5, 29–32). In "totally" diabetic animals of other species (25, 33–35), except perhaps in one instance (36), and in depancreatised humans and insulin-dependent diabetics (10), no such effect has been demonstrated and there is no evidence of significant potentiation of exogenous insulin in these studies. With occasional exceptions, again in the dog (26, 37), the sulphonylureas had no influence on tissue glucose assimilation or utilisation in studies in which they were given parenterally to eviscerated animal preparations maintained with an infusion of glucose alone or with glucose and exogenous insulin (25, 34, 35, 38–41).

The many *in vitro* studies of the effect of the addition of a sulphonylurea on the uptake of glucose from a balanced salt incubation medium and its further utilisation by muscle or adipose tissue in the presence or absence of exogenous insulin have produced very conflicting results (10, 11). In general, either no or only minimal effects were observed when the sulphonylurea was present in concentrations comparable to the blood levels (10 to 15 mg per 100 ml) required to elicit a hypoglycaemic response *in vivo*. Previous alloxanisation of the donor animal eliminated any response.

In experiments in which tolbutamide was infused directly into the brachial artery of normal and mildly diabetic patients, Butterfield et al. (42) could not detect any increase in the tissue uptake of glucose by the forearm. Com-

parable studies (43) in dogs and man have provided similar results. The above evidence, more fully detailed in previous reviews (10, 11), shows that in animals and man incapable of secreting endogenous insulin, the sulphonylureas do not act directly on the peripheral tissues or the liver in such a manner as to promote a reduction of blood glucose, even in the presence of exogenous insulin. Fowls and possibly dogs provide the only exceptions to this generalisation, and this point has been discussed previously by Duncan & Baird (10).

The hypoglycaemic action of the sulphonylureas must, therefore, depend on the presence of the pancreas and the ability of the β cells to secrete a sufficiency of endogenous insulin.

Increased release of endogenous insulin by the β cells.—The administration of a sulphonylurea to intact animals is quickly followed by β cell changes considered to indicate augmented release of insulin. Thus, degranulation was almost always observed (10, 11, 44, 45, 46). Use of the electron microscope (47, 48) has revealed changes in the cells' ultrastructure in more detail; the granule-containing membranous sacs fuse with the cell membrane then rupture and discharge the granules into the intercellular space. Prolonged treatment with a sulphonylurea may result in hypertrophy of β cell nuclei, nucleoli, and Golgi apparatus (10, 11). The histochemical changes occurring within the cell have been investigated and interpreted (45, 46).

Several workers found changes in the extractable-insulin content of the pancreas of animals given a sulphonylurea which were consistent with an early increased release of the stored hormone (49, 50, 51). That this is due to the direct action of the sulphonylurea on the β cell was suggested by the experiments of Colwell (52) and Stuhlfauth et al. (53) who directly infused the drug into the pancreatic artery, and of Bouman & Gaarnstroom (54) who showed that the insulin release of isolated pancreatic tissue was increased by incubation with a sulphonylurea.

Antoniades (55) has recently reviewed the experimental evidence that following administration of a sulphonylurea to intact animals there is a rapid increase in the insulin-like activity (ILA) of pancreatic venous blood, as determined by *in vitro* and *in vivo* techniques. Other studies confirm this increased insulin release, notably those of Pozza, Galansino & Foà (56), in cross-circulation studies using parabiotic animal preparations, and of Sobel, Rodríguiz-Inigo & Levine (57) and Richter (58) who demonstrated the hypoglycaemic effect of the sulphonylureas in hepatectomised animals having porto-caval anastomoses.

There is thus no doubt that the initial administration of a sulphonylurea, as was postulated by Loubatières in 1946, stimulates the release of endogenous insulin, by the direct action of the drug on the β cell. Antoniades (55) & Hasselblat (59) provide evidence to support their postulate that the sulphonylureas promote the splitting of insulin-protein complexes in the β cell. However, two points must be stressed.

Firstly, studies relating to pancreatic vein ILA demonstrate only that this is increased for periods of some 30 min to 3 to 4 hr (43, 60, 61) following

initial administration of the sulphonylurea; experiments by Seltzer (60) and Bellens (43) show that the increased insulin secretion elicited by glucose administration is, in comparison, considerably greater in degree and duration. Other indirect evidence, that the initial liberation of insulin is transient, is provided by Pfeiffer et al. (50) and Root (51) in studies of the extractable-insulin content of the pancreas following administration of the sulphonylureas, by La Barre & Reuse (62) in their cross-circulation studies and, by Bellens (43) who showed that the initial hypoglycaemic response was followed by a refractory period during which no further insulin release or blood glucose reduction could be induced by subsequent administration of the sulphonylurea.

Secondly, during the early phase of the sulphonylurea-induced hypoglycaemia, no increase in the ILA of peripheral blood was at first detectable either in man or the dogs (10). Moreover, Pozza, Galansino & Foá (56) in their cross-circulation studies, were unable to demonstrate any increased blood-glucose-lowering activity in the mesenteric blood of the animal to which the sulphonylurea had been administered. More recent studies have, however, shown a definite rise in the ILA of peripheral blood during the first 30 min to 3 hr following initial administration of a sulphonylurea (43, 61, 63, 64). Nevertheless, this increase was considerably less than that in the pancreatic vein, and Rodari (63) and Bellens (43) showed that it lasted for only 2 to 3 hr.

Thus, although the sulphonylureas initially promote the release of preformed insulin from the β cells, there is no unequivocal evidence that their continued long-term administration stimulates the formation and secretion of greater than usual quantities of the hormone.

It is, therefore, pertinent to examine, firstly, the kinetics of the fall in blood glucose both during the early and later phases of the initial hypoglycaemia following single administration of a sulphonylurea and during the sustained overall reduction of glycaemia brought about by its long-term therapeutic administration, and, secondly, to determine their relationship to the increased secretion of insulin or other possible actions of the sulphonylurea.

Mechanism of initial hypoglycaemic response.—Reduction of blood glucose concentration can result only from diminished hepatic release of glucose into the blood or accelerated uptake of glucose from the blood by the tissues.

Duncan & Baird (10) reviewed the three types of experimental studies germane to the problem of reduced hepatic release of glucose, namely; (*a*) those using isotopic glucose (31, 65, 66), (*b*) those in which direct transhepatic measurements of glucose release were made in animals having catheters permanently inserted into the hepatic and portal veins and the abdominal aorta (65), and (*c*) those permitting indirect determinations of net hepatic glucose released (NHGR) (67, 68). The early phase of initial blood glucose reduction was caused by marked reduction or inhibition of NHGR and there was little, if any, accelerated blood glucose outflow into the tissues. Leonards et al. (69), however, have questioned the validity of some of these

studies and concluded from their own direct transhepatic measurements in dogs given a single dose of a sulphonylurea that there was not only an appreciable increase in peripheral assimilation of glucose but that in dogs fed a high-protein—in contrast to a high-carbohydrate—diet there was no decrease in NHGR.

The most obvious cause of the reduction in NHGR would be the direct action on the liver of the additional insulin released by the β cells. However, prior to the introduction of the sulphonylureas, insulin was generally considered to have no direct effect upon the liver (70, 71). Such an influence, however, has now been demonstrated in studies previously reviewed by one of us (10), although the diet of the animal seems to influence the hepatic action of insulin (72). Madison and his colleagues, whose studies have contributed greatly to the elucidation of this question, have provided further indisputable evidence that insulin can reduce or inhibit NHGR (73).

However, there is some evidence that the sulphonylureas may themselves influence hepatic metabolism directly in the permissive presence of insulin (10, 11). Thus, several workers have demonstrated a direct effect on liver enzyme systems involved in carbohydrate metabolism (10, 74, 75, 76), and Kaldor & Pogatsa (77) showed that perfusion of isolated rat livers with a sulphonylurea reduced their release of glucose. However, although the sulphonylureas themselves may influence hepatic metabolism either directly or by reactivating endogenous insulin, such an effect cannot account for the early phase of the initial hypoglycaemic response to a sulphonylurea in intact animals.

As for accelerated uptake and utilisation of glucose, Stadie (78) and Duncan & Baird (10) concluded, from the evidence then available, that it was still uncertain as to whether or not increased peripheral tissue assimilation of blood glucose occurred during the early hypoglycaemic phase following initial sulphonylurea administration. Since then, there has been unquestionably demonstrated, during this phase, an increase in peripheral blood glucose outflow in dogs (43, 69) and man (42), a substantial fall in plasma non-esterified fatty acids (79) and in amino acids (80), and, as stated above, a definite though transient increase in the plasma ILA of peripheral blood. The presumption is that a proportion of the additional insulin released quickly passes through the liver to exert its effect on the peripheral tissues. The factors which may influence the transhepatic passage of insulin have been reviewed elsewhere (10).

From the above evidence, it can be reasonably concluded that the early phase of the lowering of glycaemia following single administration of a sulphonylurea is due mainly to inhibition of NHGR and less so to increased peripheral tissue assimilation of glucose, both of which can be attributed to the action of the additional insulin released.

However, the detectable rise in plasma ILA in the pancreatic and peripheral blood and the reduction in NHGR (31, 66) appear to last for 30 min to 3 hr only, whereas the reduction in glycaemia is more sustained. Moreover, a rapid hypoglycaemic response occurs in intact animals and man, but

the blood glucose falls only slowly in sulphonylurea-responsive diabetics (81), in fasted and moderately alloxan-diabetic animals (82) and in idiohypophyseal diabetic animals (83); the speed of reduction in glycaemia, but not its duration, seems to be related to the pancreatic reserve of stored insulin available for release. Thus, the more prolonged fall in glycaemia, following single dosage of and in consequence to daily treatment with a sulphonylurea, is unlikely to be due solely to the effects of increased insulin release and requires further explanation.

Mechanism of late and sustained hypoglycaemic response.—The metabolic and other changes noted during the late phase of the initial hypoglycaemic response to single dosage, and accompanying and consequent upon long-term treatment of diabetics with a sulphonylurea, are those associated with increased insulin-activity throughout the body (10, 84, 85). Thus, Butterfield et al. (42) have demonstrated a reduction in the peripheral-tissue blood glucose assimilation threshold in man, and Recant & Fischer (86) have demonstrated a sustained diminution of hepatic glucose release in animals given a sulphonylurea daily. Since the sulphonylureas do not act directly on peripheral tissues and because there is no firm evidence of sustained increased production of insulin by the β cells, this long-term increased insulin effect is likely, in part at least, to be attributable to decreased insulin destruction or to antagonism, or to reactivation of bound insulin.

Mirsky's view (82) that the hypoglycaemic sulphonylureas exert their later hypoglycaemic effect by reducing insulinase activity has been considered elsewhere (10, 74, 87) and is not yet entirely disproven.

An effect on insulin antibodies or antagonists is unlikely since the sulphonylureas do not alter insulin requirements or sensitivity when given to insulin-dependent or -resistant diabetics (10), nor do they alter the anti-insulin effect of β-1-lipoprotein from responsive (23), or of antibody-like protein from insulin-resistant (88) diabetics *in vitro*.

There is, however, growing evidence that the sulphonylureas may increase the activity of bound endogenous insulin in the plasma and on cells. Thus, several workers found that the addition of a sulphonylurea to plasma from normal (23, 89) or responsive diabetic (90) persons increased the glucose uptake of rat diaphragm and adipose tissue, although no such effect occurred when a balanced salt incubation medium was used. Loubatières et al. (91) transfused blood from an intact dog into a depancreatised dog and found that administration of a sulphonylurea to the recipient reduced its blood glucose concentration; this did not occur if blood from a depancreatised animal was transfused.

More recent studies by Hasselblatt (59) indicate that the sulphonylureas liberate insulin from protein complexes *in vitro*, and studies by Antoniades et al. (55) indicate that they influence the relative amounts of free insulin and insulin complex in blood and increase the rate of utilisation of the latter. Such an effect on bound endogenous insulin would explain the later blood-glucose-lowering action of the sulphonylureas and the accompanying increased insulin effect on the tissues.

Summary.—The primary action of the sulphonylureas may be the freeing of endogenous insulin from protein-bound complexes in the pancreatic β cells, the plasma, and the tissues. This could account for the proven increased release of insulin, the duration of which is uncertain, and for the increased insulin-like action on the liver and peripheral tissues. Much more evidence, however, is required to substantiate or refute this hypothesis.

THE DIGUANIDES

Although Watanabe (92) described the hypoglycaemic properties of guanidine in 1918, it was too toxic for clinical use. In 1926, the diguanidines, Synthalin A and B, were introduced by Frank & Wagner (93) as oral forms of treatment for diabetes, but were generally discarded in the 1930's on the probably unjustified grounds (10) of undue toxicity. However, in the mid-1950's, Ungar, Freedman & Shapiro (94) and Sterne (95) independently investigated the hypoglycaemic properties of the respective diguanides, phenformin (Dibotin, DBI, phenethyldiguanide), and metformin (Glucophage, dimethyldiguanide). These, as well as the more recently introduced butformin (Silubin, butylbiguanide) are now widely used in the oral treatment of diabetes.

The pharmacology and mode of hypoglycaemic action of the substituted monoguanides and the diguanidines are reviewed elsewhere (10, 11, 96) and only the diguanides are considered.

Pharmacology

Although all diguanides are absorbed from the alimentary tract and excreted in the urine within 24 hr, they differ in distribution and metabolic fate in the body. Thus, even when given parenterally, phenformin is concentrated in the gastric juice and liver (97), whereas metformin is concentrated in the intestinal walls and salivary glands (98). Only very low blood concentrations (5μg per ml) of both are achieved (99, 100), and although metformin is excreted unchanged, phenformin is metabolised into several degradation products (99, 101). Studies with butformin are being currently conducted (102).

The hypoglycaemic response depends on the drug and dose given, route of administration, the species of animal, and its nutritional and endocrine status (10). Metformin has to be given in dosages ten times that of phenformin and butformin to effect a similar reduction in blood glucose. Much larger doses of the diguanides, one third to one half the lethal dose, are needed to elicit a hypoglycaemic response in nondiabetic than in moderately diabetic animals (10), and no appreciable hypoglycaemic response occurs in nondiabetic patients given very substantial doses (11, 103). A rise in blood glucose, most marked when the drug is given intravenously, usually precedes its fall and is probably caused by stimulation of the adrenal medulla and central nervous system (104). Other pharmacological effects, such as on the blood pressure, are unimportant (10); the diguanides have chelating properties with all metals (105).

Excessive doses cause death in hypoglycaemia, preventable by glucose administration; hypophysectomy or adrenalectomy increases the hypoglycaemic response (10, 11). Long-term toxicity studies have shown no marked untoward functional or histological changes in the organs of the body (10, 11, 106).

Mechanism of Hypoglycaemic Action

The diguanides do not reduce glycaemia by influencing the alimentary absorption of glucose (107), its renal excretion, or the activity of endocrine glands (10, 11). There is good evidence that they do not increase the secretion of insulin from the pancreatic β cells in that there is no β cell degranulation (108) and perfusion studies of the isolated pancreas have not demonstrated any augmentation of insulin release (53).

Direct action on peripheral tissues.—The many early *in vitro* studies, recently reviewed in detail by Daweke & Bach (109), showed that, in the absence of added insulin, phenformin increased the glucose uptake of the rat hemidiaphragm and its liberation of lacate into the medium and decreased its glycogen content and oxygen consumption; similar inhibition of oxidation was found in studies using adipose tissue. Other *in vitro* studies using kidney or liver homogenates and mitochondrial preparations, or with adipose tissue, demonstrated that phenformin inhibited certain oxidative enzyme systems within the Krebs cycle (110–112). This led to the hypothesis that the increased uptake of glucose by these tissues was attributable to accelerated anaerobic glycolysis and that this phenomena accounted for the hypoglycaemic action of the diguanides *in vivo.* However, in all these studies, the concentration of phenformin was 4 to 300 times the maximum concentration found in the plasma of responsive diabetics given full doses of the drug. Moreover, metformin, irrespective of its concentration, does not inhibit tissue respiration (113), and several nonhypoglycaemic drugs do so (114). In any event, such a mechanism of action is incompatible with the clinical response of diguanide-treated diabetics and the metabolic accompaniments of this form of therapy (103).

Dependence of hypoglycaemic response on the presence of insulin.—The hypoglycaemic effect of the diguanides does not depend on the presence of endogenous insulin, since totally depancreatised animals and "truly" insulin-dependent diabetics receiving exogenous insulin show a hypoglycaemic response to the diguanides, and, in them, the diguanides have an insulin-sparing effect (10, 11).

Daweke & Bach (109), in careful *in vitro* studies using rat epididymal fat tissue, showed that concentrations of butformin corresponding to those in the blood of treated diabetics caused a significant increase in glucose oxidation in the presence of insulin; higher concentrations of the drug inhibited glucose oxidation, although glucose uptake still occurred. In comparable studies using the rat hemidiaphragm and in the presence of exogenous insulin, butformin in these low concentrations did not increase either the uptake or oxidation of glucose and at high concentration inhibited the glucose oxidation. These *in*

vitro studies suggest that hypoglycaemic effect of the diguanides might result from their potentiation of the effect of exogenous and endogenous insulin on adipose tissue.

The site of action.—Butterfield & Whichelow (115), in their studies of glucose disposal in forearm tissues, were unable to detect any direct effect on glucose uptake following the injection or infusion of phenformin into the brachial artery. However, after ten days of therapy with phenformin, diabetic patients were found to have a reduced peripheral tissue blood glucose assimilation threshold.

Valid studies of net hepatic glucose release in diabetics controlled by a diguanide have not been reported, although no decrease in the usual rise of glycaemia following fructose administration occurred in such patients (116).

Summary.—The blood glucose-lowering properties of the diguanides, when given in therapeutic dosage to diabetic patients, depend on the presence of either endogenous or exogenous insulin, and it is possible that they potentiate the effect of these on the tissues. The studies of Daweke & Bach (109) and the fact that diguanides are most effective in obese diabetics, suggest that adipose tissue may be the most sensitive to this action. We agree with Pometta & Plattner (116) who state in their recent review that ". . the concepts of the mechanism of action of the diguanides remain extremely confused. Review of publications up to this date reveals only fragmentary data."

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