17. F. Bloom, D. Segal, N. Ling and R. Guillemin, *Science, N.Y.* **194,** 630 (1976).
18. J. M. Walker, G. G. Berntson, C. A. Sandman, D. H. Coy, A. V. Schally and A. J. Kastin, *Science, N.Y.* **196,** 85 (1977).
19. A. F. Bradbury, D. G. Smyth, C. R. Snell, J. F. W. Deakin and S. Wendlandt, *Biochem. biophys. Res. Commun.* **74,** 748 (1977).
20. L. Graf, J. I. Szekely, A. Z. Ronai, Z. Dunai-Kovaks and S. Bajusz, *Nature, Lond.* **263,** 240 (1976).
21. G. Henderson, J. Hughes and H. W. Kosterlitz, *Br. J. Pharmac.* **46,** 764 (1972).
22. C. B. Pert, A. Pert, J. K. Chang and B. T. W. Fong, *Science, N.Y.* **194,** 330 (1976).
23. J. M. Hambrook, B. A. Morgan, M. J. Rance and C. F. C. Smith, *Nature, Lond.* **262,** 782 (1976).
24. J. D. Beluzzi, N. Grant, V. Garsky, D. Sarantakis, C. D. Wise and L. Stein, *Nature, Lond.* **260,** 625 (1976).
25. Y. F. Jaquet and N. Marks, *Science, N.Y.* **194,** 632 (1976).
26. N. Marks, A. Grynbaum and A. Neidle, *Biochem. biophys. Res. Commun.* **74,** 1552 (1977).
27. J. L. Meek, H. Y. T. Yang and E. Costa, *Neuropharmacology* **16,** 151 (1977).
28. A. F. Bradbury, D. G. Smyth and C. R. Snell, *Nature, Lond.* **260,** 165 (1976).
29. A. S. Horn and J. R. Rogers, *J. Pharm. Pharmac.* **29,** 257 (1977).

Biochemical Pharmacology, Vol. 27, pp. 2277–2278.

0006-2952/78/0915-2277$02.00/0

Enhancement of renal gluconeogenesis by clofibrate

(*Received* 17 *August* 1977; *accepted* 8 *November* 1977)

Recently, Mackerer and Haettinger [1] showed that rats treated with clofibrate (0.3% of diet) have increased rates of renal gluconeogenesis associated with increased activities of glucose 6-phosphatase (G-6-Pase), pyruvate carboxylase (PC) and phosphoenolpyruvate carboxykinase (PEPCK). The mechanism by which the drug enhances gluconeogenesis is unknown, but a possible explanation is that there is a modulation of normal hormonal activity and a homeostatic re-equilibration. Clofibric acid, the active moiety of clofibrate [2], displaces thyroxine from circulating plasma proteins, thereby causing a fall in total plasma thyroxine followed by thyroxine accumulation in both liver and kidney [3]. Administration of thyroxine to rats increases the rate of renal glucose synthesis from pyruvate and succinate [4] and increases the activities of pyruvate carboxylase [5] and glucose 6-phosphatase [6]. Administration of thyroxine to thyroidectomized rats greatly increases the activities of pyruvate carboxylase, phosphoenolpyruvate carboxykinase and glucose 6-phosphatase [7]. The following experiments were performed to determine whether the clofibrate enhancement of renal gluconeogenesis is mediated by thyroxine.

Normal and thyroidectomized rats (CR–CD strain) were obtained from Charles River Breeding Laboratories, Wilmington, Mass. and were allowed to stabilize for 1 and 6 weeks, respectively, prior to the beginning of the study. During this time the rats were individually housed and fed pellets of Rockland mouse/rat diet (complete) *ad lib.* Normal rats received tap water and thyroidectomized rats received Hank's solution [8]. After the stabilization period, the pelleted diet was replaced with powdered diet or powdered diet containing 0.3% (w/w) clofibrate. After 7

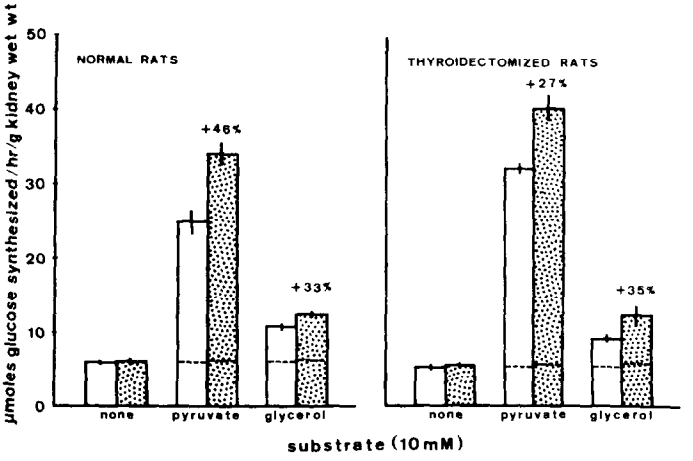

Fig. 1. Clofibrate-induced enhancement of glucose synthesis by rat kidney cortex slices from normal and thyroidectomized rats. Values are means ± S.E.M. for six rats. The per cent increase caused by clofibrate (after subtraction of basal rates in the absence of substrate) is indicated.

days of clofibrate treatment the rats were killed by decapitation, without prior fasting, between 8:00 and 11:00 a.m. Blood was collected from the wound and analyzed for thyroxine [9]. All of the thyroidectomized rats had serum thyroxine concentrations that were below detectable levels (i.e. < 1 μg/ml). The normal control rats had levels of approximately 6 μg/ml. In addition, the thyroidectomized rats had markedly decreased growth rates, and low basal metabolic rates that were not increased by intraperitoneal injections of thyrotropin-releasing factor [9].

Kidneys were rapidly excised, decapsulated, sliced in half longitudinally with a scalpel, and demedullated. The remaining cortex was then weighed and 0.5-mm slices were prepared with a Stadie-Riggs tissue slicer. Slices were blotted, weighed, trimmed to between 65 and 85 mg, and placed in cold (4°) 0.154 M NaCl for 5–10 min. Slices were then incubated in the presence of 10 mM NaCl, or the substrates pyruvate and glycerol, for 1 hr at 37° under 95% O_2–5% CO_2 in 5 ml of Krebs–Ringer bicarbonate solution containing 2.54 mM Ca^{2+} [10]. Incubations were carried out in stoppered 50-ml flasks with continuous shaking (120 oscillations/min). Reactions were stopped by adding 1 ml of 2 N $HClO_4$, slices were discarded, and samples were removed for glucose analysis [11]. Net glucose synthesis was calculated by substracting glucose formed in the absence of substrate from that formed in the presence of substrate.

The effect of clofibrate treatment on gluconeogenesis is shown in Fig. 1. Clofibrate markedly increased the rate of glucose synthesis from glycerol and pyruvate by kidney cortex slices from both normal and thyroidectomized rats and, therefore, it may be concluded that thyroxine is not involved in mediating this effect. Previous work has also eliminated metabolic acidosis as a factor [1]. It is possible that renal gluconeogensis is enhanced by a reduction of the plasma insulin/glucagon ratio. Savolainen *et al.* [12] have recently shown that clofibrate causes a fall in the rat plasma insulin level and a decreased glucose tolerance.

Acknowledgement—The author is grateful to Ms. Rosemary Auer for her excellent technical assistance.

Department of Biological Research, CARL R. MACKERER
Searle Laboratories,
Chicago, IL 60680 U.S.A.

REFERENCES

1. C. R. Mackerer and J. R. Haettinger, *J. Pharmac. exp. Ther.* **204**, 683 (1978).
2. M. N. Cayen, E. S. Ferdinandi, E. Greselin, W. T. Robinson and D. Dvornik, *J. Pharmac. exp. Ther.* **200**, 33 (1977).
3. W. R. Ruegamer, N. T. Ryan, D. A. Richert and W. W. Westerfeld, *Biochem. Pharmac.* **18**, 613 (1969).
4. P. K. Joseph and K. Subrahmanyam, *Archs Biochem. Biophys.* **127**, 288 (1968).
5. D. G. Colton, M. A. Mehlman and W. R. Ruegamer, *Endocrinology* **90**, 1521 (1972).
6. B. Szepesi and R. A. Freedland, *Am. J. Physiol.* **216**, 1054 (1969).
7. D. G. Colton, *Diss. Abstr.* **34**, 1918 (1973).
8. J. H. Hanks and R. E. Wallace, *Proc. Soc. exp. Biol. Med.* **71**, 196 (1949).
9. C. R. Mackerer, *Biochem. Pharmac.* **26**, 301 (1977).
10. H. F. DeLuca and P. P. Cohen, in *Manometric Techniques* (Eds W. W. Umbreit, R. H. Burris and J. F. Stauffer), p. 131. Burgess Publishing Co., Minneapolis (1964).
11. M. W. Slein, in *Methods of Enzymatic Analysis* (Ed. H. U. Bergmeyer), p. 117. Academic Press, New York (1965).
12. M. J. Savolainen, V. P. Jauhonen and I. E. Hassinen, *Biochem. Pharmac.* **26**, 425 (1977).

Biochemical Pharmacology, Vol. 27, pp. 2278–2280 0006-2952/78/0915-2278$02.00/0

Magnification of some enzymatic activities of brain cortex subfractions

(*Received* 21 *September* 1977; *accepted* 1 *February* 1978)

An *in vivo* investigation on cerebral energy metabolism has demonstrated that a catecholamine-like agent, 1-*p*-hydroxyphenyl-2-butylamino-ethanol or bamethan, favors post-hypoxic recovery at the level of the brain cortex motor area in the dog [1]. This interference affects the adenyl cyclase receptor, the presence of a β-blocking agent inhibiting the adenyl cyclase stimulation induced by bamethan during post-hypoxic recovery [2]. This finding confirms that cerebral adenyl cyclase behaves like a receptor with β-adrenergic characteristics [3]. The study [4], of the subcellular localization of adenyl cyclase has shown that the highest activity is found in the fractions containing nerve endings and synaptic complexes, elsewhere indicated as synaptosomal fractions [5], the myelinic and mitochondrial fractions exhibiting a lower activity. It has therefore proved interesting to examine the effect of bamethan at subcellular level on some cerebral enzymatic activities, evaluated on the mitochondrial and synaptosomal fractions of the rat brain cortex. The following enzymes were investigated: lactate dehydrogenase (*L-lactate: NAD⁺ oxidoreductase, EC 1.1.1.27*) for the glycolytic pathway; citrate synthase (*citrate oxaloacetate lyase, EC 4.1.3.7*) and malate dehydrogenase (*L-malate: NAD⁺ oxidoreductase, EC 1.1.1.37*) for the Krebs' cycle; total NADH-cytochrome *c* reductase (*NADH-cytochrome c oxidoreductase, EC 1.6.99.3*) and cytochrome oxidase (*Ferrocytochrome c: oxygen oxidoreductase, EC 1.9.3.1*) for the electron transport chain.

MATERIALS AND METHODS

Sprague–Dawley female rats (weight 300 ± 10 g) were used. The animals were selected according to randomized experimental procedures, kept from the birth under standard cycling and caging conditions (temperature, 22 ± 1°; relative humidity, 60 ± 5%; lighting cycle, 12 hr light and 12 hr darkness; low noise-disturbance) and fed a standard pellet diet. The results indicated in this paper