Effect of Sodium 2-Chloropropionate on Glucagon Secretion in the Rat

G. RIBES, G. VALETTE, J. M. DALSTEIN, AND M. M. LOUBATIERES-MARIANI

SUMMARY

We have previously shown that, in alloxan-diabetic dogs, the adjunction of sodium 2-chloropropionate (2-CP) with insulin injections resulted in a reduction of both hyperglycemia and hyperglucagonemia. The present studies were designed to more closely examine the glucagon-lowering effect of 2-CP. We investigated whether 2-CP was able to reduce elevated glucagon secretion both in vivo in streptozocin (STZ)-diabetic rats, and in vitro in the isolated, perfused rat pancreas. 2-CP (1 mmol/kg or 108 mg/kg) was given during 2 mo through esophageal tube to diabetic rats deprived of exogenous insulin. The drug induced a significant reduction of hyperglucagonemia ($P < 0.05$) of blood lactate and alanine levels ($P < 0.02$) and of plasma triglyceride levels ($P < 0.001$). Furthermore, 2-CP markedly decreased glucosuria ($P < 0.005$). In the isolated rat pancreas perfused with 2.8 mmol/L glucose, the continuous perfusion of 2-CP (1 mmol/L) starting before an infusion of arginine or alanine (5 mmol/L) considerably reduced the hypersecretion of glucagon evoked by these amino acids ($P < 0.001$). These experiments show that sodium 2-chloropropionate can reduce glucagon hypersecretion in the diabetic rat not only in vivo, but acts also directly in vitro on the isolated, perfused pancreas of normal rats. DIABETES 1985; 34:536–40.

The stimulatory action of halogenated carboxylic acid derivatives on the pyruvate dehydrogenase complex is well known.[1,2] Among these compounds, dichloroacetate (DCA) and 2-chloropropionate (2-CP) have been extensively studied. These substances, which lower blood lactate level, have been evaluated as therapeutic agents in the experimental treatment of lactic acidosis.[3-6] Moreover, the hypoglycemic effect of DCA has been subsequently demonstrated in starved and diabetic animals of several species.[7-9] However, this substance causes severe side effects,[10-12] particularly because of its catabolization to a toxic metabolite, oxalate,[13,14] which inhibits pyruvate carboxylase.[15,16] In contrast, 2-CP is not catabolized to oxalate.[13,17] We have previously shown that, in alloxan-diabetic dogs, the adjunction of 2-CP with insulin injections resulted in a marked reduction of hyperglycemia, followed by a clear decrease in hyperglucagonemia.[18]

The present work was designed to more closely study this glucagon-lowering effect of 2-CP. We investigated whether this effect could also be found in vivo in STZ-diabetic rats and in vitro on the amino acid-stimulated glucagon secretion from the isolated, perfused rat pancreas.

MATERIALS AND METHODS

In vivo experiments. Male Wistar rats (280–300 g) were randomly divided into three groups. Two groups were rendered diabetic by the intraperitoneal (i.p.) injection of streptozocin (STZ, 66 mg/kg). All the animals were fed the same diet (Extra Labo M25, Sainte Colombe, Provins, France). Glucosuria was regularly measured in the rats given STZ to ascertain their diabetic state. Fifteen days after the onset of diabetes, the rats of one diabetic group received a chronic administration of 2-CP (1 mmol/kg daily or 108 mg/kg). This drug was administered in solution form (1 ml/kg body wt of a solution at 1 mmol/ml) through esophageal intubation. This chronic treatment lasted 2 mo without adjunction of exogenous insulin. At the same time, the two other groups (normal rats and diabetic, nontreated rats) received an equal volume of water through esophageal intubation. Changes in body weight and 24-h glucosuria were checked throughout the experiment. At the end of this chronic treatment, all the rats were killed by decapitation, and the blood collected for metabolic and hormonal assays. Food was withdrawn 4 h before the decapitation and the last esophageal intubation was made 24 h before.

Blood levels of glucose,[19] lactate,[20] alanine,[21] and plasma triglyceride levels[22] were evaluated. Plasma insulin was measured by the radioimmunologic method of Hales and Randle.[23] Pancreatic plasma glucagon was measured by the

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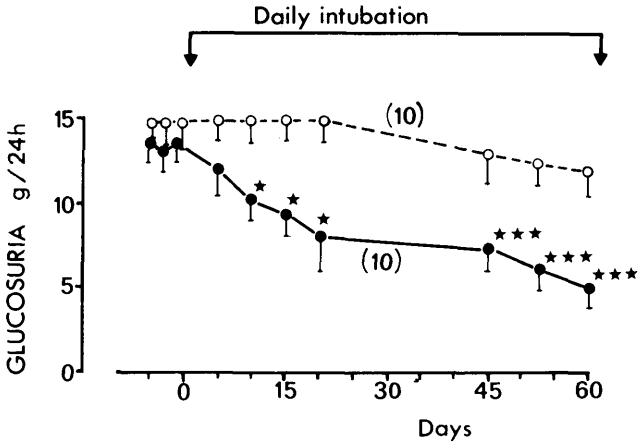

FIGURE 1. Time course of glucosuria in STZ-diabetic rats receiving either water (○----○) or sodium 2-chloropropionate, 1 mmol/kg (●——●), daily for 60 days. For each set of experiments, the number of animals is indicated in parentheses. 2-CP-treated diabetic rats versus control diabetic rats: *P < 0.05, ***P < 0.001.

method of Unger et al.[24] using BR-124 glucagon antiserum from the Institut de Biochimie Clinique, Geneva, Switzerland.[25]

In vitro experiments. *The pancreata were taken from normal* male Wistar rats fed ad libitum and weighing about 350 g. The animals were anesthetized i.p. with sodium pentobarbital (60 mg/kg), and the pancreas isolated and perfused as described previously.[26] In our preparation, the pancreas was isolated from all neighboring tissues and organs, particularly from the spleen, stomach, and duodenum. The perfusion medium was a Krebs-Ringer bicarbonate buffer containing purified bovine albumin (2 g/L). A mixture of oxygen (95%) and carbon dioxide (5%) was bubbled through the medium at atmospheric pressure. The pH of the solution was 7.35. The preparation was maintained at 37.5°C. Each organ was perfused at a constant pressure and the flow rate was about 2.4 ml/min. In all experiments, there was an equilibration period of 30 min. At the end of this period, the first sample for insulin and glucagon assay was collected. A sample taken 15 min later represented the reference sample. Throughout the experiments, glucose concentration in the perfusion medium was 2.8 mmol/L. Hormone output per minute (insulin and glucagon) was determined in the effluent by multiplying the concentration of hormone (ng/ml or pg/ml) by the flow rate (ml/min).

Two experimental protocols were used to test the effect of 2-CP on the hypersecretion of glucagon. 2-CP was perfused either after or before the stimulation of glucagon release. (1) 2-CP (108 mg/L or 1 mmol/L) was perfused 15 min after the increase in glucagon secretion induced by a continuous perfusion of L-arginine (5 mmol/L). The kinetics of glucagon secretion were compared to those obtained either in the presence of arginine alone or in its absence. (2) 2-CP (1 mmol/L) was perfused 15 min before a perfusion of amino acids stimulating glucagon secretion: L-arginine or L-alanine at the concentration of 5 mmol/L. In control experiments, L-arginine or L-alanine were perfused alone at the same concentration.

Drugs. Fluka AG (Buchs, SG, Switzerland) provided 2-chloropropionic acid. It was diluted in distilled water and adjusted to pH 7.0 with NaOH. L-Arginine and L-alanine were obtained from E. Merck, Darmstadt, FRG. Bovine serum albumin was obtained from Sigma Chemical Company, St. Louis, Missouri.

Statistical analysis. Data are expressed as absolute values for in vivo experiments and as percent of reference insulin or glucagon output rates (45 min) for in vitro experiments. Results are given as means ± SEM, and were submitted to analysis of variance using the multiple comparison test.[27]

RESULTS

IN VIVO EXPERIMENTS

Glucosuria progressively decreased in the diabetic rats chronically treated with 2-CP. This reduction became significant ($P < 0.05$) from the tenth day of treatment (Figure 1). Glucosuria decreased from 13.5 ± 1 g/24 h before 2-CP intake to 4.4 ± 1 g/24 h ($P < 0.001$) at the end of treatment.

TABLE 1
Metabolic effects of chronic oral administration of 2-CP to diabetic rats; results are compared with those of control normal rats and control diabetic rats

	Control normal rats (13)	Control diabetic rats (10)	2-CP-treated diabetic rats (10)
Body wt (g)	433.5 ± 17.7	245.0 ± 14.0	249.0 ± 21.0*
Blood lactate (mg/dl)	13.4 ± 0.8	17.1 ± 0.9	13.1 ± 1.2†
Blood alanine (mg/dl)	0.99 ± 0.07	0.91 ± 0.03	0.79 ± 0.03†
Blood glucose (mg/dl)	103.0 ± 2.0	464.0 ± 17.0	495.0 ± 55.0
Glucosuria (g/24 h)	0	12.0 ± 1.0	4.4 ± 1.0‡
Plasma insulin (ng/ml)	6.3 ± 0.8	1.4 ± 0.1	1.75 ± 0.4
Plasma glucagon (pg/ml)	158.7 ± 11.7	341.3 ± 31.4	241.5 ± 39.0*
Plasma triglycerides (g/L)	0.95 ± 0.07	2.50 ± 0.33	1.25 ± 0.22‡

2-CP (1 mmol/kg per os, daily) was administered during 2 mo to STZ-diabetic rats. Values represent means ± SEM. The number of animals is indicated in parentheses.
2-CP-treated diabetic rats versus control diabetic rats: *$P < 0.05$, †$P < 0.02$, and ‡$P < 0.001$.

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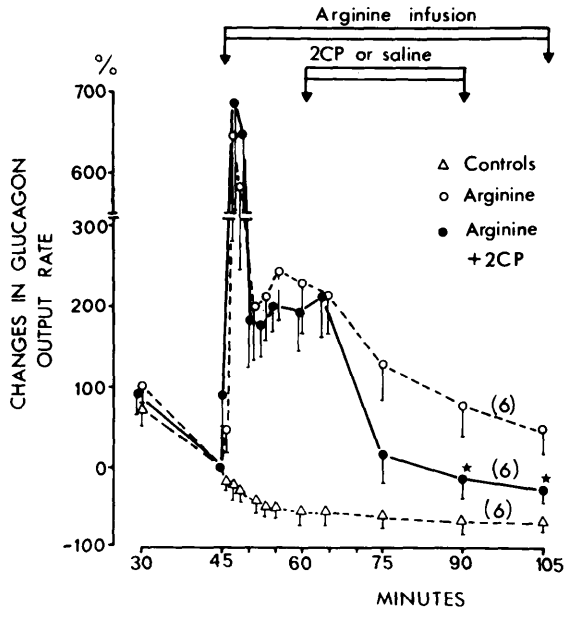

FIGURE 2. In isolated, perfused rat pancreas, effect of the infusion of sodium 2-chloropropionate (2-CP, 1 mmol/L) on glucagon hypersecretion induced by a continuous L-arginine infusion (5 mmol/L). Glucose concentration in the medium was 2.8 mmol/L throughout these experiments. Results are expressed as percent of reference output rate (45 min). Reference output was, respectively, 591 ± 112 pg/min for controls (△----△), 444.5 ± 67 pg/min for experiments with arginine alone (○----○), and 406 ± 28 pg/min for arginine plus 2-CP (●——●). For each set of experiments, the number of pancreata is indicated in parentheses. Arginine + 2-CP versus arginine alone: *P < 0.05.

Table 1 shows the results obtained after 2-mo chronic administration of 2-CP to the diabetic rats. Glucosuria was measured in the 24-h urine before decapitation. The body weight of the diabetic rats was clearly less than that of the normal control rats ($P < 0.001$), but there was no weight difference between the control diabetic and 2-CP-treated diabetic rats. 2-CP significantly reduced ($P < 0.02$) diabetic hyperlactatemia and blood alanine level. The high blood glucose level of the diabetic rats was not diminished by 2-CP treatment; in contrast, the 24-h glucosuria was markedly decreased (4 ± 1 versus 12 ± 1 g) ($P < 0.005$). Plasma insulin levels were very low in the nontreated diabetic rats compared with the normal rats ($P < 0.001$), whereas glucagon levels were significantly higher ($P < 0.001$). The administration of 2-CP to the diabetic rats did not modify circulating insulin levels, but caused a clear reduction of hyperglucagonemia ($P < 0.05$) (241 ± 39 versus 341 ± 31 pg/ml). Plasma triglyceride levels in the nontreated diabetic rats were very high compared with the normal rats ($P < 0.001$). This hypertriglyceridemia was abolished by chronic 2-CP treatment.

IN VITRO EXPERIMENTS

Effects of 2-CP on the hypersecretion of glucagon induced by a perfusion of L-arginine (Figure 2). Arginine elicited a marked increase in glucagon output rate; the response was immediate and biphasic. When 2-CP was perfused 15 min after starting the arginine perfusion, glucagon concentrations returned more rapidly toward the values observed in controls perfused without arginine. Thus, at the end of the infusion of 2-CP (min 30), the glucagon output rates of the pancreata perfused with arginine plus 2-CP were not significantly different from those of the controls. In contrast, when arginine was perfused alone, the glucagon values were still significantly higher ($P < 0.05$) at that time.

Effect of a previous perfusion of 2-CP on the increase in glucagon secretion induced by L-arginine or L-alanine. Insulin output rates were not influenced by the perfusion of amino acids alone or in association with 2-CP (Figures 3 and 4). The perfusion of 2-CP alone did not affect the basal glucagon output rate. In contrast, 2-CP totally supressed the initial peak in glucagon release elicited by L-arginine ($P < 0.001$) (Figure 3). When 2-CP was perfused before L-alanine, there was a clear reduction ($P < 0.001$) of the increase in glucagon output induced by L-alanine (Figure 4).

DISCUSSION

The results obtained in vivo in STZ-diabetic rats, chronically treated with 2-CP, confirm our previous findings in alloxan-diabetic dogs given a combined treatment with 2-CP and insulin.[18] In the diabetic rats, 2-CP caused a marked decrease in glucosuria and a clear reduction of hypergluca-

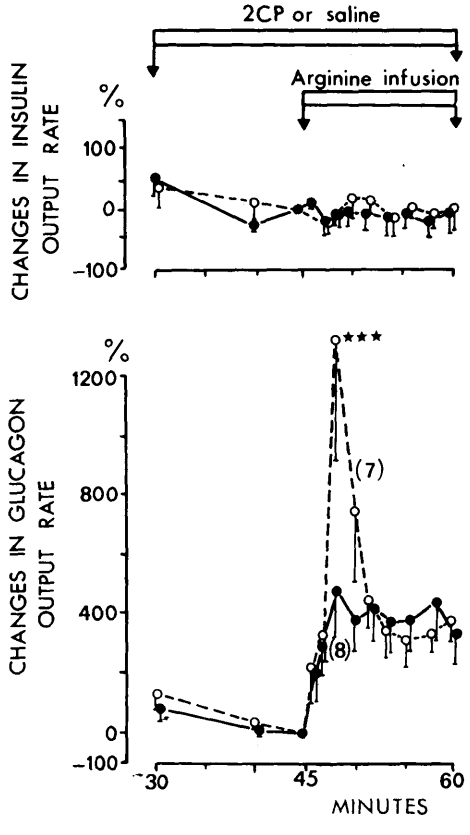

FIGURE 3. In isolated perfused rat pancreas, effects of 2-CP infusion (1 mmol/L) starting 15 min before arginine infusion (5 mmol/L) on insulin and glucagon output rates. Glucose concentration in the medium was 2.8 mmol/L throughout these experiments. Results are expressed as percent of the reference output rate (45 min). Reference output was, respectively, 2.06 ± 0.36 ng/min for insulin and 644 ± 107 pg/min for glucagon in experiments with arginine alone (○----○), and 1.50 ± 0.27 ng/min for insulin and 701 ± 102 pg/min for glucagon in experiments with 2-CP plus arginine (●——●). The number of pancreata used for each set of experiments is indicated in parentheses. 2-CP + arginine versus arginine alone: ***P < 0.001.

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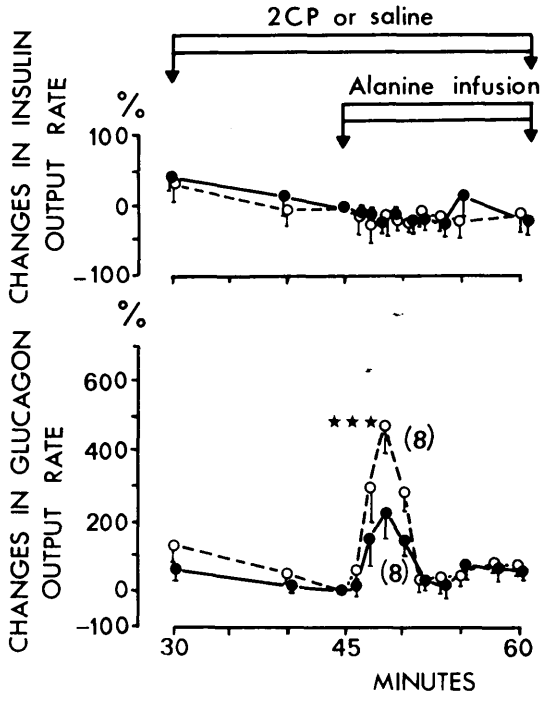

FIGURE 4. In isolated perfused rat pancreas, effects of 2-CP infusion (1 mmol/L) starting 15 min before alanine infusion (5 mmol/L) on insulin and glucagon output rates. Glucose concentration in the medium was 2.8 mmol/L throughout these experiments. Results are expressed as percent of reference output rate (45 min). Reference values were, respectively, 1.78 ± 0.41 ng/min for insulin output and 722 ± 113 pg/min for glucagon output in experiments with alanine alone (○----○), and 2.08 ± 0.54 ng/min for insulin output and 748 ± 110 pg/min for glucagon output in experiments with 2-CP and alanine (●——●). The number of pancreata used for each set of experiments is indicated in parentheses. 2 CP + alanine versus alanine alone: ***P < 0.001.

gonemia. In these experiments, treatment with exogenous insulin was not necessary to obtain these two effects. However, certain points deserve to be discussed. In the diabetic rats, hyperglycemia was not lowered by 2-CP. This may seem surprising, but it should be underlined that blood was sampled during the postprandial period. Nevertheless, the discrepancy between the effects on glucosuria and blood glucose suggests the possibility of a direct action of 2-CP on the renal handling of glucose. Although the diabetic rats did not receive exogenous insulin, a small quantity of immunoreactive insulin was still present in their plasma. This is due to the persistence of a few β-cells in the STZ-diabetic rats, as revealed by immunocytochemical study (personal unpublished data). Chronic treatment with 2-CP did not modify the insulin level; likewise, the weight of the diabetic animals was not affected by 2-CP.

2-CP provoked a decrease in blood lactate and alanine levels, which is in agreement with its previously reported stimulatory effect on the pyruvate dehydrogenase complex. A decrease in blood alanine levels after administration of sodium dichloroacetate, another activator of pyruvate dehydrogenase, has been previously observed.[3,8,28]

An interesting effect of 2-CP is the marked reduction of the elevated plasma triglyceride levels of diabetic rats. These results are in accordance with those of Yount et al.,[29] who showed that the triacylglycerol levels of 2-CP-treated normal rats, but not of dichloroacetate-treated normal rats, were significantly less than controls. These authors found that free glycerol was not significantly different from controls in either the dichloroacetate- or 2-chloropropionate-treated groups. The mechanism by which 2-CP lowers plasma triglyceride levels is not known.

In the diabetic rats, hyperglucagonemia was clearly reduced by 2-CP. This effect cannot be due to improvement in insulin levels, since they remain low in treated animals. Our in vitro experiments show that 2-CP can act directly on rat pancreas and reduce the increase in glucagon release induced either by arginine or alanine; in this case, this effect is also not concomitant with any change in insulin output. The mechanism by which 2-CP reduces the hypersecretion of pancreatic glucagon is still unexplained. A delay of about 15 min seems necessary for this effect to occur in vitro. It has been shown in the isolated, perfused rat pancreas that the hypersecretion of glucagon induced by amino acids is more pronounced as the glucose concentration in the perfusion medium is lowered.[30,31] Because of its stimulatory effect on pyruvate dehydrogenase, 2-CP increases the utilization of energetic substrates supplied to the A-cell, especially the utilization of glucose. Thus, under our experimental conditions, this cell might become less sensitive to the stimulation induced by amino acids. In the same way, the reduction of hyperglucagonemia in diabetic rats might be the consequence of the 2-CP-induced increase in the metabolization of energetic substrates by A-cells.

The lowering effects of 2-CP, both on glucagon and triglyceride levels, do not seem to be related. In fact, our experiments in vitro show that 2-CP reduces glucagon hypersecretion through a direct action on the isolated pancreas. Furthermore, several authors have shown that an increase in circulating glucagon levels induces a reduction in triglyceride levels in vivo.[32-34]

In conclusion, these experiments show that sodium 2-chloropropionate has the property of reducing glucagon hypersecretion in the rat, not only in vivo in diabetic animals, but also directly in vitro on the isolated, perfused normal rat pancreas.

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