# Effects of Chronic Administration of Sodium 2 Chloropropionate in Normal and Diabetic Dogs

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

**SUMMARY**

**Pancreatic hormonal and metabolic responses to chronic administration of sodium 2 chloropropionate (2 CP) were investigated in conscious dogs. We subcutaneously administered 2 CP daily for 7 days at the dose of 0.58 mmol/kg (62.5 mg/kg) in normal dogs and those rendered diabetic by injection of alloxan (0.24 mmol/kg, i.v.).**

**In the normal dogs, the chronic administration of 2 CP provoked a decrease in blood lactate and pyruvate but not in blood glucose concentrations. Urinary oxalate was not increased by the daily injection of 2 CP. Blood ketone body concentrations progressively increased after the third day of treatment. At the same time, plasma cholesterol slowly decreased. The 2 CP chronic administration did not change the plasma somatostatin, glucagon, and insulin levels.**

**In the alloxan-diabetic dogs, treated with insulin alone, blood glucose, ketone body concentrations, and plasma somatostatin and glucagon levels were elevated. The adjunction of 2 CP with insulin injections resulted in a fall in blood lactate and pyruvate levels and a progressive decrease of blood glucose concentrations. Blood ketone bodies, which were already high at the start, were not affected when 2 CP was combined with insulin. The hypersomatostatinemia was not decreased, whereas the hyperglucagonemia was considerably reduced. So, I/G ratio, which was strongly decreased with insulin alone, progressively returned to normal values. As to urinary compounds, 2 CP induced a marked decrease in glucosuria and did not change the elevated urinary $\beta$ hydroxybutyrate levels.**

**In conclusion, these findings show that the adjunction of sodium 2 chloropropionate to insulin in diabetic dogs results in a reduction of hyperglycemia and hyperglucagonemia. DIABETES *31*:484–488, June 1982.**

From the Laboratoire de Pharmacologie et de Pharmacodynamie, Equipe de Recherche Associée au CNRS N° 786, Institut de Biologie, Bd Henri IV, 34060 Montpellier Cedex, France.
Address reprint requests to Professor M. M. Loubatieres-Mariani at the above address.
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Among the activators of pyruvate dehydrogenase, halogenated monocarboxilic acid derivatives, sodium dichloroacetate (DCA) has been the most studied as a substance that could be used in the treatment of hyperlactatemia and lactic acidosis[1–4] or diabetes mellitus.[5–7] However, it is now known that DCA induces many untowards effects,[8] such as increase in ketone bodies[2,7] and peripheral polyneuropathy,[9] as well as its transformation into a toxic metabolite, oxalate.[10,11] Therefore, the effect of another activator of pyruvate dehydrogenase,[12] sodium 2 chloropropionate or 2 CP, was investigated in acute experiments performed either in normal rats[11,13] or in normal dogs.[14] These studies have shown that 2 CP had a hypolactatemic effect similar to that of DCA.

The aim of this study was to investigate the effects of the chronic administration of 2 CP on carbohydrate and lipid metabolism, as well as on the circulating levels of pancreatic peptide hormones in normal and diabetic dogs.

## MATERIALS AND METHODS

### GENERAL EXPERIMENTAL CONDITIONS

Our experiments were carried out in conscious dogs.

**Normal mongrel dogs** weighing 12–16 kg were kept in cages 2 wk before and during the experiment. They were fed daily with a standard usual diet (U.A.R., 121, Villemoisson-sur-Orge, France).

**Diabetic dogs.** Diabetes was produced in three mongrel dogs weighing 16–23 kg by an i.v. injection of alloxan tetrahydrate at dose of 0.24 mmol/kg (50 mg/kg). At the time of experiment, the animals were diabetic for at least 45 days and were kept in cages. A constant diet was maintained throughout the experiment (30 g meat per kilogram per day). Diabetic dogs received two subcutaneous injections of insulin per day (Endopancrine, Organon). Two dogs (A and B) received 6 IU insulin in the morning and 6 IU in the afternoon. Dog C received 4 IU insulin in the morning and 4 IU in the afternoon. The doses were chosen so that the animals displayed clear glucosuria and were mildly ketonuric.

All animals (normal and diabetic) were given 2 CP daily for 7 days. Normal dogs received a s.c. injection of 0.58 mmol/kg (62.5 mg/kg) per day with the morning meal. Diabetic dogs received subcutaneous insulin at the same doses as the preceding days and 2 CP at a dose of 0.58 mmol/kg daily with the morning meal.

METHODS

Blood was sampled from the jugular vein. Fasting venous blood was taken every morning, i.e., 18 h after the last meal.

**Measurement of carbohydrate and lipid metabolites.** Blood glucose values were recorded with a Technicon Auto-analyzer by use of the potassium ferricyanide procedure for hemolyzed blood.[15] Blood lactate and pyruvate levels were determined in whole blood according to the enzymatic methods of Hohorst[16] and Czock and Lamprecht.[17] Plasma cholesterol was evaluated using the enzymatic method of Watson.[18] The determinations of ketone bodies in whole blood were made according to enzymatic methods.[19,20] As for urinary compounds, oxalate concentrations were evaluated using the colorimetric procedure of Hodgkinson and Williams.[21] Glucosuria and urinary $\beta$ hydroxybutyrate were also assayed.[19]

**Measurement of pancreatic hormones.** Plasma insulin was measured by the method of Hales and Randle.[22] Plasma glucagon was measured by the method of Unger et al.[23] using the 30 K antiserum which is relatively specific for pancreatic glucagon. Plasma somatostatin was evaluated by a method using 80 C antiserum, a gift from Dr. R. Unger (Dallas, Texas), and the tracer, a gift from Clin Midy Laboratories (Montpellier, France). The procedure has been previously described.[24]

**Source of 2 chloropropionate.** Fluka AG (Buchs SG., Switzerland) provided 2 chloropropionic acid. It was diluted in distilled water at 6% and adjusted to pH 7.0 with NaOH.

**Statistical analysis.** The results were submitted to analysis of variance and the multiple comparison test of Student, Newman, and Keuls was used.[25]

## RESULTS

**Normal dogs.** The chronic administration of 2 CP provoked a rapid fall in blood lactate and pyruvate levels (Figure 1A). Lactatemia and pyruvicemia were clearly decreased 24 h after the first administration (from 1.05 ± 0.09 mmol/L to 0.78 ± 0.07 mmol/L and from 0.05 ± 0.005 mmol/L to 0.035 ± 0.006 mmol/L, respectively). Then these significant falls persisted for the whole period of administration and for 3 days after treatment ($P < 0.025$). Blood glucose levels were not modified by the daily injection of 2 CP. As for plasma cholesterol (Figure 1B), the administration of 2 CP induced a progressive decrease, which was significant on the 7th day ($P < 0.05$). The administration of 2 CP altered the blood $\beta$ hydroxybutyrate and acetoacetate concentrations (Figure 1B). The drug induced a progressive increase of $\beta$ hydroxybutyrate levels, which reached 0.119 ± 0.032 mmol/L the 7th day of treatment ($P < 0.05$). (Basal values were 0.035 ± 0.008 mmol/L.) The augmentation of blood acetoacetate was significant only the last day of chronic administration of 2 CP ($P < 0.01$). Urinary oxalate was not increased by the daily administration of 2 CP (Table 1). Plasma somatostatin, glucagon, and insulin levels of the normal dogs were not significantly modified by the chronic administration of 2 CP (Table 1). Thus, the I/G ratio did not significantly change (Figure 4).

**Diabetic dogs.** With insulin treatment alone, blood lactate and pyruvate levels were slightly but not significantly elevated, whereas blood glucose levels were high (Figure 2A). The addition of 2 CP resulted in a rapid and clear fall in blood lactate and pyruvate concentrations. The addition of 2 CP also induced a progressive and significant reduction of the elevated blood glucose levels (from 16.98 ± 1.44 mmol/L to 9.10 ± 1.28 mmol/L the 7th day) ($P < 0.05$) (Figure 2A). Before and during 2 CP administration, glycemia was determined every afternoon just before the second daily injection of insulin. After 4 days of 2 CP administration, the afternoon glycemia, which was 10.05 ± 0.22 mmol/L before the treatment, dropped to 5.49 ± 0.39 mmol/L. We

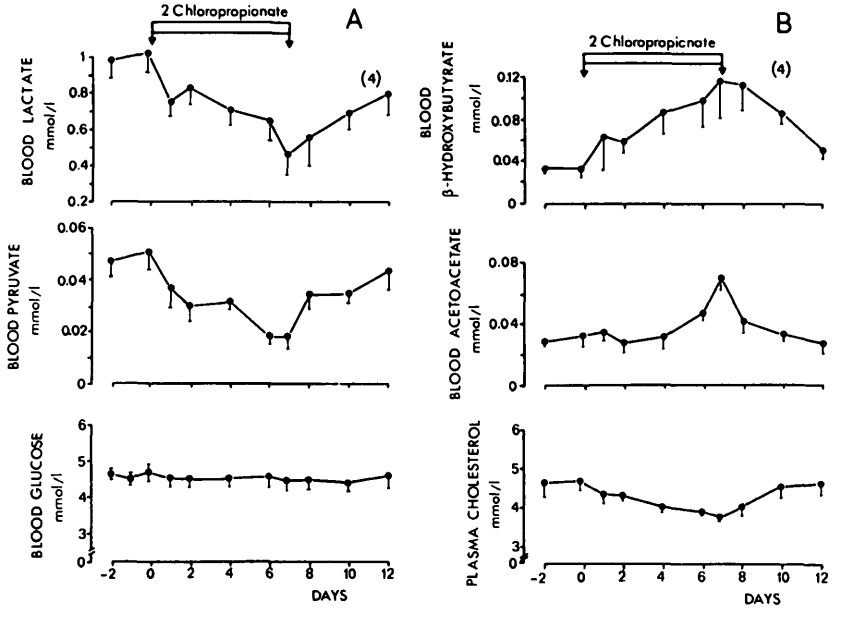


**FIGURE 1. Effects of chronic administration of 2 CP (0.58 mmol/kg daily for 7 days) in normal dogs on (A) blood lactate, pyruvate, and glucose concentrations and (B) blood acetoacetate and $\beta$ hydroxybutyrate concentrations and plasma cholesterol.**

TABLE 1
Effects of chronic administration of 2 CP (0.58 mmol/kg daily for 7 days) on urinary oxalate and plasma somatostatin, glucagon, and insulin levels in normal dogs (N = 4)

| | Days | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | 2 CP treatment | | | | | | | | | |
| | −4 | −3 | −2 | −1 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 10 | 12 |
| Plasma somatostatin (pg/ml) | 141 ±28 | | 120 ±25 | 129 ±22 | 112 ±16 | 146 ±27 | | 123 ±26 | | 143 ±25 | 130 ±21 | 123 ±31 | 132 ±17 | 141 ±32 |
| Plasma glucagon (pg/ml) | 76.5 ±11.5 | | 63.7 ±8.5 | 57.7 ±10 | 62 ±3.4 | 64 ±11 | | 53.5 ±6 | | 63 ±5 | 62.5 ±1.5 | 69 ±2.1 | 81.2 ±10.7 | 77.7 ±10.7 |
| Plasma insulin (ng/ml) | 0.65 ±0.07 | | 0.78 ±0.15 | 0.85 ±0.10 | 0.69 ±0.22 | 1.15 ±0.39 | | 1.10 ±0.30 | | 1.33 ±0.45 | 1 ±0.16 | 0.87 ±0.12 | 0.74 ±0.14 | 1.05 0.37 |
| Urinary oxalate (mmol/24 h) | 0.47 ±0.08 | 0.44 ±0.15 | 0.81 ±0.30 | 0.78 ±0.23 | | | 0.54 ±0.08 | 0.66 ±0.18 | | 0.65 ±0.16 | 0.77 ±0.20 | 0.94 ±0.43 | 0.87 ±0.19 | 0.52 ±0.27 |

therefore omitted the daily second injection of insulin on days 4, 5, 6, 7, and 8 for dogs A and B, and on days 4, 5, 6, and 7 for dog C.

When treated with insulin alone, the diabetic dogs had high blood ketone body levels ($\beta$ hydroxybutyrate and acetoacetate) (Figure 2B). The levels of these compounds were not significantly modified when 2 CP was added to insulin. As for plasma cholesterol, 2 CP did not significantly decrease the level of this metabolite, which was elevated with insulin alone (Figure 2B).

Figure 3 shows the effects of 2 CP on plasma somatostatin, glucagon, and insulin levels. When the animals received insulin alone, somatostatin and glucagon levels were significantly elevated ($P < 0.025$) : 207 ± 17 pg/ml and 116 ± 19 pg/ml, respectively, on day 0. Basal values of plasma insulin levels were diminished. The combined treatment with 2 CP and insulin caused a progressive and significant reduction in plasma glucagon levels ($P < 0.025$) which reached normal basal values at the end of treatment (53 ± 9 pg/ml). After the drug was discontinued, glucagon levels rapidly increased. Plasma insulin and somatostatin levels were not modified by the addition of 2 CP. I/G ratio was significantly decreased in diabetic animals when compared with normal dogs (Figure 4). When 2 CP was added, I/G ratio clearly increased close to normal levels.

As for urinary compounds, the combination of 2 CP with insulin induced a rapid and marked reduction of glucosuria (Table 2), but did not significantly change the elevated urinary $\beta$ hydroxybutyrate levels.

## DISCUSSION

These experiments show that the chronic administration of 2 CP lowers blood lactate and pyruvate levels both in normal and diabetic dogs. These results confirm previous studies in normal rats[13] and in normal dogs.[14]

It must be pointed out that the chronic administration of 2

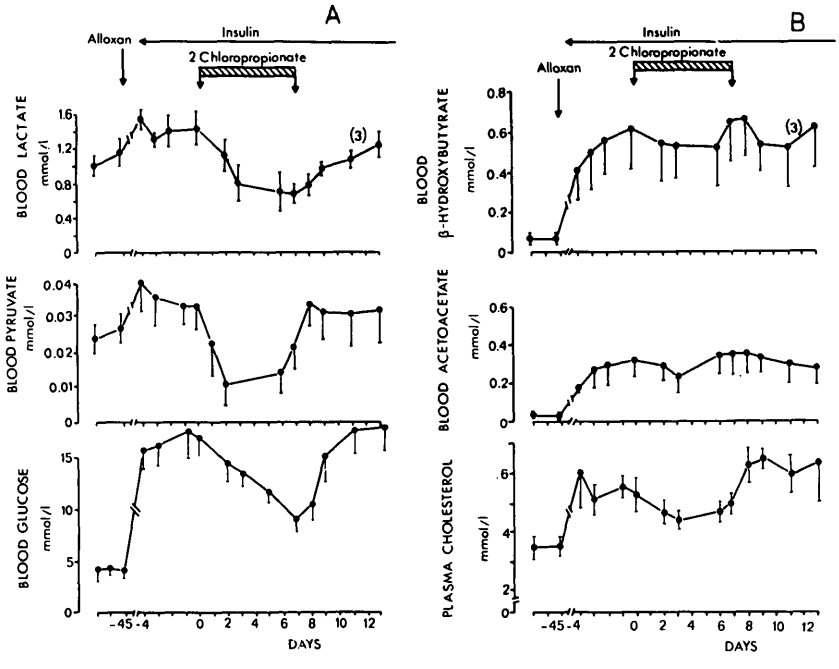


**FIGURE 2. In diabetic dogs, effects of chronic administration of 2 CP (0.58 mmol/kg daily for 7 days) added to insulin treatment on (A) blood lactate, pyruvate, and glucose concentrations and (B) blood acetoacetate and $\beta$ hydroxybutyrate concentrations and plasma cholesterol.**

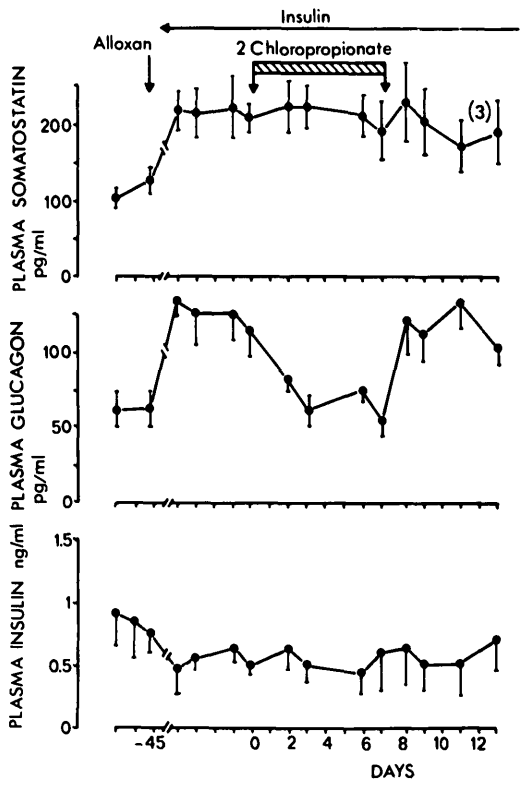


**FIGURE 3. In diabetic dogs, effects of chronic administration of 2 CP (0.58 mmol/kg daily for 7 days) added to insulin treatment on plasma somatostatin, glucagon, and insulin levels.**

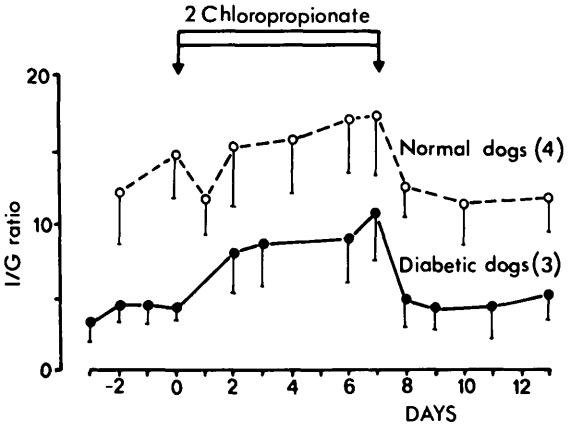


**FIGURE 4. Effect of chronic administration of 2 CP (0.58 mmol/kg daily for 7 days) on I/G ratio in normal and diabetic dogs.**

CP to the normal animals did not induce any fall in blood glucose level, contrary to what occured with DCA.[7] This compound is known to turn into a toxic metabolite, oxalate,[10,26] which inhibits pyruvate carboxylase,[27,28] this inhibition having a part in the blood glucose-lowering effect of DCA.[11] In our experiments, the urinary level of oxalate was not increased after 2 CP. This might partly explain why 2 CP did not cause any decrease in blood glucose level in normal dogs. On the contrary, in diabetic dogs, 2 CP induced a progressive and clear reduction of hyperglycemia. This phenomenon is reversible after stopping 2 CP.

In normal dogs, 2 CP induced a progressive increase of blood ketone bodies, especially β hydroxybutyrate levels. However, in diabetic dogs, the same daily dose of 2 CP did not significantly modify the high β hydroxybutyrate levels in blood and urine. In contrast, DCA at an equimolar dose (0.58 mmol/kg or 75 mg/kg) triggered a marked increase in urinary ketone bodies.[7] From these results, it follows that 2 CP is less ketogenic than DCA. In normal dogs plasma cholesterol levels are significantly reduced after 7 days of 2 CP administration. We have obtained a negative correlation between this reduction and the elevation of blood acetoacetate levels ($r = -0.67$, $P < 0.01$).

As to plasma somatostatin levels, our experiments show that alloxan-diabetic dogs have significant hypersomatostatinemia. These results are in agreement with previous reports.[29] The chronic administration of 2 CP did not change the somatostatin levels both in normal and diabetic dogs.

In normal dogs, 2 CP like DCA[30] did not modify plasma glucagon levels. Yet, the most important finding is the progressive decrease of the elevated plasma glucagon levels obtained in diabetic dogs when 2 CP was added to insulin. Thus at the end of this association, glucagon levels returned to normal values. This effect could explain the progressive reduction of hyperglycemia. So, in our experiments, I/G ratio, which is strongly decreased with insulin alone, progressively increases when 2 CP is added. On the 7th day, I/G ratio returns to normal values. This effect appears independent of plasma somatostatin levels.

In conclusion, the adjunction of 2 CP to insulin in diabetic dogs results in a reduction of hyperglycemia and hyperglucagonemia. This fact remains to be explained.

TABLE 2
Effects of chronic administration of 2 CP (0.58 mmol/kg daily for 7 days) added to insulin treatment on glucosuria and urinary β hydroxybutyrate in diabetic dogs (N = 3)

| | Days | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | 2 CP treatment | | | | | | | | | | |
| | −4 | −3 | −2 | −1 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 12 |
| Glucosuria (mmol/24 h) | 283 ±27 | 280 ±42 | 279 ±45 | 333 ±66 | 116 ±41 | 105 ±11 | 64 ±16 | 122 ±16 | 86 ±42 | 103 ±32 | 77 ±30 | 75 ±48 | 158 ±21 | | 251 ±13 |
| Urinary β hydroxybutyrate (mmol/24 h) | | 3.46 ±0.86 | 4.43 ±1.08 | 4.38 ±0.57 | | 3.03 ±0.88 | 3.07 ±0.38 | | | 3.14 ±0.79 | 2.92 ±1.08 | 2.84 ±0.94 | 3.52 ±1.05 | | 3.26 ±0.85 |

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