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Effect of Probenecid and Benzbromarone on Gluconeogenesis in Isolated Rat Liver Cells

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The effect of benzbromarone on gluconeogenesis from several gluconeogenic substrates in isolated rat liver cells is reported. Benzbromarone inhibited glucose synthesis from all substrates employed when the drug was used at concentrations half to ten times greater than its therapeutic plasma levels. This inhibition was more pronounced from lactate and pyruvate than from fructose and glycerol. The results are compared with those obtained for probenecid, a classical uricosuric drug. We found that probenecid inhibited the pathway in the same way as benzbromarone.

Key words: Gluconeogenesis, Benzbromarone, Probenecid, Hepatocytes.

Benzbromarone (2-ethyl-3-(4-hydroxy-3,5-dibromobenzoyl)-benzo-furan) is a uricosuric drug which is metabolized in the liver (3). Although some side effects on intermediate hepatic metabolism have been described (6), nothing has been reported about its interaction with carbohydrate metabolism.

We have studied the metabolic effects of this drug in isolated rat liver cells (10,12) and have found that benzbromarone decreases their ability to synthesize glucose. In this paper, the effects of benzbromarone on gluconeogenesis from lactate, fructose, pyruvate and glycerol are reported and compared with those of probenecid, a classical uricosuric drug.

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Materials and Methods

Animals. — Male Wistar rats weighing 250 to 300 g and maintained on standard laboratory diet were used. Rats were fasted for 48 h before experiments.

Chemicals. — Collagenase and reagents for glucose assays were obtained from Boehringer Gmbh (Mannheim). Probenecid (p-dipropyl-sulfamoyl benzoic acid) was purchased from Sigma. Benzbroma-

rone was generously donated by the laboratory Labaz (Barcelona, Spain). All other reagents were of analytical grade.

Isolation and incubation of hepatocytes. — Isolated hepatocytes were prepared by the method described by BERRY and FRIEND (2), modified (7, 11). Cells (10-15 mg wet wt/ml) were incubated (45 min) in Krebs-Henseleit bicarbonate buffer containing 3 mM Ca^{2+} (8) in presence and absence of the drugs (9). More than 85 % of the cells excluded trypan blue at the end of the incubation (1).

Biochemical determinations. — Glucose was determined by the glucose oxidase method according to WERNER *et al.* (14).

Statistical analysis of data was performed by Student's t-test.

Results and Discussion

Figures 1, 2 and 3 show the effect of benzbromarone, used at concentrations half to ten times greater than its therapeutic plasma levels (15), on gluconeogenesis from several substrates by isolated hepatocytes in a given time. In all cases, glucose synthesis was linear with time during the 30 min between 15 and 45 min of the incubation period studied (data not shown).

The inhibition with lactate as substrate is much higher than that obtained when pyruvate was employed. This suggests an effect of the drug on NADH/NAD^+ ratio.

In addition, the results obtained in our experiments using glycerol or fructose show that the gluconeogenic flux is also inhibited in the last steps of the pathway. The inhibition with glycerol as substrate is higher than with fructose. Again these results suggest the mentioned effect of benzbromarone on NADH/NAD^+ ratio.

Benzbromarone has been found to in-

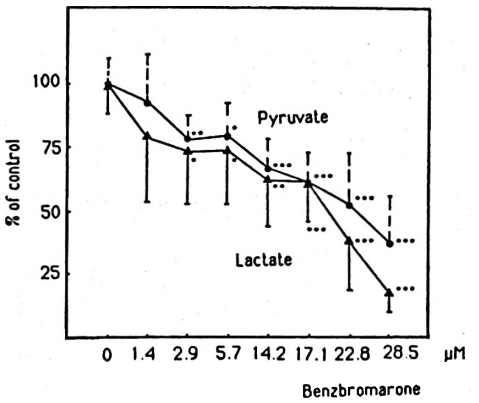

Fig. 1. *Effect (% of Control) of Benzbromarone on Gluconeogenesis from lactate and pyruvate in isolated rat liver cells.*

All precursors concentration used was 10 mM. The absolute control values (100 %) were 0.42 ± 0.04 µmol glucose/g wet wt/ min for lactate and 0.32 ± 0.03 µmol glucose/g wet wt./min for pyruvate. Results are expressed as means ± S.D. for 6 or 7 experiments. Results that are significantly different from those of control are shown: *p < 0.05, **p < 0.01, ***p < 0.001.

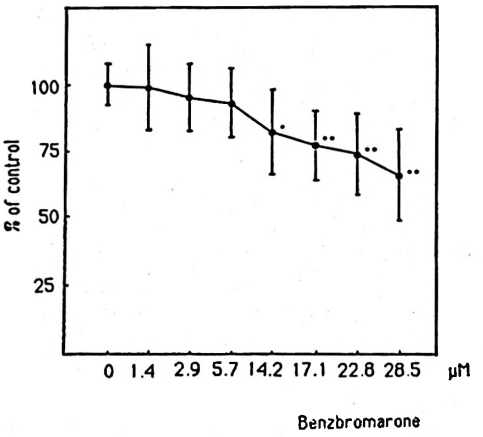

Fig. 2. *Effect (% of control) of Benzbromarone on gluconeogenesis from fructose in isolated rat liver cells.*

Fructose concentration was 10 mM. The absolute control value was 1.62 ± 0.15 µmol glucose/g wet wt./min. Results are expressed as means ± S.D. for 5 or 6 experiments. *p < 0.05, **p < 0.01.

Table I. *Effect of Probenecid on gluconeogenesis from lactate, pyruvate, glycerol and fructose in isolated rat liver cells.*

All substrates concentration used were 10 mM. Glucose synthesis is expressed as μmol/g wet wt./min. Results are expressed as means ± S.D. for 6 experiments. * $p < 0.05$, *** $p < 0.001$.

	LACTATE	PYRUVATE	GLYCEROL	FRUCTOSE
Control	0.43 ± 0.07	0.33 ± 0.02	0.47 ± 0.03	1.61 ± 0.09
Probenecid (mM)				
0.1	0.41 ± 0.05	0.31 ± 0.01	0.45 ± 0.02	1.60 ± 0.01
0.2	0.39 ± 0.06	0.30 ± 0.02*	0.44 ± 0.04	1.59 ± 0.06
0.5	0.34 ± 0.04*	0.26 ± 0.01***	0.43 ± 0.03*	1.57 ± 0.04
1.2	0.26 ± 0.04***	0.20 ± 0.01***	0.38 ± 0.03***	1.50 ± 0.05*
2.4	0.18 ± 0.04***	0.17 ± 0.02***	0.28 ± 0.05***	1.40 ± 0.08***

hibit the urea synthesis in isolated rat hepatocytes (12). Both processes, gluconeogenesis and ureogenesis, require ATP. Consequently, the inhibition of both pathways by benzbromarone could be due to a possible effect of the drug on oxidative phosphorylation, as it has been shown for several drugs with a similar effect on gluconeogenesis (4, 5) and ureogenesis (9). It could act as an uncoupler of oxidative phosphorylation since benzbromarone shares some of the structural features of other standard uncouplers, i.e. an aromatic ring associated with a weak acidic group.

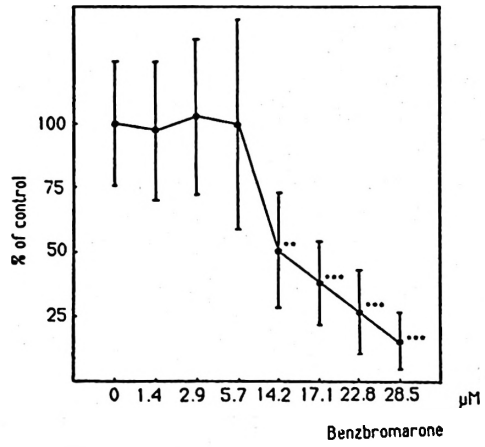

Fig. 3 *Effect (% of control) of Benzbromarone on gluconeogenesis from glycerol in isolated hepatocytes*

Glycerol concentration was 10 mM. The absolute control value was 0.42 ± 0.11 μmol glucose/g wet wt./min. Results are expressed as means ± S.D. for 5 or 6 experiments. **$p < 0.01$, ***$p < 0.001$.

Table I shows the effect of probenecid, used at the same relative levels as benzbromarone (13), on gluconeogenesis in isolated hepatocytes. This drug inhibited the pathway in the same way as benzbromarone. This suggests a similar effect to that of benzbromarone on NADH/NAD^+ ratio, since, in addition, probenecid also inhibits ureogenesis (12). In fact, this drug presents the features mentioned above for uncouplers of oxidative phosphorylation. In contrast, we found that allopurinol, another hypouricemic drug, has no effect on gluconeogenesis (results not shown) and ureogenesis (12).

The potential clinical implications of our study deserve comment. A substantial inhibition of glucose synthesis might occur during the treatment with benzbromarone or probenecid, having noxious consequences.

Resumen

Se estudia el efecto de la benzobromarona sobre la gluconeogénesis, a partir de diferentes sustratos, en hepatocitos aislados de rata. La benzobromarona, a concentraciones entre la mitad y diez veces su concentración plasmática terapéutica, inhibe la síntesis de glucosa a partir de todos los sustratos estudiados. La inhibición es mayor a partir del lactato y piruvato

que de fructosa y glicerol. Los resultados, comparados con los del probenecid, muestran que ambos fármacos inhiben la vía en un grado similar.

Palabras clave: Gluconeogénesis, Benzobromarona, Probenecid, Hepatocitos.

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