tion and/or control mechanisms of muscarinic receptors or a distinct susceptibility of the two strains of rat to 6-OH dopamine (the drug efficacy can, indeed, be modified by the circulatory flow and by the importance of catecholamine uptake in nerve endings [16], two parameters that might also differ in SHR and WKY rats).

Acknowledgements—Aided by Grant 3317 from I.R.S.I.A. (Belgium) and a grant from the Ministère de la Politique Scientifique (Belgium).

Department of Biochemistry and Nutrition
Medical School
Université Libre de Bruxelles
Bld de Waterloo 115
B-1000 Brussels
Belgium
MAGALI WAELBROECK
PATRICK ROBBERECHT
JEAN CHRISTOPHE*

Continental Pharma Research Laboratories
B-1348 Mont-Saint-Guibert
Belgium
PIERRE CHATELAIN
ANH NGUYEN HUU
JOSEPH ROBA

REFERENCES

1. F. J. Ehlert, W. R. Roeske and H. I. Yamamura, *Fedn Proc.* **40**, 153 (1981).
2. S. Yamada, H. I. Yamamura and W. R. Roeske, *Molec. Pharmac.* **18**, 185 (1980).
3. V. K. Sharma and S. P. Banerjee, *Nature, Lond.* **272**, 276 (1978).
4. C. Sachs and G. Jonsson, *Biochem. Pharmac.* **24**, 1 (1975).
5. S. Z. Langer, *Biochem. Pharmac.* **23**, 1793 (1974).
6. S. Z. Langer, *Pharmac. Rev.* **32**, 337 (1981).
7. J. M. Saavedra, H. Grobecker and J. Axelrod, *Circulation Res.* **42**, 529 (1978).
8. R. D. Ekas, Jr. and M. F. Lokhandwala, *Am. J. Physiol.* **241**, 379 (1981).
9. C. Limas and C. J. Limas, *Biochem. biophys. Res. Commun.* **83**, 710 (1978).
10. P. Chatelain, M. Waelbroeck, J. C. Camus, P. de Neef, P. Robberecht, J. Roba and J. Christophe, *Eur. J. Pharmac.* **72**, 17 (1981).
11. P. Robberecht, J. Winand, P. Chatelain, P. Poloczek, J. C. Camus, P. De Neef and J. Christophe, *Biochem. Pharmac.* **30**, 385 (1981).
12. M. Waelbroeck, P. Robberecht, P. Chatelain and J. Christophe, *Molec. Pharmac.* **21**, 581 (1982).
13. P. Robberecht, M. Waelbroeck, M. Claeys, A. Nguyen Huu, P. Chatelain and J. Christophe, *Molec. Pharmac.* **21**, 589 (1982).
14. P. Chatelain, P. Robberecht, P. De Neef, M. Claeys and J. Christophe, *FEBS Lett.* **107**, 86 (1979).
15. D. D. Story, M. S. Briley and S. Z. Langer, *Eur. J. Pharmac.* **57**, 423 (1979).
16. R. M. Kostrzewa and D. M. Jacobowitz, *Pharmac. Rev.* **26**, 199 (1974).

* To whom correspondence should be addressed.

Biochemical Pharmacology, Vol. 32, No. 11, pp. 1807–1809, 1983.
Printed in Great Britain.

0006-2952/83 $3.00 + .00

Effects of antilipolytic agents on peroxisomal β-oxidation of fatty acids in rat liver

(*Received* 4 *October* 1982; *accepted* 29 *December* 1982)

Peroxisomes can perform β-oxidation of fatty acids [1]. There are now many reports on the induction of this oxiditive activity in the liver after the administration of hypolipidemic drugs (see e.g. [2]) or under conditions of sustained high hepatic influx of fatty acids (see e.g. [3, 4]). In this paper we report on the effects of two different antilipolytic drugs on this activity in comparison with the effects of the natural antilipolytic agent glucose.

Materials and methods

Experiments were performed on random groups of conventional male Sprague–Dawley rats (190–220 g body weight) housed at 18–25° in plastic cages with a drinking bottle and fed a Randoin–Causeret diet (Piccioni, Brescia, Italy) containing 3.6% lipid. 3,5-Dimethylpyrazole (DMP) was given by intraperitoneal injection of 12 mg/kg (in 0.2 ml saline) every 3 hr [5]. ACIPIMOX (5-methylpyrazine carboxilic acid 4-oxide)—a new antilipolytic drug [6, 7] marketed by Carlo Erba S.p.A. (Milano, Italy)(25 mg/kg body weight, in 1.0 ml saline, pH 2.13) was given intraperitoneally every 3 hr. In both instances, dosages had been found to be maximally active on peroxisomal β-oxidative activity (see also [8]). Glucose (3 g/kg body wt) was administered by stomach tube as a 40% solution (w/v) in water every hour. Controls were given only the vehicles.

After a fasting period of 12 hr, the animals were rapidly bled under nembutal anaesthesia (50 mg/kg body weight) by cutting the femoral vessels, and the blood was collected and then centrifuged. The livers were rapidly removed and weighed. The right lobe was homogenized (1:10, w/v) in 0.25 M sucrose with a Teflon-pestle glass-vessel homogenizer. The peroxisomal β-oxidative activity was measured according to Inestrosa *et al.* [9]. In the normal rat this procedure also assays the acyl-CoA oxidase activity. The homogenate (0.2 ml) was added to 2.8 ml of assay mixture. Final concentrations were: 100 mM Tris–Cl pH 8.3; 100 mM methanol; 1.0 mM Na palmitate (added as a methanol solution); 0.1 mM Coa; 2.5 mM ATP; 5 mM $MgCl_2$; 33 mM nicotinamide; 6.6 mM semicarbazide; 0.2 mM NAD. After 5, 10, 15 and 20 min, aliquots of the assay mixture were transferred into centrifuge tubes containing ice-cold 5% TCA. After centrifugation, formaldehyde was detected in the supernatant according to Nash [10]. Results are given as μmole of formaldehyde/min produced by the liver corresponding to 100 g body wt.

Plasma glucose was assayed by the glucose-oxidase/peroxidase method using commercially available kits (Glucosio Test SCLAVO, Siena, Italy). The levels of plasma FFA and triglycerides were assayed according to [11] and [12], respectively. Liver glycogen was purified and determined spectrophotometrically according to [13]. Liver triglycerides were extracted according to [14] and assayed by a fully enzymatic procedure [12].

All products used were of analytical grade.

Results and discussion

Table 1 shows the changes in the peroxisomal β-oxiditive activity in the liver homogenate after the administration of antilipolytic agents at a maximally active dosage form an appropriate time (see [8]). Data have been given in absolute terms (i.e. as enzyme units in the liver wet weight corresponding to 100 g body weight) to compensate for the

Table 1. Effects of glucose (3 g/kg body weight every hr), DMP (12 mg/kg body weight every 3 hr or fraction) and ACIPIMOX (25 mg/kg body weight every 3 hr or fraction) administration (for 4 hr) on the wet weight, and the peroxisomal β-oxidative activity and on the triglyceride content of the rat liver

Glucose (3 g/kg body weight)	DMP (12 mg/kg body weight)	ACIPIMOX (25/kg body weight)	Liver weight (g/100 g body weight)	β-Oxidative activity (μmole/min per unit wet weight)	Triglycerides (mg/unit wet weight)
−	−	−	3.3 ± 0.18(6)	1130 ± 67(6)	90.2 ± 19.7(6)
+	−	−	4.18 ± 0.15(6)	1590 ± 110(6)	85.7 ± 19.6(6)
−	+	−	3.08 ± 0.05(6)	870 ± 36(6)	46.2 ± 19.1(6)
−	−	+	3.57 ± 0.17(6)	990 ± 95(6)	58.5 ± 6.1(6)
+	+	−	3.83 ± 0.06(6)	1290 ± 77(6)	76.2 ± 12.1(6)
+	−	+	3.83 ± 0.10(6)	1470 ± 140(6)	75.5 ± 10.1(6)
−	+	+	3.46 ± 0.19(6)	910 ± 75(6)	39.0 ± 6.6(6)
+	+	+	3.73 ± 0.16(6)	1320 ± 70(6)	63.4 ± 11.2(6)

The oxidative activity and the triglycerides content were assayed as described (see Materials and Methods). Results are given as μmole formaldehyde produced during 1 min incubation at 37° by the liver per 100 g body weight and as mg in the liver per 100 g body weight, respectively. Means ± S.E. are given. The number of cases are given in parentheses. Statistical analysis (*F* test and Bonferroni's *t*-multiple test): the effects of glucose and of DMP on the liver weight are significant ($P < 0.01$ and $P < 0.05$ respectively; with regards to the oxidative activity, the effect of glucose is highly significant ($P < 0.01$) and those of DMP and of ACIPIMOX are significant ($P < 0.01$ and $P < 0.05$, respectively) whereas interactions are not; with regards to triglycerides, the effects of DMP and of ACIPIMOX are significant ($P < 0.01$); the effect of glucose is not but interactions between glucose and DMP, and glucose and ACIPIMOX are significant $P < 0.05$ both).

fluctuations of the liver size because of the experimental treatment (the administration of glucose greatly enhances the liver weight whereas that of DMP can decrease it significantly, see Table 1), It is clearly apparent that the administration of antilipolytic drugs causes a significant decrease in the peroxisomal β-oxiditive activity (−22%, $P < 0.01$ and −10%, $P < 0.05$ after DMP or ACIPIMOX administration, respectively). At these dosages, the effects of the two drugs are not additive. On the other hand, it should be emphasized that the administration of glucose enhances the peroxisomal β-oxiditive activity (+42%, $P \ll 0.01$) and that this latter effect prevails when glucose and antilipolytic drugs are given together.

Figures 1 and 2 show that antilipolytic drugs and glucose all decrease the FFA plasma levels to a similar extent. Therefore, the load of this lipid to the liver cannot be the (only) factor regulating the peroxisomal β-oxiditive activity. Perhaps, attention should be focused on the role of the liver lipid. It is known that glucose administration stimulates lipogenesis in the liver [15] and that different fatty acids may have different inductive effects on the peroxisomal acyl-CoA oxidase activity [16]. Table 1 shows that both antilipolytic drugs and glucose affect lipid metabolism in the liver (antilipolytic drugs decrease the liver content in triglycerides; glucose has no effect on the basal levels but increases the triglycerides in the liver of DMP- or

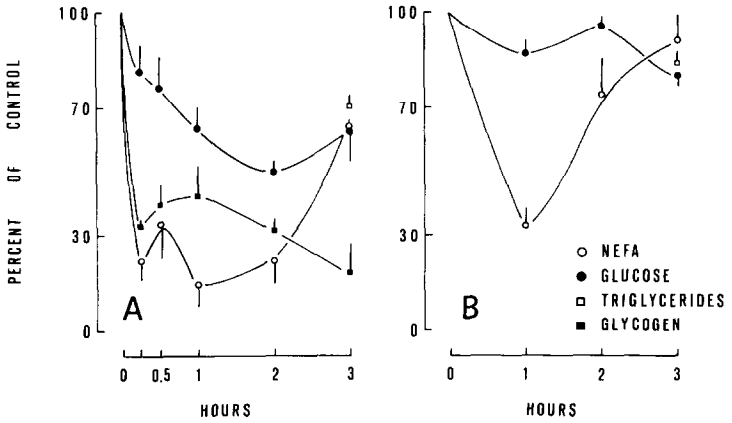

Fig. 1. Per cent changes in NEFA, glucose and triglyceride plasma levels and glycogen content in the liver after DMP (A) or ACIPIMOX (B) administration. Triglyceride plasma levels were assayed in controls and in the 3-hr groups only. Means of six cases are given. On the abscissa: hours after the injection of the drug (see Materials and Methods). The control values were: (A): NEFA 47 ± 11 μmole/100 ml; plasma glucose: 133 ± 6 mg/100 ml; plasma triglycerides: 84 ± 16 mg/100 ml; liver glycogen 0.45 ± 0.13 g/100 g tissue wet weight. (B): 51 ± 8 μmole /100 ml; 110 ± 4.5 mg/100 ml and 101 ± 7.8 mg/100 ml, respectively. Glycogen was not assayed but in a similar experiment we obtained the following results: control 0.57 ± 0.062 g/100 wet tissue (4 cases); ACIPIMOX 1 hr, 0.43 ± 0.056(4); ACIPIMOX 2 hr, 0.33 ± 0.017(4); ACIPIMOX 3 hr, 0.34 ± 0.015(4). Statistical analysis was performed on the original values. After DMP, all changes were highly significant ($P < 0.01$); after ACIPIMOX the decrease in NEFA levels is highly significant ($P < 0.01$ both at 1 and 2 hr) and the changes in glucose and triglyceride levels are significant ($P < 0.05$).

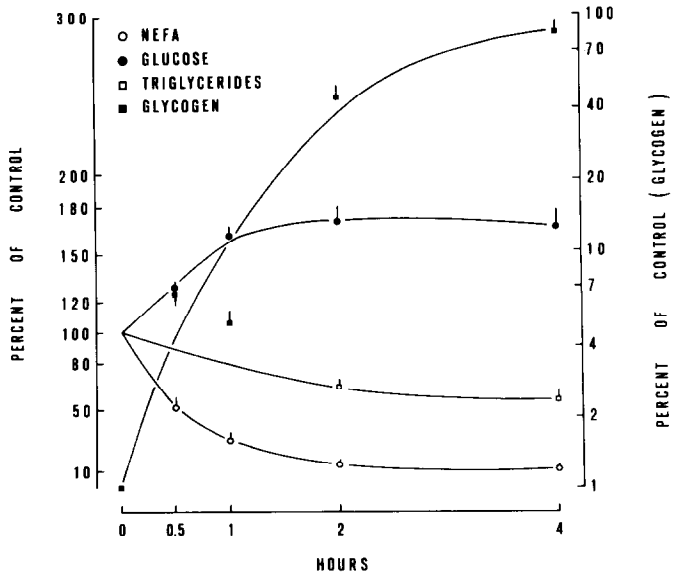

Fig. 2. Per cent changes in NEFA, glucose and triglyceride plasma levels and changes in liver glycogen (on the logarithmic scale) after glucose administration by the gastric tube (see Materials and Methods). Means of six cases. On the ordinate: see Fig. 1 legend. On the abscissa: hours after the first administration of glucose. All changes after glucose are highly significant ($P < 0.01$).

ACIPIMOX-treated rats). However, at present we have no data on the liver content in fatty acid and on the composition of this lipid fraction.

The mechanisms of these different enzyme changes deserve the final comment. Antilipolytic agents and glucose have no '*in vitro*' effect on peroxisomal β-oxiditive activity [8]. In the '*in vivo*' experiments, the enzyme changes are preceded by several metabolic events (e.g. the changes in blood glucose levels and in the concentration of liver glycogen, see Figs. 1 and 2). Therefore, it may be appropriate to mention that these experimental treatments are likely to affect the endocrine status of the animals (glucose administration increases insulin and decreases glucagon levels in plasma and antilipolytic drugs decrease blood glucose and should cause opposite hormonal changes, see e.g. [17]) and that changes in enzyme content seem to occur in hepatic peroxisomes in diabetic rats [18].

In summary, the administration of antilipolytic agents can affect the peroxisomal β-oxiditive activity of rat liver. These enzyme changes may not always be a direct consequence of the lower influx of free fatty acids; they can also be an effect of local changes in the lipid metabolism.

Acknowledgements—This research was supported in part by grants from the C.N.R. (Gruppo di Endocrinologia) and the Ministero della Pubblica Istruzione.

Istituto di Patologia Generale T. LOCCI-CUBEDDU
dell'Università di Pisa E. BERGAMINI
II Cattedra, Italy

REFERENCES

1. P. B. Lazarow, *J. biol. Chem.* **255**, 1522 (1978).
2. P. B. Lazarow and C. De Duve, *Proc. natn. Acad. Sci. U.S.A.* **73**, 2043 (1976).
3. H. Ishii, N. Fukumori, S. Horie and T. Suga, *Biochim. biophys. Acta* **617**, 1 (1980).
4. H. Ishii, S. Horie and T. Suga, *J. Biochem.* **87**, 1855 (1980).
5. P. S. Schein, K. G. M. M. Alberti and D. H. Williamson, *Endocrinology* **89**, 827 (1971).
6. P. P. Lovisolo, G. Briatico-Vangosa, G. Orsini, R. Ronchi and R. Angelucci, *Pharmac. Res. Commun.* **13**, 151 (1981).
7. P. P. Lovisolo, G. Briatico-Vangosa, G. Orsini, R. Ronchi and P. Angelucci, *Pharmac. Res. Commun.* **13**, 163 (1981).
8. T. Locci-Cubeddu and E. Bergamini, *Bull. molec. Biol. Med.* **7**, 47 (1982).
9. N. C. Inestrosa, M. Bronfman and F. Leighton, *Biochem. J.* **182**, 779 (1979).
10. T. Nash, *Biochem. J.* **55**, 416 (1953).
11. S. W. Smith, Analyt. Biochem. **67**, 531 (1975).
12. A. W. Wahlenfeld, in *Methods in Enzymatic Analysis* (Ed. H. U. Bergmeyer), Vol. 4, p. 1831. Academic Press, New York (1974).
13. W. Z. Hassid and S. Abraham, in *Methods in Enzymology* (Eds. S. P. Colowick and N. O. Kaplan), Vol. 3, p. 34. Academic Press, New York (1957).
14. J. Folch, M. Lees, G. H. Sloane Stanley, *J. biol. Chem.* **226**, 497 (1957).
15. A. G. Goodridge, *Fedn Proc.* **34**, 117 (1975).
16. H. Osmundsen, in *Peroxisomes and Glyoxysomes, Ann. N.Y. Acad. Sci.* (Eds. H. Kindl and P. B. Lazarow), Vol. 386, p. 13 (1982).
17. A. E. Renolds, D. H. Mintz, W. A. Muller and G. F. Cahill, Jr., in *The Metabolic Basis of Inherited Disease* (Eds. J. B. Standbury, J. B. Wyngaarden and D. S. Fredrickson), p. 80. McGraw-Hill, New York (1978).
18. S. Horie, H. Ishii and T. Suga, *J. Biochem.* **90**, 1691 (1981).