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*Review*

# The fiftieth anniversary of hypoglycaemic sulphonamides. How did the mother compound work?

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Probably over 20 million patients world-wide suffering from Type 2 (non-insulin-dependent) diabetes mellitus are treated with hypoglycaemic sulphonylureas. In fact, all antidiabetic drugs currently used with the aim of stimulating insulin release belong to this family.

Soon after the discovery of insulin, Abel and Geiling [1] showed that the hormone contains large amounts of sulphur which is essential for its biological action. These observations prompted several studies on the possible effects of sulphur itself on glucose homeostasis. Oral and parenteral administration of colloidal sulphur caused a small decrease in blood glucose levels in normal rabbits and humans [2–4] and slightly lowered glycaemia and glucosuria in certain diabetic patients [4, 5]. The mechanisms of action of sulphur have not been clearly identified.

The first report that a synthetic sulphur-containing compound may lower blood glucose probably dates from 1930 [6]. Ruiz and collaborators observed that oral or intravenous administration of 4- or 5-methyl, 2-thiolimidazole (Fig. 1) to normal fasting rabbits was followed by a slight decrease of glycaemia, but the mechanism involved was not elucidated [6].

## From a clinical observation with compound 2254 RP ...

The story of the hypoglycaemic sulphonamides started in France, 50 years ago. In the spring of 1942, Marcel Janbon from the Clinic of the Montpellier Medical School evaluated the efficacy of a sulphonamide, compound 2254 RP (p-aminobenzenesulphamido-isopropylthiodiazol) (Fig. 1) in the treatment of typhoid fever. This compound (also known as VK 57 or IPTD) had been synthesized one year earlier by Vonkennel and Kimmig [7], and found to have a slight bacteriostatic effect on the typhoid bacillus. After the unexplained death of some of their patients, Janbon and his collaborators realized that 2254 RP was causing hypoglycaemia [8, 9]. In June 1942, this "side-effect" of the drug was confirmed experimentally by Auguste Loubatières [10], who then undertook a careful study of the mechanisms underlying this hypoglycaemia [11–13].

He demonstrated that, whatever the route of administration, 2254 RP decreased blood glucose levels in normal dogs. This hypoglycaemic effect was unaffected by vagotomy, persisted in partially pancreatectomized animals, but disappeared when the pancreatectomy was complete. The degree of hypoglycaemia was dependent on the sulphonamide concentration in plasma, but low doses were sufficient to cause a marked fall in blood glucose when they were injected directly into the pancreatic artery [11–13].

These observations led Loubatières to propose that the hypoglycaemic property of 2254 RP was due to its ability to stimulate insulin release through a direct action on Beta cells. Further support for this interpretation was obtained in cross-circulation experiments. When the pancreaticoduodenal vein of a normal donor dog was anastomosed to the jugular vein of a receiver dog made diabetic by alloxan, injection of the drug to the donor was followed by a decrease in blood glucose levels in the receiver [12]. The hypoglycaemic effects of 2254 RP and of a related com-

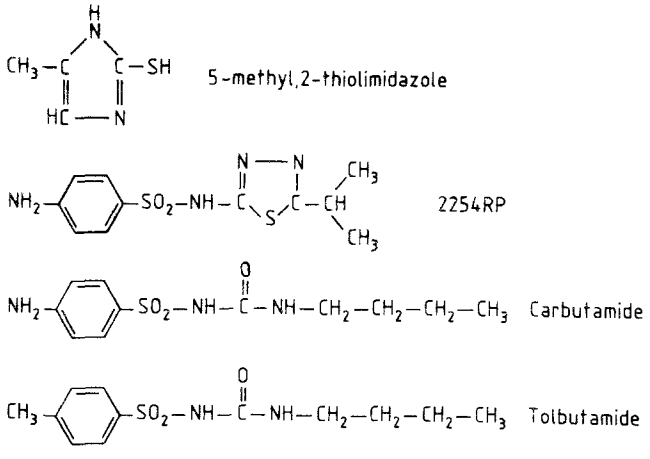


**Fig. 1.** Structural formulae of 4-methyl,2-thiolimidazole, compound 2254 RP, carbutamide and tolbutamide

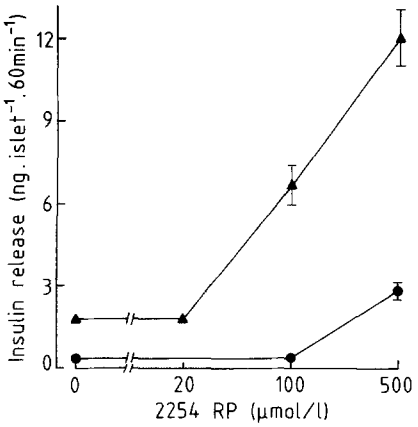


**Fig. 2.** Effects of various concentrations of 2254 RP on insulin release by mouse islets incubated in a medium containing 3 (●) or 10 (▲) mmol/l glucose. Values are means ± SEM for 12 batches of islets

pound were rapidly confirmed by Chen et al. [14] and by La Barre and Reuse [15]. These authors further showed that the drug remained effective in alloxan-diabetic dogs provided the destruction of Beta cells was not complete.

In the mid-1950s, morphological studies of the pancreas of animals treated with 2254 RP temporarily cast doubt on Loubatières' interpretation of the mode of action of the drug. These studies suggested that the hypoglycaemic property was due to a destruction of glucagon-secreting Alpha cells [16, 17]. That 2254 RP affected the morphological appearance of Alpha cells was confirmed [18, 19], but Gepts and collaborators [19] pointed out that the cells were degranulated, not severely damaged. They also noted that Beta cells were degranulated by the treatment [19].

Structure-activity studies were carried out very early in man by Janbon [20], in dogs by Bovet and Dubost [21], and in rabbits by Loubatières [22]. The results suggested that both the sulphonamide group and the nature of the side chain were important for the hypoglycaemic action.

Although Loubatières had already suggested in his thesis published in 1946 [12] that "such hypoglycaemic drugs could be useful in the treatment of certain forms of functional diabetes characterized by a sluggishness of the insulin-secretory mechanisms", only few clinical assays were performed up to 1955 [23–25]. These assays showed that 2254 RP lowered glycaemia and glucosuria in patients whose diabetes did not require insulin treatment, but could never be substituted for insulin in patients with more severe diabetes.

## ... to the sulphonylureas ...

In the spring of 1954, a similar story was taking place in Berlin. Franke and Fuchs [26] noted that a new sulphonamide, that was being tested in the treatment of various bacterial infections, caused hypoglycaemia in normal subjects. This compound, known as BZ 55 or carbutamide (Fig. 1), differed from 2254 RP by the substitution of the isopropylthiodiazol moiety with an n-butylurea group. The hypoglycaemic effects first observed in normal human subjects were confirmed in animals [27] and the drug was rapidly tested in diabetic patients. Carbutamide was found to lower blood glucose levels and decrease glucosuria in adult diabetic subjects who did not require insulin treatment [26, 28]. It has been claimed by Kleinsorge that the hypoglycaemic effects of carbutamide (also known as compound Ca 1022) had already been studied in a large number of patients in 1952–1953. However, this information was not published in the scientific literature until 1956 [29].

Shortly after, tolbutamide (Fig. 1) was synthesized [30] and found to possess no bacteriostatic but clear hypoglycaemic properties. A number of experimental and clinical studies established its usefulness in the treatment of certain forms of diabetes [31]. Glibenclamide, the first of the more potent sulphonylureas of the "second generation" became available in 1966 [32].

At the end of 1956, despite the general agreement that sulphonylureas had no effect on blood glucose in the complete absence of insulin, it was not at all clear that their primary action is to stimulate insulin release [33, 34]. The major steps leading to this demonstration were the following.

The first studies were directly inspired by those performed 10 years earlier by Loubatières with 2254 RP. Cross-circulation experiments between hepatectomized donor dogs and normal recipients showed that sulphonylurea injection to the donor caused hypoglycaemia in the recipient [35]. It was also observed that low doses of sulphonylurea, which were ineffective when injected in a peripheral vein, caused hypoglycaemia when injected in the pancreatic artery [36].

The initial attempts to demonstrate an increase in plasma insulin levels after sulphonylurea administration were not conclusive largely because of the limitations of the insulin bioassays which were then available. These assays, however, detected an increase in plasma insulin when blood was sampled from the pancreatic vein of rats [37], and subsequently in peripheral blood from human subjects [38–40] receiving sulphonylureas. The advent of radioimmunoassays then made it clear that these drugs acutely increase the plasma insulin concentration [41].

In vivo treatment with sulphonylureas was soon found to cause degranulation of Beta cells [42, 43] and to lower the insulin content of the pancreas [44, 45]. It was, however, not immediately clear whether this reflected decreased production or increased secretion of insulin. Convincing support for the latter interpretation was provided by ultrastructural studies showing margination of insulin granules and abundant exocytotic figures in Beta cells of animals treated with tolbutamide [46].

In vitro techniques to study insulin release were developed in the 1960s. Using a paper chromatography method to measure insulin, it was first shown that carbutamide increases insulin release from the isolated and perfused dog pancreas [47]. This was then confirmed with an insulin bioassay [48], that was also used to demonstrate that tolbutamide stimulates insulin release from fragments of rat pancreas [49]. Insulin radioimmunoassays eventually made it possible to establish the stimulatory ef-

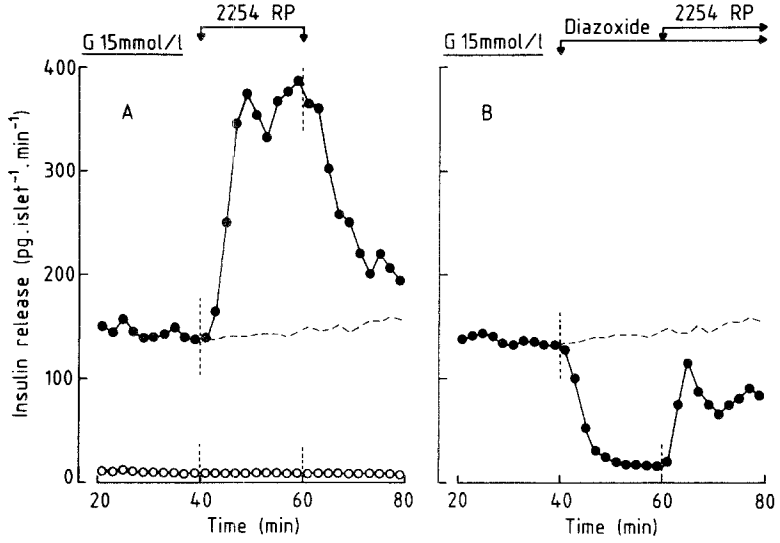


**Fig. 3 A, B.** Effects of 2254 RP on insulin release by mouse islets perifused with a medium containing 15 mmol/l glucose (G). In **A** one series of experiments (○) was made in the absence of $Ca^{2+}$. 2254 RP (100 µmol/l) and diazoxide (50 µmol/l) were added for the indicated periods. Control release in the presence of glucose alone is shown by the broken lines. Values are means of two experiments

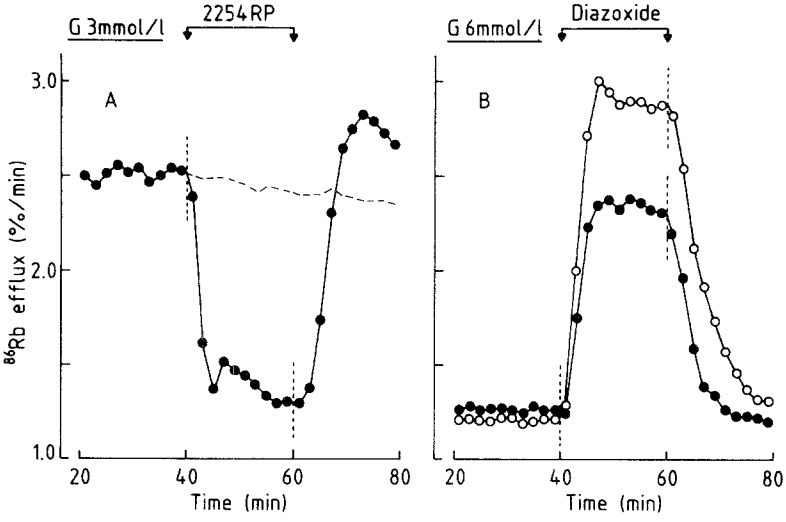


**Fig. 4 A, B.** Effects of 2254 RP on the efflux of $^{86}$Rb (used as a tracer for potassium) from perifused mouse islets **A.** The concentration of glucose (G) was 3 mmol/l and 2254 RP (500 µmol/l) was added between 40 and 60 min. Control experiments without 2254 RP are shown by the broken line **B.** The concentration of glucose (G) was 6 mmol/l and diazoxide (100 µmol/l) was added between 40 and 60 min. In one series (●) 2254 RP (100 µmol/l) was present throughout. Values are means of two experiments. The methods used in these experiments have been described previously [60]

fects of sulphonylureas on Beta cells in pieces of rabbit pancreas [50], in the perfused rat pancreas [51] and in isolated rat islets [52].

Since then numerous studies have been devoted to the elucidation of the cellular mechanisms by which sulphonylureas influence Beta cell function. These will not be reviewed again here because several recent articles have dealt with this question [53–57]. I found it more interesting and amusing to determine if and how compound 2254 RP, the molecule with which the whole story started, affects Beta cells.

### ... and back

The remainder of a batch (No 4443) of 2254 RP used by Loubatières himself was found, well-preserved by the dry climate of Montpellier, and was generously made available to me by Prof. M. M. Loubatières-Mariani. The effects of the drug were investigated in vitro with islets isolated from normal mice.

Figure 2 shows that basal insulin release by islets incubated in a medium containing 3 mmol/l glucose was unaffected by 100 µmol/l and increased about seven-fold by 500 µmol/l 2254 RP. It also shows that insulin release induced by 10 mmol/l glucose was not modified by 20 µmol/l 2254 RP, but was potentiated approximately four- and seven-fold by 100 and 500 µmol/l 2254 RP, respectively. In dogs acutely treated with 2254 RP Loubatières usually measured a total sulphonamidaemia between 30 and 150 mg/l [12], which corresponds to 100–500 µmol/l 2254 RP. Although the assay also measured metabolites of the drug, the concentrations were well within the range of those that are effective on Beta cells in vitro. These observations thus establish that Loubatières was correct in his assumption that 2254 RP directly stimulates pancreatic Beta cells to release insulin.

Experiments with perifused islets showed that the increase in insulin release brought about by 2254 RP is rapid and reversible, and that omission of calcium from the medium prevented both glucose and 2254 RP from stimulating insulin release (Fig. 3).

In the presence of a low concentration of glucose (3 mmol/l), the potassium permeability of the Beta-cell membrane is high because many ATP-sensitive $K^+$ chan-

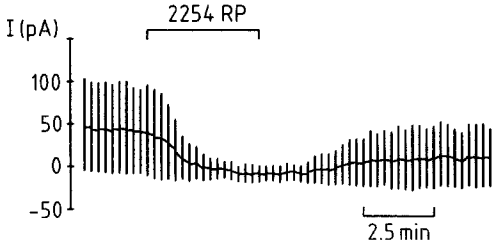


**Fig. 5.** Effects of 2254 RP on ATP-sensitive $K^+$ currents in a mouse Beta cell. Currents were recorded with the whole cell mode of the patch clamp technique as previously described [63]. Currents were evoked by pulses to −60 and −80 mV from a holding potential of 70 mV. The size of the upward and downward deflections indicates the size of the current (I). The recording starts 2 min after establishment of the whole cell configuration. 2254 RP (500 µmol/l) was added for the indicated period. The recording is representative of results obtained in three different cells

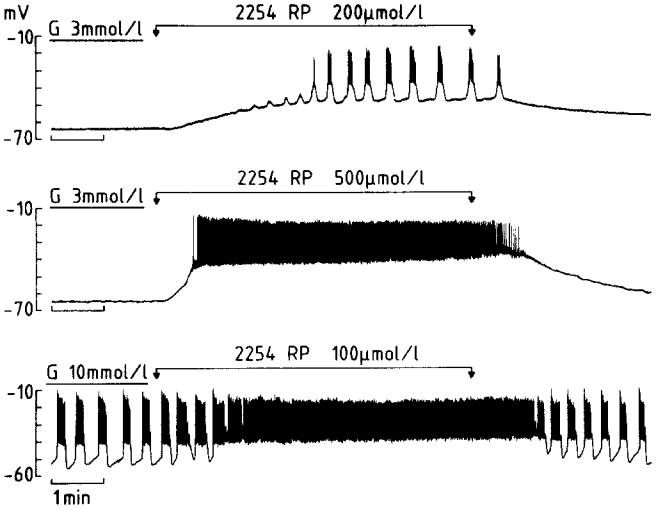


**Fig. 6.** Effects of 2254 RP on the membrane potential of mouse Beta cells perifused with a medium containing 3 or 10 mmol/l glucose (G). 2254 RP was added at the concentrations and for the periods indicated on top of the panels. The recordings were obtained with an intracellular microelectrode technique [64]. Similar results have been obtained in two experiments

nels are open [58, 59]. This explains why the efflux rate of $^{86}Rb$ (a tracer of potassium [60]) from islet cells is high (Fig. 4). Addition of 2254 RP rapidly and reversibly decreased this rate of efflux. In the presence of 6 mmol/l glucose, the rate of $^{86}Rb$ efflux is low because most ATP-sensitive $K^+$ channels are closed [58, 59]. Diazoxide, a selective opener of these channels [61], caused a marked acceleration of $^{86}Rb$ efflux which could be partially antagonized by an equimolar concentration of 2254 RP (Fig. 4). The opening of ATP-sensitive $K^+$ channels by diazoxide repolarizes the Beta-cell membrane [62] and inhibits glucose-induced insulin release. This inhibition was partially reversed by 2254 RP (Fig. 3). All these observations indirectly indicate that 2254 RP closes ATP-sensitive $K^+$ channels.

This closure was directly demonstrated by the patch-clamp technique. When a Beta cell is dialysed with a pipette solution containing a low concentration of ATP and its membrane potential held at −70 mV (resting level), ATP-sensitive $K^+$ currents can be selectively triggered by pulses to −60 and −80 mV [61, 63]. As shown in Figure 5, 2254 RP rapidly and reversibly inhibited these currents.

The decrease in potassium conductance resulting from the closure of ATP-sensitive $K^+$ channels may be expected to cause depolarization of the Beta-cell membrane. When islets were perifused with a medium containing only 3 mmol/l glucose, the resting potential of Beta cells was about −65 mV. Addition of 2254 RP was followed by a depolarization and appearance of electrical activity the intensity of which increased with the concentration of the drug (Fig. 6). In the presence of 10 mmol/l glucose, Beta cells exhibited their typical electrical activity consisting of slow waves of the membrane potential with bursts of spikes superimposed on the plateau [64]. After addition of 2254 RP the duration of slow waves increased before the depolarization at the plateau potential became sustained and the spike activity became continuous (Fig. 6).

Since this electrical activity reflects $Ca^{2+}$ influx through voltage-dependent $Ca^{2+}$ channels [65], the effects of 2254 RP on the concentration of cytoplasmic $Ca^{2+}$ in Beta cells were measured. In non-stimulated Beta cells (3 mmol/l glucose), $Ca^{2+}{}_i$ was low and stable; it was markedly increased by 2254 RP (Fig. 7). In the presence of 15 mmol/l glucose, $Ca^{2+}{}_i$ was higher and displayed regular oscillations [66]. Addition of 2254 RP was followed by a sustained increase in $Ca^{2+}{}_i$ (Fig. 7).

## Conclusions

The mode of action of 2245 RP in Beta cells can be schematically represented as follows (Fig. 8). By closing ATP-sensitive $K^+$ channels, 2254 RP causes a decrease in potassium conductance of the Beta-cell membrane that leads to depolarization with activation of voltage-dependent $Ca^{2+}$ channels. The ensuing influx of $Ca^{2+}$ raises the concentration of free cytoplasmic $Ca^{2+}$, which activates an effector system eventually responsible for exocytosis of insulin granules. This sequence of events is similar to that triggered by sulphonylureas of the first or second generation.

Given their central role in the control of Beta-cell membrane potential, ATP-sensitive $K^+$ channels are an

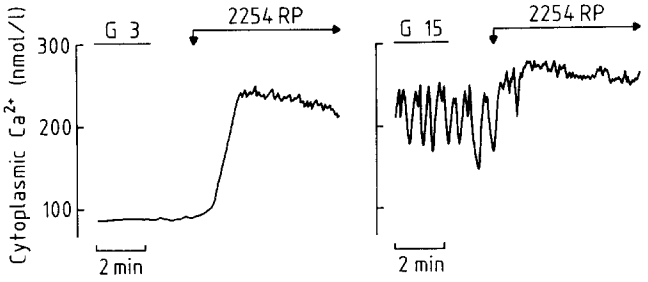


**Fig. 7.** Effects of 2254 RP on the concentration of cytoplasmic $Ca^{2+}$ in mouse Beta cells. The concentration of $Ca^{2+}$ was measured in islets loaded with the $Ca^{2+}$ fluorochrome fura-2 [66] and perifused with a medium containing 3 or 15 mmol/l glucose (G). 2254 RP was added at the concentrations of 500 µmol/l (left) and 250 µmol/l (right) for the periods indicated at the top of the panels. The traces are representative of results obtained with three islets

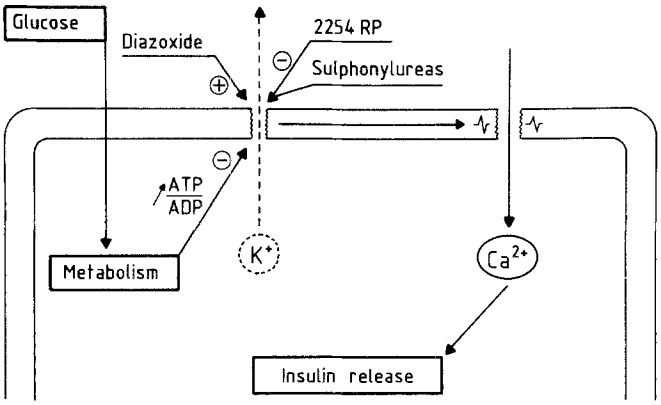


**Fig. 8.** Schematic representation of the major mechanisms by which glucose, compound 2254 RP and sulphonylureas stimulate insulin release

exquisitely sensitive target for pharmacological agents purported to affect Beta-cell function. All hypoglycaemic drugs that we currently use to correct the insufficient release of insulin in Type 2 diabetic patients act on this target. New substances, be they sulphonamides or not, able to cause a rapid and short-term inhibition of ATP-sensitive $K^+$ channels would certainly enrich our therapeutic arsenal. However, with the improvement of our understanding of Beta-cell physiology and pathophysiology has come the time to look for drugs acting at other sites [53]. It would be unwise to deliberately restrict our therapeutic means to compounds aiming at the same single target for another 50 years.

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