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Effect of a New Hypoglycaemic Agent (HB 699) on the In Vivo Secretion of Pancreatic Hormones in the Dog

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Summary. The effects of HB 699, a non-sulphonyl urea acyl-amino-alcyl benzoic acid derivative, were studied in unanaesthetized dogs. Changes in blood glucose and plasma insulin, glucagon, pancreatic polypeptide and somatostatin were measured after a single intravenous injection. HB 699 caused hypoglycaemia and stimulated insulin secretion in a dose-dependent manner. The effects of HB 699 (40 mg/kg) on pancreatic hormone secretion were compared to those of tolbutamide given at a dose (12 mg/kg) which induced a similar maximal hypoglycaemia. Both drugs caused a similar increase in insulin release (180 ± 32% for tolbutamide and 240 ± 41% for HB 699) lasting for approximately 1 hour. Despite hypoglycaemia, plasma glucagon concentrations were unaltered by either substance. HB 699 caused a marked increase in the secretion of pancreatic polypeptide (220 ± 60% at 30 min) for up to 2 hours, whereas tolbutamide caused no significant change in plasma pancreatic polypeptide levels. In contrast, while tolbutamide caused a significant (45 ± 12%) but short-lived increase in plasma somatostatin concentrations, HB 699 had no significant effect.

Key words: Acyl-amino-alcyl benzoic acid derivative, hypoglycaemic agent, insulin release, pancreatic polypeptide, glucagon, somatostatin, tolbutamide, unanaesthetized dogs.

Certain acyl-amino-alcyl benzoic and phenyl-propionic acid derivatives were previously described as having hypoglycaemic properties [1, 2, 3, 4]. These substances are devoid of the structural requirements regarded as essential for the insulin releasing action of hypoglycaemic sulphonamides, the sulphamido-thiodiazol and sulphonylurea groups. Two compounds in particular have received attention, HB 699 or 4-[2-(5-chloro-2-methoxybenzamido)-ethyl]-benzoic acid [1, 2, 4], and HB 093 or 3-(4-[2-(5-chloro-2-methoxybenzamido)-ethyl]-phenyl)-propionic acid [1]. The HB 699 molecule (Fig. 1) is identical with one part of the glibenclamide molecule, a second generation hypoglycaemic sulphonamide that has been extensively studied.

In vitro studies using the isolated perfused rat pancreas have shown that HB 699 stimulates insulin release without affecting glucagon release in the presence of glucose [4, 5]. In the first part of the present study the effects of HB 699 on blood glucose and plasma insulin concentrations were examined in conscious dogs. In the second part of the study the effects of HB 699 on plasma glucagon, pancreatic polypeptide (PP) and somatostatin concentrations were measured. In all experiments tolbutamide was used as a reference hypoglycaemic agent.

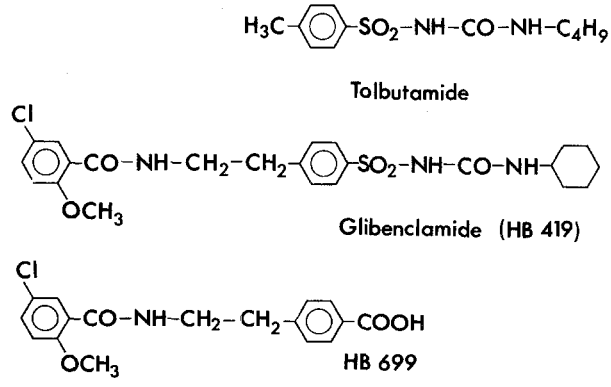

Fig. 1. Structural formulae of tolbutamide, glibenclamide and HB 699

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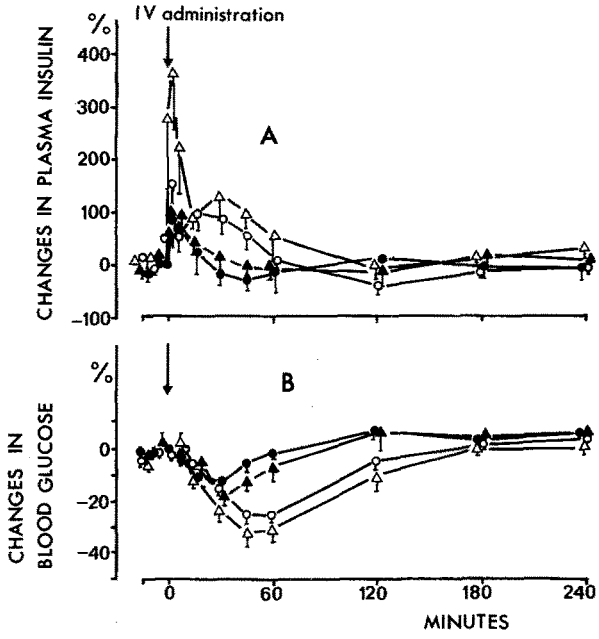

Fig. 2. Changes in plasma insulin (A) and blood glucose (B) levels after different doses of HB 699 administered IV: ●—● 5 mg/kg (n = 5), ▲—▲ 10 mg/kg (n = 5), ○—○ 20 mg/kg (n = 5), Δ—Δ 40 mg/kg (n = 5). The basal absolute values were for blood glucose: 4.03 ± 0.11, 4.2 ± 0.06, 4.2 ± 0.11, 4.31 ± 0.17 mmol/l and for plasma insulin: 1.10 ± 0.22, 1.49 ± 0.3, 1.45 ± 0.24, 1.16 ± 0.23 ng/ml. Results are presented as mean ± SEM

Materials and Methods

Mongrel male dogs weighing 10 to 15 kg were used. Before the experiment the animals had a daily free access to a standard balanced diet (UAR 121, Villemoisson-sur-Orge, France). They were fasted for 18 h before the experiment. Test agents were injected into one jugular vein and blood sampling was carried out from the contralateral jugular vein. Both studies were performed in conscious dogs habituated to the blood sampling technique. In the first study HB 699 was administered IV as a bolus injection at doses of 5, 10, 20 and 40 mg/kg body weight. This substance was dissolved in NaCl solution (0.154 mol/l) using an equimolar amount of 1 mol/l NaOH. Blood glucose and plasma insulin concentrations were measured. In the second study the effects of HB 699 on release of pancreatic hormones were examined and compared with results obtained with tolbutamide at a dose which had a similar maximum hypoglycaemic effect. The animals were given an IV injection of either HB 699 (40 mg/kg) or tolbutamide (12 mg/kg). Control animals were given 0.154 mol/l NaCl solution. Blood sampling times were −15, −5, −1, 1, 3, 5, 15, 30, 45, 60, 120, 180, 240 minutes.

Blood glucose was measured by the potassium ferricyanide method using a Technicon auto-analyser [6]. Plasma insulin was measured by the method of Hales and Randle [7]. Plasma glucagon was measured by the method of Unger et al. [8], using the 30 K antiserum which is relatively specific for pancreatic glucagon. Plasma pancreatic polypeptide (PP) was measured in samples which had been extracted by the Heding technique [9]. The recovery of PP by this extraction procedure ranged from 93 to 98%. The standard was purified natural bovine pancreatic polypeptide (bPP). The same bPP, iodinated by the chloramine-T method [10] and purified using Sephadex QAE A25, was used as tracer. The bPP antiserum and the purified natural bPP were both gifts from Dr. R. E. Chance (Eli Lilly, Indianapolis). Bound and unbound hormone were separated by the dextran-coated charcoal method [11]. The sensitivity of this assay is 2 pg/ml. Plasma immunoreactive somatostatin was measured by a method using the 80 C antiserum of Unger, relatively specific for pancreatic somatostatin. The antiserum was a gift from Dr. R. Unger (Dallas) and the tracer a gift from Clin Midy Laboratories (Montpellier). In the method used (Casellas P, Paul R, Lusseau D, 1980, personal communication) the tracer stability was controlled by measuring the immunoreactivity after incubation with dog plasma in the conditions of the assay. The intra-assay coefficient of variation was 10% and interassay 14.4%. The sensitivity was 10 pg/ml (1 pg/tube) with 95% confidence interval. Plasma dilution curves are parallel to standard curves.

Statistical Analysis

Results were expressed as percent of the basal and as mean ± SEM. The results were submitted to analysis of variance using the multiple comparison test [12].

Results

1. Hypoglycaemic and Insulin Secretory Effects of HB 699 (Fig. 2)

At all doses, HB 699 caused an immediate rise in plasma insulin levels (Fig. 2 A). Plasma insulin levels rose from the first minute after injection, reaching a maximum between the third and fifth minute and returning towards basal values which were reached within 1 to 2 h. The increase in peripheral insulin levels was dose-dependent.

The hypoglycaemic effect (Fig. 2 B) was delayed until the 15 min, and the maximum decrease was reached between 30 and 45 min. The hypoglycaemic effect was also dose-dependent. In all cases blood glucose returned to basal levels within 3 h.

When the dose of HB 699 was expressed logarithmically, significant correlations were found between the dose of HB 699 and both the increase in plasma insulin ($r = 0.6$, $p < 0.01$) and the decrease in blood glucose ($r = 0.7$, $p < 0.001$). For these calculations the total integrated areas of plasma insulin above basal values and of blood glucose below basal values were used.

In order to determine the relative potency of HB 699 in relation to sulphonylureas, we established a dose-response curve. The maximum hypoglycaemic drop obtained with each dose and each animal was plotted as a function of the logarithm of the doses (Fig. 3) and compared with those previously obtained with sulphonylureas [13]. The calculated regression lines did not significantly deviate from parallelism [14]. We established that HB 699 was about three times less potent than tolbutamide. Consequently we

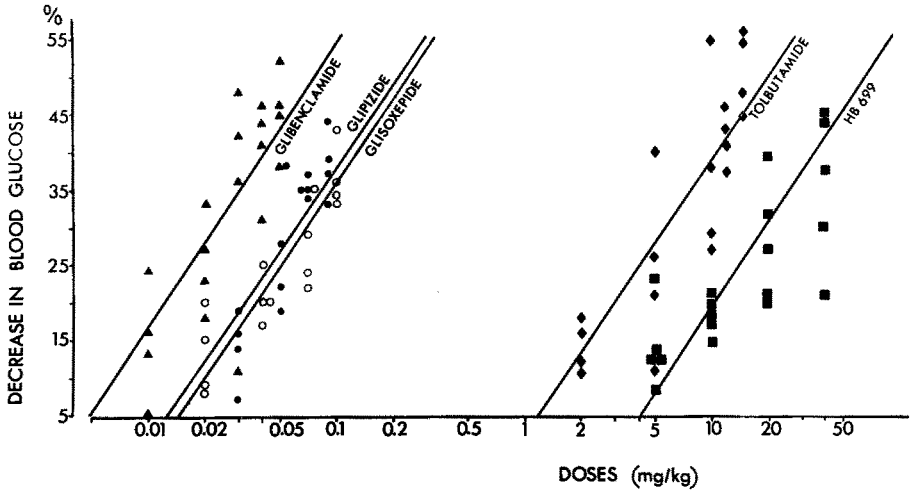

Fig. 3. Comparative study of the hypoglycaemic activity of HB 699 in relation to other sulphonylureas. Each point represents the maximal percentage blood glucose fall obtained in each animal after administration of one dose. (Data for the other sulphonylureas are from [13])

could choose equipotent doses of HB 699 (40 mg/kg) and tolbutamide (12 mg/kg) for further IV studies on blood glucose and pancreatic hormones.

2. Comparison of the Effects of HB 699 and Tolbutamide

Effect on Blood Glucose. Both substances produced a marked decrease in blood glucose levels (Fig. 4). The decreases at the 60 min were 30% for HB 699 (from 4.1 ± 0.1 mmol/l to 2.9 ± 0.06 mmol/l) and 32% for tolbutamide (from 3.9 ± 0.2 mmol/l to 2.7 ± 0.2 mmol/l). With HB 699, the blood glucose levels returned to basal at 3 h. In contrast, tolbutamide-induced hypoglycaemia persisted throughout the 4 h experimental period.

Effect on Plasma Insulin. Both HB 699 and tolbutamide caused immediate increases in plasma insulin concentrations, the maximum level being reached at the third minute (Fig. 4). Those levels were 3.49 ± 0.77 ng/ml (+180%) for tolbutamide and 3.92 ± 0.61 ng/ml (+240%) for HB 699. The basal values were 1.26 ± 0.30 ng/ml and 1.16 ± 0.23 ng/ml respectively. With both substances the first peak of insulin release was followed by a second phase of insulin secretion. The plasma insulin levels during the first and second phases of secretion were similar with tolbutamide and HB 699.

Effect on Plasma Pancreatic Polypeptide (PP) Concentrations. HB 699 caused an immediate increase in plasma PP levels (Fig. 4). From the first minute, the PP level rose to 363 ± 88 pg/ml at 30 min (224% above the basal value of 112 ± 20 pg/ml). The PP level then fell but remained raised about 100% above basal values for 2 h.

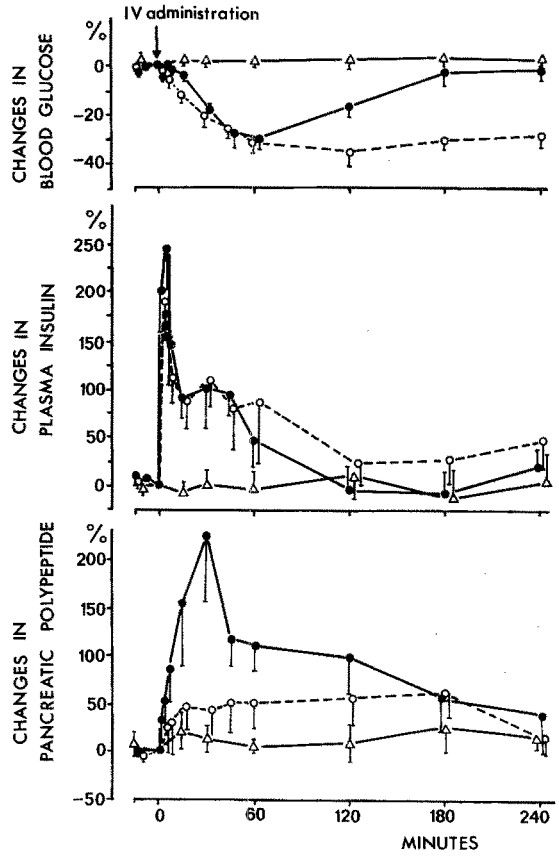

Fig. 4. Changes in blood glucose, plasma insulin and plasma pancreatic polypeptide (PP) levels after IV injection of HB 699 (40 mg/kg) (●—●) (n = 9), tolbutamide (12 mg/kg) (○----○) (n = 6), or 0.154 mol/l saline (controls) (Δ—Δ) (n = 4). Basal values of controls, HB 699 and tolbutamide treated animals were respectively for blood glucose: 3.8 ± 0.2, 4.1 ± 0.1, 3.9 ± 0.2 mmol/l, for plasma insulin: 1.20 ± 0.1, 1.16 ± 0.23, 1.26 ± 0.3 ng/ml and for plasma PP: 87.5 ± 9.0, 112 ± 20, 98 ± 8.5 pg/ml. Results are presented as mean ± SEM

The tolbutamide injection produced a slight and progressive increase in plasma PP levels which reached 127 ± 19 pg/ml at the 90 min (basal value was 98 ± 8.5 pg/ml). This rise was not significant with respect to control values.

Effect on Plasma Glucagon Concentrations. HB 699 and tolbutamide injections did not produce any significant change in the plasma glucagon levels. These ranged from 116 ± 23 pg/ml to 161 ± 71 pg/ml for tolbutamide treated animals and from 96.8 ± 22 pg/ml to 106 ± 29.3 pg/ml for HB 699-treated animals.

Effect on Plasma Somatostatin Concentrations. HB 699 caused no significant change in the concentrations of somatostatin in the peripheral plasma. These ranged from 94 ± 21.5 pg/ml to 110 ± 17.5 pg/ml. Tolbutamide injection induced a definite increase in plasma somatostatin ($p < 0.05$). Basal values of 161 ± 34 pg/ml before tolbutamide reached 228 ± 19 pg/ml at 30 min (45% above basal value). The increase was transient and by 60 min somatostatin levels had returned to basal values.

Discussion

The experiments presented here show that in vivo HB 699, an acyl-amino-alcyl benzoic acid derivative, has insulin releasing and hypoglycaemic properties like sulphonylureas. However, HB 699 is three times less potent (on a weight basis) and has a more transient hypoglycaemic effect than tolbutamide. A direct insulin-releasing action of HB 699 has been shown in isolated rat islets [15]. Furthermore, HB 699 was inactive in streptozotocin diabetic, alloxan diabetic and pancreatectomized animals [1, 2].

HB 699 caused a marked increase in plasma PP levels before any significant change in blood glucose occurred. This suggests that hypoglycaemia could not be responsible for the initial rise in PP levels. It is, however, possible that hypoglycaemia could have contributed to the raised PP levels found later on during the experiment since hypoglycaemia has been reported to enhance plasma PP levels [16, 17]. Under our experimental conditions and at the dose used, tolbutamide did not cause a significant increase in plasma PP levels. In view of this, and since HB 699 and tolbutamide elicited a similar insulin release, the increase of plasma PP levels following HB 699 injection does not seem to be mediated by an effect of insulin on the PP cell.

Glucagon secretion was not affected by HB 699. There are several reports that hypoglycaemic sulphonylureas are without effect on glucagon secretion [18, 19, 20]. The results obtained with tolbutamide in this study are in accord with previous reports.

Finally, while HB 699 failed to cause somatostatin secretion, there was a small but significant increase of somatostatin release following the injection of tolbutamide. The results with tolbutamide are in agreement with previous reports on somatostatin release in response to sulphonylureas [21, 22].

Our results suggest that HB 699 meets the basic requirements for a potentially new class of antidiabetic agent. One particularity of this drug lies in its ability to induce a rapid and considerable increase in PP levels.

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References

1. Geisen K, Hübner M, Hitzel V, Hrstka VE, Pfaff W, Bosies E, Regitz G, Kuhnle HF, Schmidt FH, Weyer R (1978) Acyl-amino-alkylsubstituierte Benzoe und Phenyl-alkansäure mit blut-glucosesenkender Wirkung. Arzneim Forsch 28: 1081–1083
2. Geisen K, Hitzel V, Hrstka VE, Pfaff W, Regitz G, Weyer R (1977) Acylaminoalkyl-Benzoesäuren, eine neue Klasse blutzuckersenkender Substanzen. 12. Kongreß der Deutschen Diabetes Gesellschaft, Homburg/Saar. Novo-Kongreß-Band und Ergänzungsband mit Dia-Reproduktionen, Vortrag Nr 38
3. Kühnle HF, Hrstka VE, Schmidt FH, Bosies F, Heerdt R, Geisen K (1977) Phenylalkancarbonsäuren als blutzuckersenkende Substanzen. 12. Kongreß der Deutschen Diabetes Gesellschaft, Homburg/Saar. Novo-Kongreß-Band und Ergänzungsband mit Dia-Reproduktionen, Vortrag Nr 18
4. Fussgänger RD, Wojcikowski C (1977) Stimulation of insulin secretion of the isolated perfused rat pancreas by a new hypoglycaemic agent, an acyl-amino-alcyl-benzoic acid derivative. Diabetologia 13: 394–395
5. Blayac JP, Loubatières-Mariani MM, Ribes G (1979) Effets in vitro d'un dérivé acyl-amino-alkyl de l'acide benzoïque: le HB 699, sur la sécrétion d'insuline et de glucagon. J Pharmacol (Paris) 10: 229–238
6. Alric R, Mariani MM, Loubatières A (1965) Importance de l'état des éléments figurés du sang et en particulier de celui des globules rouges sur les valeurs du glucose sanguin mesuré par l'auto-analyseur Technicon. Pathol Biol (Paris) 13: 506–511
7. Hales CN, Randle PJ (1963) Immunoassay of insulin with insulin antibody precipitate. Biochem J 88: 137–146
8. Unger RH, Aguilar-Parada E, Müller W, Eisentraut AM (1970) Studies of pancreatic alpha-cell function in normal and diabetic subjects. J Clin Invest 49: 837–848
9. Heding LG (1971) Radioimmunological determination of pancreatic and gut glucagon in plasma. Diabetologia 7: 10–19
10. Greenwood FC, Hunter WM, Glover JS (1963) The preparation of I^{131} labelled human growth hormone of high specific radioactivity. Biochem J 89: 114–123

11. Herbert V, Lau KS, Gottlieb CW, Bleicher SJ (1965) Coated charcoal immunoassay of insulin. J Clin Endocrinol Metab 25: 1375–1384
12. Zar JH (1974) Biostatistical analysis. Prentice-Hall, Englewood Cliffs NJ, p 151
13. Loubatières A, Mariani MM, Ribes G, Alric R (1973) Pharmacological comparison between tolbutamide and two second generation hypoglycemic sulfonylureas (glibenclamide and glisoxepide). Acta Diabetol Lat 10: 261–282
14. Finney DJ (1964) Statistical method in biological assay, 2nd ed. Griffin and Co, London, p 174
15. Glatt M, Schatz H (1979) Studies on the insulinotropic action of a new hypoglycemic agent, an acyl-amino-alcyl-benzoic acid (HB 699). In: Waldhäusl W, Alberti KGMM (eds) 10th Congress of the International Diabetes Federation, Vienna, Austria, Int Congr Series 481. Excerpta Medica, Amsterdam Oxford, p 75
16. Adrian TE, Bloom SR, Besterman AS, Barnes AJ, Cooke TJ, Russel RC, Faber RG (1977) Mechanism of pancreatic polypeptide release in man. Lancet I: 161–163
17. Marco J, Hedo JA, Villanueva ML (1978) Control of pancreatic polypeptide secretion by glucose in man. J Clin Endocrinol Metab 46: 140–145
18. Marco D, Valverde I (1973) Unaltered glucagon secretion after seven days of sulfonylurea administration in normal subjects. Diabetologia 9: 317–319
19. Loubatières A, Loubatières-Mariani MM, Alric R, Ribes G (1974) Tolbutamide and glucagon secretion. Diabetologia 10: 271–276
20. Lecomte MJ, Luyckx AS, Lefevre PJ (1977) Plasma glucagon and clinical control of maturity-onset type diabetes. Effects of diet, placebo and glipizide. Diabète Métab 3: 239–243
21. Ipp E, Dobbs RE, Arimura A, Vale W, Harris V, Unger RH (1977) Release of immunoreactive somatostatin from the pancreas in response to glucose, aminoacids, pancreozymin, cholecystokinin. J Clin Invest 60: 760–765
22. Efendic S, Enzmann F, Nylen A, Uvnäs-Wallensten K, Luft R (1980) Sulphonylurea (glibenclamide) enhances somatostatin and inhibits glucagon release induced by arginine. Acta Physiol Scand 108: 231–233

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