J. Pharm. Pharmacol. 1987, 39: 169–172
Received July 14, 1986

Effects of glucagon on cAMP accumulation and ketogenesis in hepatocytes from euthyroid and hypothyroid rats

OMAR SHAHEEN, SADEQ OTHAM†, WALID A. AL-TURK†* AND NAIF SLIMAN‡

Departments of Pharmacology, †Pharmaceutical Chemistry and ‡Internal Medicine, College of Medicine and College of Pharmacy, University of Jordan, Amman, Jordan

The resistance to the effects of glucagon was studied in isolated hepatocytes prepared from male rats treated with 6*N*-propyl-2-thiouracil (PTU). Incorporation of [^{14}C]oleate into ketone bodies in response to various concentrations of glucagon (10^{-5} to 10^{-10} M) was reduced in hepatocytes from hypothyroid rats compared with the euthyroid group. The reduced sensitivity to the effects of glucagon on ketogenesis after treatment with PTU was associated with a reduced ability of those hepatocytes to maintain cyclic adenosine-3′,5′-monophosphate (cAMP) at levels required to stimulate ketogenesis. The concentration of cAMP in response to glucagon (10^{-5} to 10^{-10} M) was diminished in hepatocytes from hypothyroid rats, compared with those from euthyroid animals.

The response of target tissues to various endocrine factors and neurotransmitters has been shown to be affected by changes in thyroid status (Broadus et al 1970; Elkeles et al 1975; Gutler et al 1977; Shaheen et al 1982). The increased sensitivity of the myocardium in rats from hyperthyroid subjects to the β-adrenergic agonists is perhaps the most widely known example (Heimberg et al 1964; Berry & Friend 1969). However, liver and adipose tissue which are involved in the regulation of fuel homeostasis and metabolism are also markedly affected by thyroid status of both man and animals (Broadus et al 1970; Elkeles et al 1975; Gutler et al 1977). In previous studies (Weiner & Berry 1974) glucagon-dependent regulation of ketogenesis and triglyceride output was examined in perfused livers from rats made hypothyroid by 6*N*-propyl-2-thiouracil (PTU) (1 mg/100 g daily). The effects of glucagon on ketogenesis and triglyceride output was modulated by altered thyroid status. Glucagon caused a marked decrease in ketogenesis and triglyceride output in perfused livers from hypothyroid animals, while livers from hyperthyroid animals showed additional increase in ketogenesis and triglyceride output.

The mechanism(s) by which thyroid status modulates the effect of glucagon to regulate intracellular ketogenesis and triglyceride output may include alteration of intracellular cAMP response to glucagon (Elkeles et al 1975; Gutler et al 1977) and/or glucagon receptors (Madsen & Sonne 1976; Sperling et al 1980). Furthermore, plasma cAMP responses to glucagon were augmented in hyperthyroid (Gutler et al 1977) and diminished in hypothyroid patients (Elkeles et al 1975).

In this work, the effects of thyroid status on the glucagon-dependent cAMP response were investigated. Isolated hepatocytes were used to study the regulation of cAMP and fatty acid metabolism.

MATERIALS AND METHODS

Male rats (125–150 g) were maintained under standard conditions for at least one week before treatment with 6*N*-propyl-2-thiouracil (PTU) (1 mg/100 g s.c.) in 0·9% NaCl at pH 8·5 and administered in the dorsolumbar region daily for seven days.

At the end of the treatment the animals were anaesthetized with ether and the livers were removed as described by Kohout et al (1971) except that the posthepatic inferior vena cava and bile duct were not cannulated. The livers were then mounted in the perfusion apparatus described by Heimberg et al (1964) and which had a hydrostatic pressure of 12 mL water, and were perfused with a Ca^{2+}-free Krebs-Henseleit bicarbonate buffer containing 300 mg glucose dL^{-1}, pH 7·4 at 37 °C in an atmosphere of O_2/CO_2 (95 : 5%) (Berry & Friend 1969; Weiner & Berry 1974). Collagenase was added to give a final concentration of 0·5 mg mL^{-1}, and after 30 min the livers were removed and were placed in beakers with the perfusion medium. They were then minced and shaken at 80–90 oscillations min^{-1} at 37 °C for 10 min. The preparation was filtered through silk sieves and the cell suspension centrifuged at 45*g* for 3 min. The cells were counted using a

* Correspondence.

Coulter Counter and then adjusted to 1·5–2·5 million cells mL^{-1} suspension.

Cell suspensions were shaken at 80–90 oscillations min^{-1} at 37 °C in the presence of FFA-albumin complex, 0·5 mM oleate, 1% bovine serum albumin and 0·0675 μCi mL^{-1} [^{14}C]oleate. Glucagon in glycine buffer (pH 9·0), or glycine buffer alone, was added to the medium, when indicated, to give a glucagon concentration of 10^{-11} to 10^{-5} M. Incubations were for 60 min for studies of ketogenesis at which time 0·5 mL aliquots of cell suspension were assayed for ketone bodies. In companion studies, cells were incubated without radioactive oleate in the medium, and when indicated 100 μL of those cell suspensions were taken for cAMP assay.

The aliquots of cell suspension for analysis of ketone bodies were treated with $Ba(OH)_2$ (0·1 mL, 0·15 M) and $ZnSO_4$ (1·0 mL, 2·5%) (Van Harken et al 1969; Michaels et al 1951). Incorporation of [^{14}C]oleate was measured by mercury precipitation of ketones (Beibendorf et al 1970).

For determination of cAMP, 100 μL of cell suspension was added to 100 μL of 0·3 M perchloric acid and centrifuged. The supernatant was neutralized with 20% KOH. Aliquots were then taken for determination of cAMP by radioimmunoassay using the method of Steiner et al (1969, 1972).

DNA was measured using a modification of the diphenylamine reaction as described by Burton (1956) and Richards (1974).

RESULTS

The rate of ketogenesis and the accumulation of cyclic adenosine-3′,5′-monophosphate (cAMP) in response to glucagon were studied in hepatocytes from euthyroid and hypothyroid rats. The concentrations of cAMP in hepatocytes from either euthyroid or hypothyroid animals generally reached a maximum within 5 min after the addition of glucagon and returned to the basal levels within 15–30 min, depending on the concentration of glucagon in the incubation medium (Figs 1, 2).

The cAMP response to glucagon was first observed at 10^{-9} M and reached a maximal effect at 10^{-6} M in hepatocytes from both euthyroid and hypothyroid animals. Accumulation of cAMP in response to glucagon, however, was substantially reduced in hepatocytes from hypothyroid rats compared with euthyroid controls. The EC50 for glucagon appears to be similar in both groups.

The data in Fig. 3 indicate that accumulation of cAMP by hepatocytes from hypothyroid rats in response to glucagon was less than the euthyroid and never achieved the same maximum. Furthermore, the unchanged EC50 suggests the potency of the hormone was not altered by hypothyroidism.

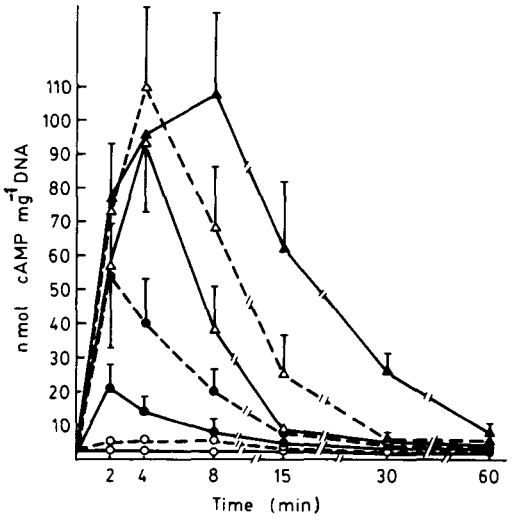

FIG. 1. Time-course and dose-course effects of glucagon on cAMP accumulation in hepatocytes from euthyroid rats. Glucagon concentration: ——▲——, 10^{-5} M; - - -△- - -, 10^{-6} M; ——△——, 10^{-7} M; - - -●- - -, 10^{-8} M; ——●——, 10^{-9} M; - - -○- - -, 10^{-10} M; ——○——, basal level.

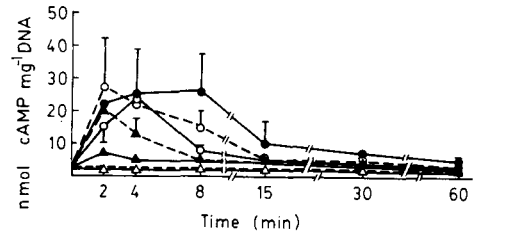

FIG. 2. Time-course and dose-course effect of glucagon on cAMP accumulation in hepatocytes from hypothyroid rats. Glucagon concentrations: ——●——, 10^{-5} M; - - -○- - -, 10^{-6} M; ——○——, 10^{-7} M; - - -▲- - -, 10^{-8} M; ——▲——, 10^{-9} M; - - -△- - -, 10^{-10} M; ——△——, basal level.

The basal rate of ketogenesis by rat isolated hepatocytes was not altered by the mild hypothyroid state induced by treatment with PTU (euthyroid, 4·99 ± 0·47 compared with hypothyroid, 4·56 ± 0·36 μmol [^{14}C]oleate mg^{-1} DNA). Incorporation of [^{14}C]oleate into ketone bodies was increased by addition of glucagon in both groups (Fig. 4). The ketogenic response to glucagon was observed at 10^{-9} M concentration. Maximal rate, at 10^{-5} M concentration, observed for the euthyroid rats was 9·40 ± 0·45 and for the hypothyroid rats 6·63 ± 0·27 μmol

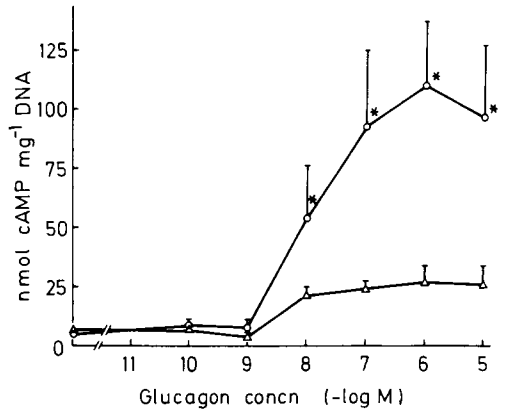

FIG. 3. Dose-response curve of glucagon-stimulated cAMP accumulation in hepatocytes from: euthyroid ——○——; hypothyroid ——△—— rats.

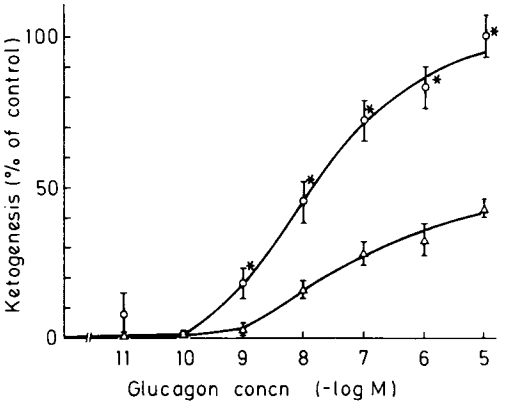

FIG. 4. Modulation by hypothyroidism of the effects of glucagon on hepatic ketogenesis. Euthyroid ——○——; hypothyroid ——△—— rats.

[^{14}C]oleate mg^{-1} DNA. However, the maximal change in the rate of ketogenesis due to glucagon was approximately two-fold in the euthyroid rats compared with the basal rate. Stimulation of ketogenesis in response to glucagon was reduced in hepatocytes from hypothyroid rats compared with euthyroid rats at all concentrations of glucagon; the response was approximately 50% at the maximal glucagon concentration compared with that of the euthyroid group. The data suggest that the maximal effect of glucagon was reduced by hypothyroidism rather than the potency of the hormone, since the EC50 was similar in both groups.

DISCUSSION

The mechanism(s) through which thyroid status influence the effects of glucagon to regulate intracellular ketogenesis and triglyceride output may include alteration by thyroid status of intracellular cyclic adenosine-3′,5′-monophosphate (cAMP) response to glucagon (Elkeles et al 1975; Gutler et al 1977) and for glucagon receptors (Madsen & Sonne 1976; Sperling et al 1980). Also, plasma cAMP responses to glucagon were augmented in hyperthyroid (Gutler et al 1977) and diminished in hypothyroid patients (Elkeles et al 1975). This is relevant to hepatic lipid metabolism because plasma and urinary cAMP are thought to be derived primarily from hepatic production of the nucleotide (Broadus et al 1970). The number of glucagon receptors were found to be increased in adipocytes from hypothyroid rats (Madsen & Sonne 1976), while the number of functional glucagon receptors in livers from hypothyroid rats were reported to be reduced (Sperling et al 1980).

The resistance to the effects of glucagon on hepatic lipid metabolism observed previously in perfused livers from hypothyroid rats (Shaheen et al 1982) was further investigated with isolated hepatocytes in the present study. In confirmation and extension of those studies with perfused livers, incorporation of [^{14}C]oleate into ketone bodes was reduced in hepatocytes from hypothyroid rats compared with the euthyroid group, at all concentrations of glucagon. Furthermore, production of cAMP in response to glucagon was diminished severely in hepatocytes from hypothyroid rats.

The data reported herein suggest that one means by which thyroid hormones modulate the effect of glucagon may be through metabolism or subsequent action of cyclic nucleotidase. Several workers have investigated the effects of thyroid status on glucagon and on adenylate cyclase activity in many tissues in an attempt to explain the modulatory effects of thyroid hormones. The results have been inconclusive and conflicting (Levey et al 1969a, b; Malbon et al 1978; Malbon 1980a, b). Malbon et al (1978) and Malbon (1980b) observed an increase (35%) in [^{125}I]glucagon binding to purified membranes obtained from hypothyroid rat hepatocytes. This increase in binding, however, had no apparent effect on cAMP accumulation or adenylate cyclase activity. In contrast, Sperling et al (1980) observed a decrease in the number of functional liver glucagon receptors in hypothyroid rats.

The evidence presented in this study supports the hypothesis that thyroid hormones and glucagon are related through cAMP metabolism, and that thyroid status appears to regulate, at least in part, the intracellular cAMP concentrations.

REFERENCES

Beibendorf, F. A., Chernick, S. S., Scow, T. O. (1970) J. Clin. Invest. 49: 1685–1689

Berry, M. N., Friend, D. S. (1969) J. Cell. Biol. 43: 506–509

Broadus, A. E., Kaminsky, N. I., Northcutt, R. C., Hardman, J. G., Sutherland, E. W., Liddle, G. W. (1970) J. Clin. Invest. 49: 2237–2242

Burton, K. (1956) Biochem. J. 62: 315–319

Elkeles, R. S., Lazarus, J. H., Siddle, K., Campbell, A. K. (1975) Clin. Sci. Mod. Med. 48: 27–29

Gutler, R. B., Croxson, M. S., DeQuattro, V. L., Warren, D. W., Otis, C. L., Nicoloff, J. T. (1977) Metab. Clin. Exp. 26: 1155–1158

Heimberg, M., Figette, N., Klansner, H. (1964) J. Am. Oil Chem. Soc. 41: 774–779

Kohout, M., Kohoutova, B., Heimberg, M. (1971) J. Biol. Chem. 264: 5067–5073

Levey, G. S., Skelton, C. L., Epstein, S. E. (1969a) Endocrinology 85: 1004–1008

Levey, G. S., Skelton, C. L., Epstein, S. E. (1969b) J. Clin. Invest. 48: 1663–1669

Madsen, S. N., Sonne, D. (1976) Nature 262: 793–798

Malbon, C. C. (1980a) J. Biol. Chem. 255: 8692–8694

Malbon, C. C. (1980b) Mol. Pharmacol. 18: 193–197

Malbon, C. C., Moreno, F. J., Cabelli, R. J., Fain, J. N. (1978) J. Biol. Chem. 253: 671–677

Michaels, G. D., Morgen, S., Liebert, G., Kinsell, L. W. (1951) J. Clin. Invest. 30: 1483–1486

Richards, M. G. (1974) Anal. Biochem. 57: 369–375

Shaheen, O., Morgan, D. W., Wilcox, H. G., Keyes, W. G., Heimberg, M. (1982) Endocrinology 115: 250–257

Sperling, M., Ganguli, S., Voina, S., Kaptein, E., Nicoloff, S. (1980) Ibid. 107: 684–689

Steiner, C. A., Kipnis, M. D., Utiger, R., Parker, C. (1969) Proc. Natl. Acad. USA 64: 367–372

Steiner, C. A., Wehmann, E. R., Parker, W. C., Kipnis, M. D. (1972) Adv. Cyclic Nucl. Res. 2: 51–57

Van Harken, D. B., Dixon, C. W., Heimberg, M. (1969) J. Biol. Chem. 244: 2278–2282

Weiner, H. V., Berry, M. N. (1974) Eur. J. Biochem. 42: 315–321