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# *IN VITRO* EFFECTS OF BAY K 8644, A DIHYDROPYRIDINE DERIVATIVE WITH HYPOGLYCAEMIC PROPERTIES, ON HEPATIC GLUCOSE PRODUCTION AND PANCREATIC HORMONE SECRETION

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**Abstract**—In rats, oral administration of BAY K 8644 (methyl 1,4-dihydro-2,6-dimethyl-3-nitro-4-(2-trifluoromethylphenyl)-pyridine-5-carboxylate), a dihydropyridine derivative, $Ca^{2+}$-channel activator, lowers fasting glycaemia and improves glucose tolerance to carbohydrate loading without elevating peripheral plasma insulin. To study the hypoglycaemic mechanism of this compound, we have examined its effects on glucose production by isolated rat hepatocytes and on hormone secretion by the perfused rat pancreas. Incorporation of BAY K 8644 (0.2–10 μM) into the hepatocyte incubation medium failed to significantly modify glycogenolysis, gluconeogenesis or L-lactate production. Hepatocyte glycogen phosphorylase *a* (EC 2.4.1.1) activity and fructose 2,6-bisphosphate levels were also unaffected by BAY K 8644. In the perfused rat pancreas, BAY K 8644 markedly stimulated insulin release without modifying glucagon or somatostatin output. Thus, the possibility that this compound exerts its hypoglycaemic effect by provoking insulin secretion should be further investigated.

BAY K 8644 (methyl 1,4-dihydro-2,6-dimethyl-3-nitro - 4 - (2 - trifluoromethylphenyl) - pyridine - 5 - carboxylate) is a novel dihydropyridine derivative with $Ca^{2+}$-agonistic properties since it behaves as a $Ca^{2+}$-channel activator [1, 2]. Puls and Bischoff [3] have recently reported that, in rats, oral administration of this compound lowers fasting glycaemia and improves glucose tolerance to oral or intravenous carbohydrates loading. This effect, however, was not accompanied by an elevation of circulating insulin.

To gain further insight into the mechanism of action of BAY K 8644, we have investigated its influence on hepatic glucose production and on pancreatic hormone secretion. For this purpose, we tested: (1) the effect of this compound on glycogenolysis, gluconeogenesis and glycolysis in isolated rat hepatocytes, as well as the activity of glycogen phosphorylase *a* (EC 2.4.1.1) and the concentration of fructose 2,6-bisphosphate; and (2) the effect of BAY K 8644 on insulin, glucagon and somatostatin release by the isolated perfused rat pancreas.

## MATERIALS AND METHODS

BAY K 8644, kindly supplied by Dr F. Hoffmeister and Dr W. Puls (Institute of Pharmacology, Bayer AG, Wuppertal, F.R.G.), was dissolved in dimethylsulfoxide; the same amount of the solvent (1 μl/ml) was present in both test and control experiments. Handling of BAY K 8644 was carried out under light from a sodium vapour lamp in order to prevent its degradation. Full biological activity of the BAY K 8644 batch employed in our study was confirmed by determining its capability to potentiate contractility of the isolated rat aortic strip [1].

*Incubations of rat hepatocytes and measurements of glycogenolysis, gluconeogenesis and glycolysis.* Hepatocytes were isolated from fed or 24-hr fasted male Wistar rats (200–250 g body weight) by perfusion of the liver with collagenase [4], and subsequently incubated in Krebs–Henseleit medium as described elsewhere [5].

Glycogenolysis was measured in hepatocytes from fed rats by the rate of glucose production [6]. In these experiments, neither glucose nor gluconeogenic precursor were added to the incubation medium. The activity of glycogen phosphorylase *a* was assayed at 37°, as described by Hue *et al.* [4]. Gluconeogenesis was estimated by the rate of radioactive glucose formation [5] using 4 mM L-(U-$^{14}$C)lactate (0.25 μCi/ml) (Amersham International, Amersham, U.K.). Glycolysis was measured by the rate of net L-lactate production in hepatocytes from 24-hr fasted rats. The amount of L-lactate produced by the cells and the hepatocyte content of fructose 2,6-bisphosphate were measured as described elsewhere [7]. All the above measurements are expressed per gram (wet weight) of hepatocytes; 1 g packed hepatocytes corresponds to 220 ± 5 mg protein (N = 12). Protein was measured by the method of Bradford (Bio-Rad Protein Assay, Bio-Rad Laboratories, München, F.R.G.) using bovine serum albumin as standard [8].

*Perfused rat pancreas system and hormone determination.* Fed male Wistar rats (200–250 g body weight) were used as donors. After anaesthesia of the rat with pentobarbital sodium (50 mg/kg, i.p.),

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the pancreas was dissected and perfused *in situ*, according to the procedure of Leclercq-Meyer *et al.* [9] as adapted in our laboratory [10]. Briefly, the pancreas was perfused through the celiac and superior mesenteric arteries, via a cannula inserted into the aorta. Effluent samples were collected from the portal vein, without recycling, at 2-min intervals (flow rate, 2 ml/min) in tubes containing 2000 kallikrein inhibitor units of aprotinin (Bayer AG, Leverkusen, F.R.G.), and frozen at −20° until the time of assay.

The perfusion medium consisted of a Krebs–Henseleit buffer (gas phase 95:5, $O_2$:$CO_2$; pH 7.4) supplemented with 4% (w/v) dextran T-70, 0.5% (w/v) bovine albumin (Cohn fraction V) and glucose (5.5 mM). After an equilibration period of 25 min, baseline samples were collected for 12 min. At zero time, BAY K 8644 was infused through a sidearm cannula as a constant infusion (20 μM for 20 min). An identical volume of perfusion medium was infused in control experiments.

Radioimmunoassay was employed to measure insulin [11], glucagon [12] and somatostatin [13]. Antiglucagon serum (30K) and antisomatostatin serum (80C) were kindly donated by Dr R. H. Unger (University of Texas Health Science Center at Dallas, TX).

*Expression of results.* Results are presented as the mean ± SEM. Statistical study was performed by analysis of variance and by the Student's *t*-test for paired or unpaired observations.

## RESULTS

### *Effect of BAY K 8644 on glycogenolysis and glycogen phosphorylase a activity*

As shown in Table 1, addition of BAY K 8644 (0.2 μM and 2 μM) to the incubation medium did not significantly modify glycogenolysis or glycogen phosphorylase *a* activity in hepatocytes isolated from fed rats. In the same experiments, phenylephrine ($10^{-4}$ M) showed its well-known stimulatory effect on both glycogenolysis and glycogen phosphorylase *a* activity. In addition, as can be seen in Table 2, when hepatocytes were incubated in the presence of 10 mM glucose, incorporation of BAY K 8644 (10 μM) into the incubation medium failed to modify glycogen phosphorylase *a* activity when this enzyme was stimulated by a suboptimal ($10^{-10}$ M) or a saturating ($10^{-8}$ M) concentration of glucagon.

### *Effect of BAY K 8644 on gluconeogenesis, L-lactate production and fructose 2,6-bisphosphate concentration*

Table 2 shows that, in hepatocytes incubated with 10 mM glucose, the presence of BAY K 8644 (10 μM) did not significantly affect gluconeogenesis, measured as glucose production from L-(U-$^{14}$C)lactate. Furthermore, BAY K 8644 did not significantly modify gluconeogenesis when it was stimulated by glucagon at either a suboptimal or a saturating concentration. We also examined the

Table 1. Effect of BAY K 8644 on glycogenolysis and glycogen phosphorylase *a* activity in isolated rat hepatocytes

| Additions | Glucose production (μmol/g of cells × 40 min) | Glycogen phosphorylase *a* (units/g of cells) |
|---|---|---|
| Saline | 47.2 ± 4.0 | 5.64 ± 0.8 |
| BAY K 8644 (0.2 μM) | 49.7 ± 2.1 (NS) | 5.90 ± 1.17 (NS) |
| BAY K 8644 (2 μM) | 48.7 ± 2.7 (NS) | 5.71 ± 0.98 (NS) |
| Phenylephrine $10^{-4}$ M | 75.5 ± 4.0* | 12.10 ± 1.1* |

Glucose production was measured in fed rat hepatocytes incubated for 40 min with no added glucose. Glycogen phosphorylase *a* activity was measured after 30 min preincubation with 10 mM glucose, and 5 min after addition of BAY K 8644, saline or phenylephrine to the incubation medium. The values represent the means of 3–6 incubations ± SEM. *P values versus saline incubation less than 0.05; NS: nonsignificant.

Table 2. Effect of BAY K 8644 on glycogen phosphorylase *a*, gluconeogenesis and fructose 2,6-bisphosphate levels in glucagon-treated isolated rat hepatocytes

| Additions | Glycogen phosphorylase *a* (units/g of cells) | L-(U-$^{14}$C)lactate converted to glucose (μmol/g of cells × 20 min) | Fructose 2,6-bisphosphate (nmol/g of cells) |
|---|---|---|---|
| Saline | 2.44 ± 0.3 | 6.4 ± 0.4 | 11.6 ± 0.4 |
| Saline +BAY K 8644 (10μM) | 2.60 ± 0.1 (NS) | 5.8 ± 0.5 (NS) | 12.5 ± 0.8 (NS) |
| Glucagon ($10^{-10}$ M) | 9.26 ± 0.3 | 13.9 ± 1.3 | 2.19 ± 0.25 |
| Glucagon ($10^{-10}$ M) +BAY K 8644 (10 μM) | 9.48 ± 0.5 (NS) | 13.6 ± 1.8 (NS) | 2.11 ± 0.35 (NS) |
| Glucagon ($10^{-8}$ M) | 10.1 ± 0.5 | 16.5 ± 1.2 | 2.37 ± 0.20 |
| Glucagon ($10^{-8}$ M) +BAY K 8644 (10 μM) | 10.1 ± 0.3 (NS) | 16.0 ± 1.0 (NS) | 2.50 ± 0.19 (NS) |

Hepatocytes were incubated in the presence of 10 mM glucose. Glycogen phosphorylase *a* activity and fructose 2,6-bisphosphate levels were measured in samples taken 5 min after saline, BAY K 8644 or glucagon addition. Values are the means of 6 incubations ± SEM. NS: nonsignificant differences between values of incubations in the presence and in the absence of BAY K 8644.

effect of BAY K 8644 on glycolysis by measuring L-lactate production in hepatocytes obtained from 24-hr fasted rats, incubated in the presence of different glucose concentrations. As can be seen in Fig. 1A, the presence of the dihydropyridine derivative did not alter L-lactate production by hepatocytes at any glucose concentration assayed.

In good agreement with these results, the hepatocyte content of fructose 2,6-bisphosphate was not affected by the presence of BAY K 8644 in the incubation medium under any condition assayed (Table 2 and Fig. 1B).

*Effect of BAY K 8644 on insulin, glucagon and somatostatin release by the perfused rat pancreas (Fig. 2)*

As expected, in control perfusions, insulin output did not vary significantly throughout the experimental period. Addition of BAY K 8644 (20 μM) to the perfusate markedly stimulated insulin output ($F = 4.02$, $P < 0.01$). The insulin curve showed a distinct biphasic pattern, with a first peak at 4 min ($14 \pm 1.9$ ng/4 ml/2 min vs $3 \pm 0.4$ ng/4 ml/2 min, mean preinfusion value; $P < 0.001$) and a second peak at 20 min ($11 \pm 1.3$ ng/4 ml/2 min; $P < 0.001$).

BAY K 8644 incorporation into the perfusate failed to significantly modify glucagon ($F = 1.09$) and somatostatin ($F = 1.13$) release.

## DISCUSSION

The foregoing results indicate that BAY K 8644, at concentrations 5–100-fold higher than those capable of stimulating contractility of rabbit aortic strips [1] and catecholamine release by cat chromaffin cells [2], does not modify glycogenolysis or glycogen phosphorylase *a* activity in isolated rat hepatocytes. Gluconeogenesis, L-lactate production, and hepatocyte concentration of fructose 2,6-bisphosphate, a regulatory metabolite that plays an important role in the control of glycolysis and gluconeogenesis in the liver [14], were also unaffected by BAY K 8644. This leads us to propose that the reported hypoglycaemic action of this drug is not mediated by a direct effect on hepatic glucose metabolism.

Hepatic glucose production can be affected by

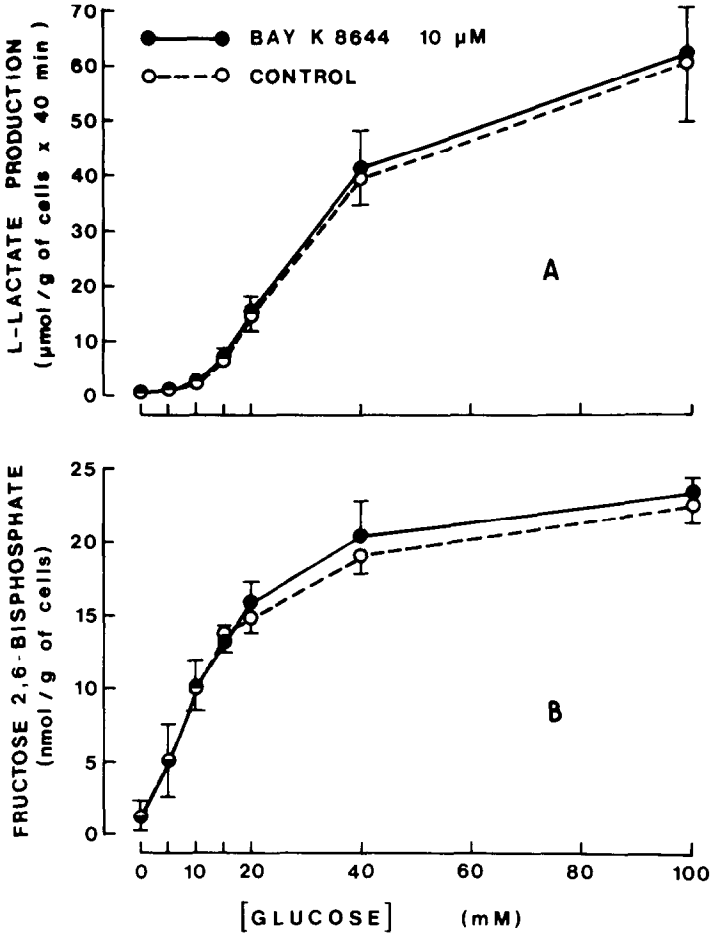


Fig. 1. Effect of BAY K 8644 on L-lactate production (A) and on fructose 2,6-bisphosphate concentration (B) in isolated rat hepatocytes incubated in the presence of different glucose concentrations. Fructose 2,6-bisphosphate was measured in samples taken after 20 min of incubation. The values represent the means of 4 different experiments ± SEM.

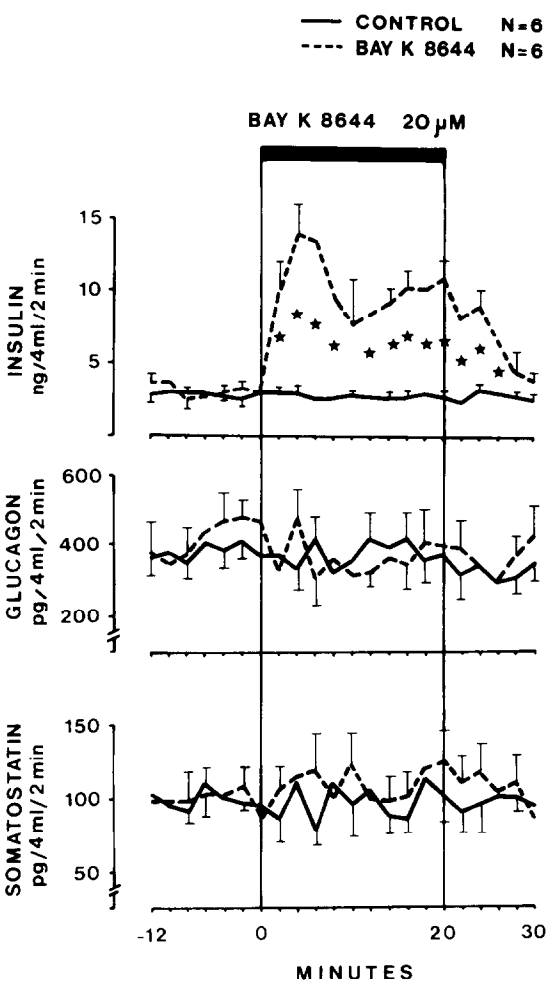


Fig. 2. Effect of BAY K 8644 (20 μM) insulin, glucagon and somatostatin secretion by the perfused rat pancreas. Solid and broken lines correspond to control and BAY K 8644 experiments, respectively (means ± SEM). Asterisks represent statistically significant differences ($P < 0.05$) between BAY K 8644 and control perfusions at a given time.

substances which modify the intracytoplasmic calcium concentration [14]. It has been demonstrated that BAY K 8644 increases $^{45}Ca$ uptake in different types of cells [2, 15], although it remains undetermined whether or not this compound promotes $Ca^{2+}$ uptake in hepatocytes. While studies on the binding of BAY K 8644 to the liver have not been reported, the binding to liver of other dihydropyridine derivatives has been shown to be very low [16, 17]. This is in accordance with the ineffectiveness of BAY K 8644 observed in our preparation of rat hepatocytes.

As for the influence of BAY K 8644 on pancreatic endocrine secretion, our results demonstrate that it displays insulinotropic activity in the perfused rat pancreas. This finding is in agreement with previous observations of Malaisse-Lagae *et al.* [18], who found a stimulatory effect of BAY K 8644 on insulin output in isolated rat pancreatic islets. In our pancreas preparation, BAY K 8644 did not modify glucagon or somatostatin secretion. This would suggest that BAY K 8644 stimulation of insulin release represents a direct effect of this compound on the B cell, not paracrine-mediated through the A or D cells.

To sum up, BAY K 8644 stimulates insulin secretion by the perfused rat pancreas, without modifying glucagon or somatostatin output, while it does not affect glucose production in rat hepatocytes. Thus, although preliminary observations have shown a lack of effect of BAY K 8644 on insulin secretion *in vivo* [3], the possibility that this compound exerts its hypoglycaemic effect by stimulating insulin release should be further investigated.

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