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Effect of carbenoxolone on lipolysis in rat adipose tissue

B. LAMBERT*, C. GODARD AND C. JACQUEMIN

Laboratoire de Biochimie
Centre de Biologie et de Biochimie du Developpement Faculte des Sciences de Reims, France

Carbenoxolone slightly but significantly decreased the release of FFA from rat epididymal fat pads. The antilipolytic action of carbenoxolone was not blocked by 10^{-3}M 3-isobutyl-1-methylxanthine, a potent inhibitor of phosphodiesterase. The findings suggest that carbenoxolone exerts its antilipolytic activity by acting on adenylate cyclase, thereby decreasing cyclic AMP concentrations and the activity of the hormone-sensitive lipase in adipose tissue.

Many investigators have shown that prostaglandin E_1 decreases hormone stimulated lipolysis *in vitro* in the adipose tissue of rat (Steinberg, Vaughan & others, 1963, 1964), rabbit (Boberg, Micheli & Rammer, 1970) bird (Langslow, 1971) and man (Bergström & Carlson, 1965; Micheli, 1970). It has been also demonstrated that lipolysis, induced by hormones and by nerve stimulation, is accompanied by the release of prostaglandins which may modulate the action of the original stimulus by a negative feedback mechanism (Shaw & Ramwell, 1968; Christ & Nugteren, 1970; Ramwell & Shaw, 1970). Other authors have shown that the anti-inflammatory drug indomethacin is an inhibitor of prostaglandin biosynthesis *in vitro* (Ferreira, Moncada & Vane, 1971; Smith & Willis, 1971; Vane, 1971) so that this drug has often been used to evaluate the physiological role of prostaglandins. However reported results are often contradictory.

In some reports indomethacin increased basal or adrenaline-stimulated lipolysis by inhibiting prostaglandin biosynthesis (Illiano & Cuatrecasas, 1971) from arachidonic acid. In others, no modification of lipolysis was observed, so that a role for prostaglandins in the regulation of basal or activated lipolysis has not been confirmed (Dalton & Hope, 1973; Fain, Psychoyos & others, 1973; Fredholm & Hedqvist, 1975). It is also known that prostaglandins are metabolized by 15-hydroxyprostaglandin dehydrogenase (for a review, see Hansen, 1976). The triterpenoid carbenoxolone has been reported to be an inhibitor of 15-PG dehydrogenase (Peskar, Holland & Peskar, 1976). We have therefore examined the effect of this antiulcer drug on basal and stimulated lipolysis in rat adipose tissue.

* Correspondence.

MATERIALS AND METHODS

Chemicals. (−)-Adrenaline bitartrate and adrenocorticotropic hormone peptide (ACTH 150 U mg^{-1}) were obtained from Sigma. Crystalline beef insulin (B. grade, 25·9 IU mg^{-1}), glucagon and N^6O^2 dibutyryl-cyclic adenosine-3′,5′-monophosphate sodium salt, 2½ hydrate, B. grade) were purchased from Calbiochem. Bovine serum albumin (Pentex, fraction V, Miles Laboratories) was purified free of fatty acids by the method of Chen (1967). 3-Isobutyl-1-methylxanthine was obtained from Regis Chemical Co. and carbenoxolone sodium was a gift from Biorex Laboratories Ltd, London.

Animals. Male Wistar rats, 180–230 g, from Animalabo (Paris-France) had free access to food and water.

Lipolysis studies. The rats were decapitated and the epididymal fat pads were removed, rinsed in ice-cold physiological saline (NaCl 0·9% w/v), cut in small pieces (80–100 mg) and incubated for 1 h at 37° in vials containing 1 ml of Krebs-Ringer bicarbonate buffer pH 7·4 with Ca^{2+} (1·3 mM) and 4% (w/v) albumin (fatty acid free). At the end of the incubation, the contents of each flask were homogenized at 0° in a Polytron homogenizer at maximum speed for 1 min. Free fatty acids were extracted according to Dole & Meinertz (1960) and assayed by the method of Novak (1965). The data were evaluated statistically by Student's *t*-test. *P* values of less than 0·05 were accepted as indicating a significant difference between compared values.

RESULTS

When adipose tissue from rats was incubated with carbenoxolone alone, basal lipolytic activity was not

Table 1. *Effect of concentrations of carbenoxolone (CBX) on basal lipolysis in rat adipose tisue* (mean ± s.e., n = 6, *P* values *vs* basal).

Lipolysis: μmol FFA g^{-1} wet tissue h^{-1} (basal 3·44 ± 0·22)			
CBX 10^{-6} M	CBX 10^{-5} M	CBX 10^{-4} M	CBX 10^{-3} M
3·22 ± 0·15 N.S.	2·92 ± 0·14 N.S.	2·72 ± 0·16 $0·02 < P < 0·05$	2·54 ± 0·27 $0·02 < P < 0·05$

significantly modified at concentrations from 10^{-6} to 10^{-5} M. A slight but significant decrease of fatty acid release could be observed (Table 1) at 10^{-3} to 10^{-4} M.

The release of fatty acids produced by 1 μg ml^{-1} adrenaline was inhibited by carbenoxolone, but its lowest effective concentration was 10^{-4} M (Table 2). A similar antagonistic effect could be achieved with carbenoxlone (10^{-3} M) when lipolysis was stimulated by 50 mU ml^{-1} ACTH or 5 μg ml^{-1} glucagon but not by 2 × 10^{-3} M dibutyryl-AMP (Table 3).

To examine the effect of carbenoxolone on phosphodiesterase activity, cAMP breakdown was inhibited by 10^{-3} M 3-isobutyl-1-methylxanthine, and the effects of insulin (100 μU ml^{-1}) and carbenoxolone on induced lipolysis investigated. Although the concentration of insulin used was sufficient to inhibit the rate of release of free fatty acids, no effect on lipolysis was seen. In contrast, carbenoxolone (10^{-3} M) significantly decreased lipolysis (Table 4).

DISCUSSION

The results show that carbenoxolone decreases basal (Table 1) and stimulated lipolysis in a dose-dependent manner. In the rat, adipose tissue lipolysis may be stimulated *in vitro* in three ways (a) stimulation of adenylate cyclase by various hormones (e.g. adrenaline, ACTH, glucagon) (Butcher, Baird & Sutherland, 1968; Fain, 1973; Schwabe, Ebert & Erbler, 1973), (b) inhibition of phosphodiesterase by methylxanthines (Beavo, Rogers & others, 1970) or (c) by direct stimulation of the activation process of triglyceride lipase by dibutyryl-cAMP.

Studies on the effects of carbenoxolone (10^{-3} M) on the elevated lipolysis induced by stimulation of adenylate cyclase with hormones (e.g. adrenaline, glucagon, ACTH, (Tables 2, 3) reveal that in each case the drug reduces hormone-induced lipolysis and hence could be acting by reducing cAMP concentrations or by interfering with the action of cAMP on triglyceride lipase. However, when lipolysis is stimulated by exogenous dibutyryl cAMP (2 × 10^{-3} M) (Table 3) no effect of carbenoxolone is seen, suggesting no interaction with the cascade of reactions leading to the activation of triglyceride lipase. In addition, carbenoxolone at 10^{-3} M significantly decreased free fatty acid release whether or not the phosphodiesterase inhibitor 3-isobutyl-1-methylxan-

Table 2. *Effect of different doses of carbenoxolone (CBX)* (M) *on adrenaline (AD) (1 mg ml^{-1})-induced free fatty acids release in segments of rat adipose tissue.* (mean ± s.e., n = 6, *P* values as shown).

Lipolysis: μmol FFA g^{-1} wet tissue h^{-1} (basal 3·44 ± 0·22)				
AD I	AD + CBX 10^{-6} II	AD + CBX 10^{-5} III	AD + CBX 10^{-4} IV	AD + CBX 10^{-3} V
21·23 ±1·51	20·52 ±1·46 II *vs* I N.S.	18·37 ±0·71 III *vs* I N.S.	15·65 ±1·44 IV *vs* I $0·02 < P < 0·05$	13·04 ±1·01 V *vs* I $0·001 < P < 0·01$

Table 3. *Effect of carbenoxolone (CBX) (10^{-3} M) on basal and stimulated lipolysis (μmol FFA g^{-1} wet tissue h^{-1}) by adrenaline (AD) (1 mg ml^{-1}), glucagon (G) (5 mg ml^{-1}), ACTH (50 mU ml^{-1}) and DBcAMP (2 × 10^{-3} M) on rat adipose tissue.* (Mean ± s.e., n = 6; *P* values as shown).

Basal I	CBX II	AD III	AD + CBX IV	G V
3·44 ±0·20	4·30 ±0·24 II *vs* I $0·02 < P < 0·05$	25·96 ±0·52 III *vs* II $P < 0·001$	18·43 ±0·90 IV *vs* III $P < 0·001$	15·00 ±0·35 V *vs* II $P < 0·001$
G + CBX VI	ACTH VII	ACTH + CBX VIII	DBcAMP IX	DBcAMP + CBX X
10·74 ±0·29 VI *vs* V $P < 0·001$	16·99 ±0·97 VII *vs* II $P < 0·001$	11·94 ±0·96 VIII *vs* VII $0·01 < P < 0·001$	21·67 ±1·13 IX *vs* II $P < 0·001$	21·15 ±1·79 X *vs* IX NS

Table 4. *Effect of insulin (In) (100 μU ml^{-1}) and carbenoxolone (CBX) (10^{-3} M) on basal and 3-isobutyl-1-methylxanthine (IBMX) (10^{-3} M) induced lipolysis in rat pads* (mean ± s.e., n = 6, *P* values as shown).

Lipolysis: μmol FFA g^{-1} wet tissue h^{-1} (basal 3·44 ± 0·22)					
Basal I	In II	CBX III	IBMX IV	IBMX+In V	IBMX+In+CBX VI
3·44 ±0·22	3·45 ±0·25 II *vs* I N.S.	2·85 ±0·12 III *vs* I $0·02 < P < 0·05$	22·86 ±1·80 IV *vs* I $P < 0·001$	22·59 ±1·32 V *vs* IV N.S.	18·40 ±0·99 VI *vs* V $0·02 < P < 0·05$ VI *vs* IV $P < 0·05$

thine was present in the system, implying that carbenoxolone has no effect on the degradation of cAMP.

These results would suggest that carbenoxolone inhibits lipolysis by altering the rate of cAMP production. As mentioned previously, prostaglandins have been thought to be antilipolytic through a proposed negative feed-back effect on cAMP production. Carbenoxolone has recently been reported to inhibit 15-hydroxy-prostaglandin dehydrogenase in the guinea-pig lung (Peskar & others, 1976) and hence if the mode of action of this drug were the same in adipose tissue, then it is possible that this antiulcer drug could be effective by elevating tissue prostaglandin concentrations, which in turn would reduce both basal and hormone-stimulated lipolysis.

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