Plasma adenosine 3':5'-cyclic monophosphate response to glucagon in thyroid disease

R. S. ELKELES, (1) J. H. LAZARUS, K. SIDDLE and A. K. CAMPBELL

Departments of Medicine and Medical Biochemistry, Welsh National School of Medicine

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Summary

1. The plasma adenosine 3':5'-cyclic monophosphate (cyclic AMP) response to intravenously administered glucagon has been studied in nine hyperthyroid, five hypothyroid and ten euthyroid patients.

2. Concentrations of the nucleotide rose to a peak at 15 min and declined to near basal values by 120 min, the response being greatest in hyperthyroid and least in hypothyroid subjects. The mean peak concentrations were 1028 nmol/l in the hyperthyroid group, 252 nmol/l in the hypothyroid group and 534 nmol/l in the euthyroid group.

3. These results could not be accounted for by differences in serum insulin response.

4. It is suggested that the response of plasma cyclic AMP to glucagon may serve as an index of tissue thyroid status.

Key words: plasma cyclic AMP, glucagon, hyperthyroidism, hypothyroidism.

Introduction

It has been suggested that some of the metabolic abnormalities in thyroid disease may be secondary to alterations in the metabolism of adenosine 3':5'-cyclic monophosphate (cyclic AMP) in tissues (Krishna, Hynie & Brodie, 1968; Levey, Skelton & Epstein, 1969a; Caldwell & Fain, 1971). Investigations of this type have largely been concerned with heart and adipose tissue of experimental animals, although metabolic changes have also been studied in the liver in thyroid disease (Freedland, 1965; Dooner, Parada, Aliaga & Hoyl, 1967; Klion, Segal & Schaffner, 1971). Cyclic AMP is widely accepted as the intracellular mediator of the hepatic effects of glucagon (Robison, Butcher & Sutherland, 1971). Glucagon increases the content of cyclic AMP in liver in vivo (Pauk & Reddy, 1971), in perfused liver in vitro (Exton, Robison, Sutherland & Park, 1971), and in organ culture (Siddle, Kane-Maguire & Campbell, 1973). It has also been shown that intravenous glucagon in man produces a large increase in plasma cyclic AMP concentration (Broads, Kaminsky, Northcutt, Hardman, Sutherland & Liddle, 1970) and that the liver is the source of this cyclic AMP (Broads et al., 1970; Liljenquist, Bomboy, Lewis, Sinclair-Smith, Felts, Lacy, Crofford & Liddle, 1974).

Campbell & Kane-Maguire (1973) found that the rise in intracellular cyclic AMP produced by glucagon in rat liver in organ culture was increased by previous incubation with thyroid hormones. In order to ascertain whether thyroid status similarly affects hepatic production of cyclic AMP in man we have investigated plasma cyclic AMP concentration after administration of glucagon to patients with thyroid disease. Measurements have also been made of blood glucose, serum insulin and plasma non-esterified fatty acids (NEFA), both to aid in the interpretation of the cyclic AMP results and to compare with previous data (Levy, Adesman & Spergel, 1970).

Patients and methods

Nine hyperthyroid patients, six female and three...
female (mean age 54.5 years; range 31–79), and five hypothyroid patients, four female and one male (mean age 53.8 years; range 34–65), were studied. The control group comprised ten euthyroid convalescent patients, seven female and three male (mean age 42.2 years; range 23–57). None of the patients had diabetes and all were within 10% of their ideal body weight. Informed consent for the study was obtained from all patients. The thyroid status of the patients was assessed clinically, by measurement of serum thyroxine iodine (Technicon), serum tri-iodothyronine (T3) (Hufner & Hesch, 1973), thyopac-3 (Abbott), free thyroxine index (FTI) and by standard radioiodine uptake tests where appropriate. The values for all indices measured fell within the range appropriate to the assigned thyroid status for the individual patient. Full details of these measurements have been deposited as Clinical Science and Molecular Medicine Table 74/11 with the Librarian, the Royal Society of Medicine, 1 Wimpole Street, London W17 8AE, from whom copies may be obtained on request.

After the patient had fasted overnight, a cannula was inserted into an antecubital vein, which was kept patent by a slow infusion of 154 mmol/l sodium chloride. Patients were then rested for 20 min before the base-line blood sample was drawn. After the intravenous injection of 1 mg of glucagon (Lilly) further blood samples were taken at 5, 15, 30, 60, 90 and 120 min. Blood for cyclic AMP measurement was taken into lithium sequestrene tubes, which were immediately placed on ice. A portion (2 ml) of 0.61 mol/l trichloroacetic acid was added to 2 ml of the separated plasma and the precipitated protein was removed by centrifugation. Trichloroacetic acid was removed from the deproteinized plasma with 4 x 10 ml of ether, and samples were stored at −20°C before assay. Standards were prepared in cyclic AMP-free plasma and treated in the same way as the samples. Cyclic AMP was measured by radioimmunoassay (Siddle et al., 1973). Blood glucose was measured by a glucose oxidase method using an Autoanalyser, serum insulin by the double antibody method of Hales & Randle (1963) and plasma NEFA by the method of Dole & Meinertz (1960).

Results

Plasma cyclic AMP

There were no significant differences in the fasting plasma cyclic AMP concentrations between any of the groups studied. After intravenous injection of

![Fig. 1. Plasma cyclic AMP response to glucagon administered intravenously at 0 min in hyperthyroid (●), euthyroid (●) and hypothyroid (○) subjects. Each point is the mean concentration ±1 standard error.](image-url)
Plasma cyclic AMP in thyroid disease

The mean fasting NEFA concentration (Fig. 4) was higher in the hyperthyroid group (702 ± 81 μmol/l, mean ± SEM; P < 0.02) than in the euthyroid group (362 ± 33 μmol/l), and the concentration in the hypothyroid group was intermediate (512 ± 64 μmol/l; P < 0.05 relative to the euthyroid group). After glucagon injection plasma NEFA concentration fell to a minimum at 30–60 min in the euthyroid and hyperthyroid groups, and then rose above the initial values by 120 min. In the hypothyroid group the fall in NEFA concentration was smaller and delayed, and there was no secondary rise by 120 min.

Discussion

This study has shown that the rise in plasma cyclic AMP concentration in response to intravenous glucagon is increased in hyperthyroid and decreased in...
hypo- and euthyroid rats, but neither tissue cyclic AMP concentrations nor the effects of glucagon were studied. In rat adipose tissue, effects of thyroid hormones in increasing cyclic AMP formation with adrenaline have been demonstrated (Challoner & Allen, 1970; Caldwell & Fain, 1971) and may explain the increased lipolysis associated with hyperthyroidism. Studies of effects of thyroid hormones on adenylate cyclase and cyclic AMP phosphodiesterase of adipose tissue and heart in animals have produced conflicting and inconclusive results (Krishna et al., 1968; Mandel & Kuehl, 1967; Levey & Epstein, 1969; Frazer, Hess & Shanfield, 1969; Sobel, Dempsey & Cooper, 1969; Levey et al., 1969a, b; Broekhuysen & Ghislain, 1972). However, it appears possible from the present results and those of some previous authors that thyroid hormones modulate responsiveness of tissues to acutely acting hormones, as measured by cyclic AMP production.

The increased fasting blood glucose and NEFA concentrations in hyperthyroid subjects (Fig. 2 and Fig. 4) confirm previous results (Levy et al., 1970; Marks, Kiem & Hills, 1960; Hales & Hyams, 1964; Doar, Stamp, Wynn & Audhya, 1969). Stimulation of hepatic glucose production by glucagon is thought to be mediated by cyclic AMP (Robison et al., 1971). However, the increase in blood glucose after glucagon was least in hyperthyroid subjects, as previously reported (Levy et al., 1970), although this group showed the greatest cyclic AMP response (Fig. 1).

There has been controversy concerning serum insulin concentrations and response to glucose in thyroid disease (Hales & Hyams, 1964; Doar et al., 1969; Malaise, Malaise-Lagae & McCraw, 1967; Andreani, Menzinger, Fallucca, Aliberti, Tamburrano & Cassano, 1970; Cavagnini, Peracchi, Raggi, Bana, Pontirolli, Malinverni & Pinto, 1974). The insulin responses to glucagon in the present study (Fig. 3) appear to differ from those reported by Levy et al. (1970), who found that after treatment to render patients euthyroid the insulin response was increased in previously hyperthyroid subjects and decreased in previously hypothyroid subjects.

It seems likely that plasma cyclic AMP response to glucagon reflects an hepatic effect of thyroid hormone. Cyclic AMP measurement after intravenous glucagon could provide a tissue index of thyroid status more sensitive than those in current use.

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References


