CONCURRENT MEASUREMENTS OF ADENOSINE 3', 5'-MONOPHOSPHATE, TYROSINE AMINOTRANSFERASE AND α-AMINOISOBUTYRATE UPTAKE IN RAT LIVER AFTER INJECTION OF GLUCAGON*

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Received 9 January 1971

1. Introduction

Cyclic AMP has been proposed as the active agent in induction of tyrosine aminotransferase ([1,2]; TAT: EC 2.6.1.5), increased uptake of α-aminoisobutyrate [3–5], AIB) or both [6,7]. However, none of these studies have demonstrated that cyclic AMP levels change either before or co-incident with the increases in either TAT activity or AIB uptake. This is an essential requirement which must be fulfilled before a role for cyclic AMP can be established [8]. This paper reports the results of concurrent measurements of cyclic AMP, TAT, and AIB uptake in rat livers at various times after the injection of glucagon. These results indicate that hepatic cyclic AMP levels do increase after injections of glucagon before increases in TAT activity and AIB uptake are observed.

2. Materials and methods

Rats were obtained from the Charles River Breeding Laboratories (Wilmington, Massachusetts, USA) at 36 days of age. The rats were housed in a windowless room with the lighting regulated to give alternating 12 hr periods of light and dark and were given a 30% protein diet on an '8 + 16' feeding schedule [9]. No food was available for 18 hr before the experiments were begun, and food was withheld during the experiments. The rats were injected with either 0.2 mg glucagon in 0.5 ml of isotonic NaCl per 100 g of body weight, given subcutaneously instead of intraperitoneally on the basis of previous experiments [12], 9.0 mg theophylline in 1.0 ml of saline per 100 g of body weight, given intraperitoneally; or both glucagon and theophylline. Those animals not receiving injections of both glucagon and theophylline were given a balancing injection of saline such that each animal received the same number of injections and the same volume per 100 g body weight. Rats were killed by decapitation and the blood was collected in beakers containing heparin. The livers were quickly excised and frozen in liquid nitrogen. The livers were powdered, weighed and rapidly homogenized in either 5% TCA (v/v), for cyclic AMP samples or in 0.15 M NaCl for TAT samples. The injection of AIB as well as the preparation of the enzyme and plasma samples was as previously described [6]. TAT was assayed by the procedure of Diamondstone [10]. The preparation and subsequent assay of the cyclic AMP was as described elsewhere [11]. The AIB data are expressed as the ratio between the dpm per g liver and the dpm per ml of plasma.

* Financial support was provided by departmental grant CA-07175, Training Grant T01-CA 5002 and USPHS Post-doctoral Fellowship, 1-F02-CA-32,836 (DFS) and 1-F02-CA-43,880 (FRB) through the National Cancer Institute.

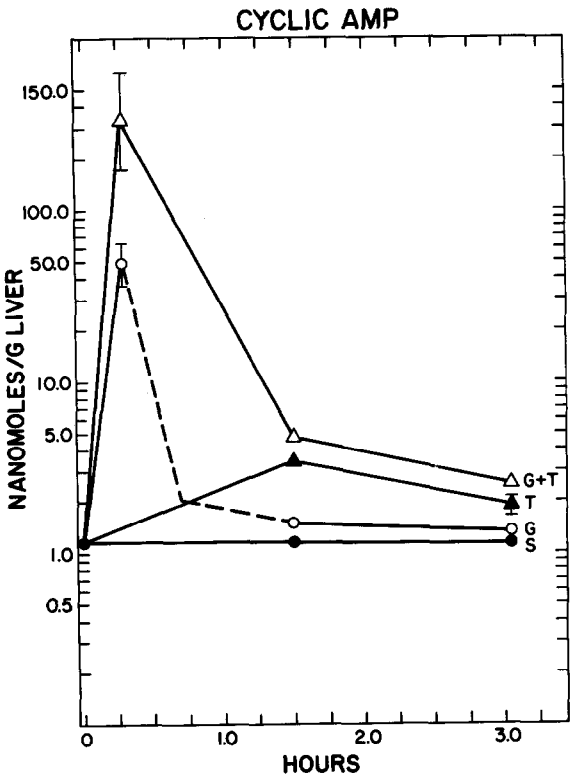

Fig. 1. Time course following cyclic AMP levels in rat liver after injection of: glucagon (G), theophylline (T), glucagon plus theophylline (G+T), or saline (S). The doses are given in the text. The data are expressed as the mean ± standard error of values from 3 animals per time point. The standard error falls within the character for those points where no standard error is indicated. The dashed lines between the 20 min and 1.5 hr time points indicate the decline of cyclic AMP concentration, after injection of glucagon, which we have observed in other experiments.

3. Results

The results in fig. 1 show that glucagon increases the hepatic level of cyclic AMP 50-fold above the control at 20 min. If theophylline, an inhibitor of the phosphodiesterase [8], is injected with the glucagon the hepatic cyclic AMP levels are increased to 120 times the control value at 20 min. Subsequent to the 20 min time point the levels decline to 1.4 and 4.5 times the control at 1.5 hr for the animals receiving glucagon and glucagon plus theophylline respectively. Theophylline increased the level of hepatic cyclic AMP to three times the control at 1.5 hr. A detailed time course for the effect of theophylline or glucagon on hepatic AMP levels will be reported in detail elsewhere [12].

Data in fig. 2 shows the level of TAT and of AIB uptake at 1.5 hr and 3.0 hr after the various treatments. Measurements of AIB uptake at 20 min (not shown) have previously established that there is no effect of glucagon and a slight effect of glucagon plus theophylline on the liver/plasma ratio due to a drop in the plasma level of AIB. Similarly, there is no effect of glucagon plus theophylline on TAT activity at 20 min (not shown). The differences in the means of the AIB uptake or of TAT activity at 1.5 or at 3 hr are not significantly different, within either of the individual time points, except for AIB uptake at 1.5 hr after injection of glucagon.

Comparison of data in figs. 1 and 2 demonstrates that the hepatic cyclic AMP levels change in response to glucagon or glucagon plus theophylline before significant changes are observed in either TAT of AIB.

4. Discussion

Sutherland et al. [8] have proposed a series of criteria which must be fulfilled before cyclic AMP can be considered the mediator of a hormonal response. One of these criteria requires that the intracellular concentration of cyclic AMP should either increase before or at least coincident with the response studied. In the present report the responses studied were TAT activity and AIB uptake. It was demonstrated that the hepatic cyclic AMP levels increase in response to glucagon or glucagon plus theophylline before an increase in either TAT or AIB uptake is observed; thus, satisfying one of the criteria stated by Sutherland et al. [8].

A previous report from this laboratory [6] has stated that injection of dibutyryl AMP increases both TAT and AIB uptake. These findings satisfy another criterion of Sutherland et al. [8] which states that cyclic AMP or dibutyryl cyclic AMP should elicit the response(s) directly.

Another criterion of Sutherland [8] states that the responses studied should be potentiated by the addition of phosphodiesterase inhibitors. We do not

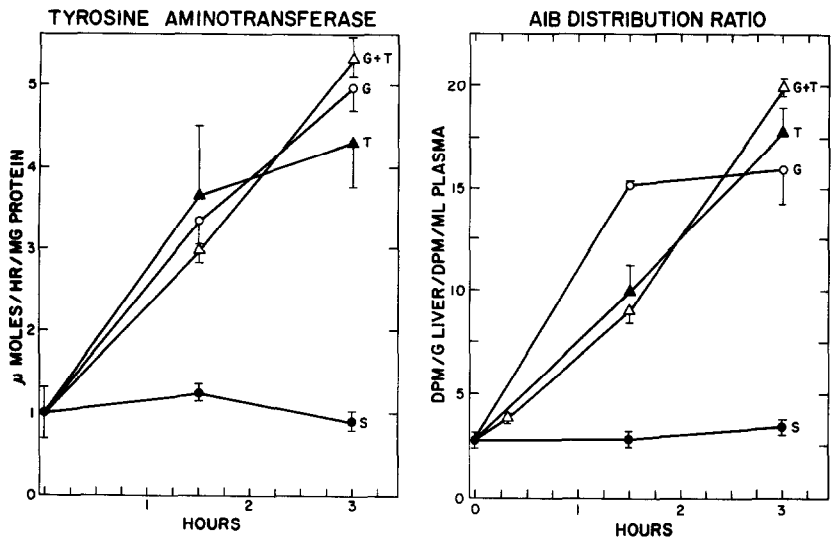

Fig. 2. Time course following changes in TAT activity and AIB distribution ratio. Characters are as described in legend to fig. 1. The data are expressed as the mean ± the standard error of values from 3 animals per group.

obtain a potentiation of the effect of glucagon on TAT activity or on AIB uptake when theophylline, an inhibitor of the phosphodiesterase, is given with glucagon; however, theophylline plus glucagon gives a much greater increase in hepatic cyclic AMP levels than does glucagon alone. This can be explained if one assumes that the systems responsible for induction of TAT and increased AIB uptake can be saturated with respect to cyclic AMP. Thus, increasing the concentration of cyclic AMP above the saturating concentration would not lead to a greater response. If, in the present study, glucagon alone gave a concentration of cyclic AMP which would maximally induce TAT and AIB uptake, then a further increase in the concentration of cyclic AMP should not give a higher level of TAT activity or AIB uptake.

Although the data of this report and of a previous report from this laboratory [6] strongly suggest cyclic AMP as the mediator of the induction of TAT and of increased AIB uptake by glucagon, alternative approaches should still be pursued. Such approaches should consider the possible involvement of calcium as has been proposed by Rasmussen [13]. In addition reagents which will block the action of glucagon on adenyl cyclase or inhibit adenyl cyclase directly should be sought as an alternative approach.

Acknowledgements

The authors wish to acknowledge the excellent technical help of Mr. M. Mecikalski.

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