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EFFECT OF CARBENOXOLONE ON GLUCOSE METABOLISM IN RAT ADIPOSE TISSUE

JOSÉ A. GOMEZ-CAPILLA,* CAROLINA GUTIERREZ and JOSÉ M. FERNÁNDEZ-FERNÁNDEZ

Departamento de Bioquimica, Facultad de Medicina, Universidad de Granada, Granada, 18071 Spain

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Abstract—Carbenoxolone significantly decreased the glucose uptake and the incorporation of glucose into triglycerides and CO_2 in rat epididymal fat pads. The effect produced by insulin on these metabolic pathways was reduced when adipose tissue was incubated with insulin in the presence of carbenoxolone (10^{-3} M). On the other hand the drug (10^{-3} M) produced a decrease in cyclic AMP concentration in adipose tissue similar to that produced by insulin (100 ng/ml).

Carbenoxolone is a semi-synthetic triterpenoid used in the treatment of gastric and duodenal ulcer, with an effectiveness similar to cimetidine [1, 2].

The mode of action is uncertain although it has been shown that this drug contributes to an improved barrier function of the gastric mucosa by stimulating the production of gastric mucus [3, 4].

More recently it has been published that carbenoxolone (10^{-3} M) has inhibitory effects on the catecholamine stimulated lipolysis in adipose tissue of normal rats [5], in adipose tissue of rats in different states of insulin resistance [6], in chicken adipose tissue [7] and in normal or insulin-resistant human adipose tissue (unpublished results).

Carbenoxolone has, in this respect, some insulin-like effects, and it seems that it inhibits lipolysis by altering the rate of cyclic AMP production [5, 8] in the same way as insulin does, although the hormone effect is mediated by a more complex membrane system [9]. In accordance with the above considerations it would be interesting to know if carbenoxolone modify *in vitro* other metabolic pathways such as glucose uptake or CO_2 production and triglyceride synthesis from glucose in adipose tissue, well-known to be modified by insulin.

MATERIAL AND METHODS

Chemicals. Crystallin beef insulin was obtained from Burroughs Wellcome Co. (23.9 UI mg^{-1}). Glucose-U-^{14}C and cyclic AMP kit from The Radiochemical Centre, Amersham, U.K. Carbenoxolone sodium was a gift from Laboratorios Leo S.A. Madrid, Spain. Other chemicals were obtained from commercial sources.

Animals. Male Wistar rats, 180–220 g weight, were obtained from The Animals Service of the University of Granada. They were housed in quarters at 20–25° and 12 hr of photoperiod. Before use they had free access to food and water.

Metabolic studies. The rats were decapitated and fat was obtained from the distal segment of epididymal fat pads, cut in small pieces (100–200 mg) and incubated for 1 hr at 37° in 1 ml of Krebs–Ringer bicarbonate (KRB) buffer pH = 7.4 supplemented with albumin at a concentration of 2%. The stock solution of bicarbonate used to make the Krebs–Ringer was previously gassed with O_2 at 95% and CO_2 at 5%. All the receptacles that contained KRB were kept in an atmosphere of this gas. At the end of the incubation, the glucose uptake was measured as described previously [10]. ^{14}C-carbon dioxide production from U-^{14}C-glucose by Goodridge's [11] method. The U-^{14}C-glucose incorporation into triglycerides was measured as described previously [12]. For cyclic AMP analysis, samples were extracted with 2 ml of a solution of cold 10% (w/v) trichloroacetic acid, centrifuged (3000 *g*) at 4° for 15 min and the supernatant was extracted five times with 5 ml of diethyl ether.

A 50 μl aliquot was used for cyclic AMP analysis, using a commercial kit from The Radiochemical Centre, Amersham. The radioactive samples were counted in a LKB Wallac scintillation spectrometer.

Statistical analysis. Significance was determined by an ANOVA-2 test.

RESULTS

Insulin produced a 167% increase of the lipogenesis, although when the hormone was in the presence of carbenoxolone (10^{-3} M), the lipogenesis increased only 88% compared with the control results. On the other hand, carbenoxolone alone had an inhibitory effect (43%) on the lipogenesis in adipose tissue, which is consistent with the observed inhibition of the insulin effects when the hormone was in the presence of carbenoxolone (Table 1).

One of the clearest effects of insulin on glucose metabolism in adipose tissue is the stimulation of the sugar oxidation to CO_2. The studies of this pathway show (Table 1) that the effect produced by insulin (84% of increased) was reduced when adipose tissue was incubated with insulin in the presence of car-

* To whom correspondence should be addressed.

Table 1. Effect of carbenoxolone (CBX) (10^{-3} M) and insulin (100 ng/ml) on the glucose uptake and on the incorporation of glucose-U-^{14}C into triglycerides and CO_2 in rat adipose tissue

Treatment	Triglycerides (μmol glucose/g wet tissue)	CO_2 (μmol glucose/g wet tissue)	Glucose uptake (μmol glucose/g wet tissue)
Control	5.32 ± 0.83	3.70 ± 1.02	11.16 ± 1.78
Insulin	14.22 ± 1.72*	6.79 ± 1.79*	34.00 ± 2.78*
Insulin + CBX	10.02 ± 1.33*	5.65 ± 1.70*	27.94 ± 3.16*
CBX	3.05 ± 0.62*	2.51 ± 1.01*	6.72 ± 1.22*

The incubation medium was Krebs–Ringer bicarbonate, 2% albumin, with 0.4 μCi/ml of glucose-U-^{14}C and glucose at a concentration of 5.5 mM. Values are mean ± SEM of six quadruplicate experiments.
* $P < 0.05$ when compared to control.

benoxolone (10^{-3} M). In this case, the increase in the production of CO_2 from glucose was only 53%. Carbenoxolone alone (10^{-3} M) inhibited the CO_2 production (32%).

Similar results were observed when the glucose uptake was studied. The increased glucose uptake produced by insulin (205%) was inhibited by carbenoxolone (150%) and carbenoxolone alone (10^{-3} M) had an inhibitory effect on the glucose uptake (40%) (Table 1).

Furthermore, carbenoxolone produced a decrease in cyclic AMP similar to that produced by insulin (Table 2).

DISCUSSION

Our results show for the first time that carbenoxolone inhibits glucose metabolism in adipose tissue and has an opposite effect from that of insulin.

Following the initial observations that insulin stimulates the metabolism of glucose in a variety of peripheral tissues [13], it was soon recognized that this stimulatory effect was produced at the level of glucose uptake [14, 15] (for a review see Simpson and Cushman [16]).

Thus, the inhibition of glucose uptake produced by carbenoxolone (Table 1) can, in part, justify the observed inhibition by carbenoxolone of the glucose incorporation into triglycerides and CO_2 in rat adipose tissue.

As previously mentioned, carbenoxolone has a clear antilipolytic effect in adipose tissue similar to that of insulin [6, 7] and it has been suggested that the drug exerts its antilipolytic activity by decreasing cyclic AMP concentration [5]. Our results confirm this suggestion showing that carbenoxolone decreases cyclic AMP concentration in adipose tissue in a similar manner to that of insulin (Table 2). Although carbenoxolone decreases cAMP as well as insulin, the drug has an opposite effect from that of the hormone (Table 1) on glucose uptake in adipose tissue and, in turn, on glucose intracellular incorporation into triglycerides and CO_2.

Table 2. Effect of carbenoxolone (CBX) (10^{-3} M) and insulin (100 ng/ml) on intracellular levels of cyclic AMP in rat adipose tissue

Treatment	pmol cAMP/g wet tissue	Decrease (%) vs control
Control	72.35 ± 14.28	
Insulin	21.07 ± 5.03*	−71
Insulin + CBX	9.14 ± 2.70*	−87
CBX	28.30 ± 6.80*	−61

The incubation medium was Krebs–Ringer bicarbonate, 2% albumin, with glucose at a concentration of 5.5 mM. Values are mean ± SEM of four quadruplicate experiments.
* $P < 0.05$ when compared to control.

The concept that the decrease in intracellular cAMP levels mediates most actions of insulin has not been demonstrated [17]; however, there are several instances in which the action of insulin can be dissociated from its ability to decrease cAMP concentration [18–21]. A decrease in the intracellular concentration of cyclic AMP by insulin under certain conditions may explain some, but not all, of the effects of insulin.

With respect to activation of glucose transport by insulin, it has been published that it is clearly mediated independently of alterations in the intracellular concentration of cyclic AMP [22, 23]. Since carbenoxolone decreases cAMP concentration (similar to insulin) and simultaneously inhibits glucose uptake (contrary to insulin), this suggests that the effect of insulin stimulating glucose uptake is mediated by an additional or cAMP-independent change. In this way our results supply (by a different approach) new data that confirm the existence of a divorce between cAMP and glucose uptake in adipose tissue and are consistent with the above considerations.

From our results, however, we cannot establish the mechanism by which carbenoxolone (10^{-3} M) inhibits *in vitro* the uptake of glucose. Nevertheless, the results obtained agree with previously published data and thereby suggest a possible mechanism of action.

It is known that prostaglandins are metabolized by 15-hydroxyprostaglandin dehydrogenase [24] and that carbenoxolone inhibits this enzyme in guinea pig lungs [25]. Carbenoxolone concentrations higher than 0.5 mM also stimulates prostaglandin synthase in rabbit kidney medulla [8]. On the other hand, it has been reported that prostaglandin E_1 inhibits the transport of hexoses in chinese hamster ovary cells [25]. Therefore, if the mode of action of carbenoxolone were the same in adipose tissue, then it would be possible that this antiulcer drug could be effective by elevating tissue prostaglandin concentration, which, in turn, would inhibit glucose

uptake. Nevertheless further investigations will be necessary to clarify this aspect, and such experiments are now under way in our laboratory.

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