POTENTIATING EFFECT OF A PHYSIOLOGICAL DOSE OF CORTISONE ACETATE ON THE DIBUTYRYL CYCLIC AMP-MEDIATED INDUCTION OF TYROSINE AMINOTRANSFERASE IN RAT LIVER

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1. Introduction

The stimulatory effect of glucocorticoids and cyclic AMP (or glucagon) on the induction of hepatic tyrosine aminotransferase (EC 2.6.1.5) (TAT) has well been documented and its de novo synthesis was evidenced in rat liver [1,2] and in cultured cells [3]. Given together with glucocorticoids to animals or to cultured cells, cyclic AMP exerts a more than additive effect in the induction of TAT [2–4]. Recent work of Krone et al. [5] suggests a role of the pharmacological dose of hydrocortisone in maintaining the induced level of TAT and phosphenolpyruvate carboxykinase (GTP) (EC 4.1.1.32) brought about by N^6, $O^{2'}$-dibutyryl cyclic AMP (DBcAMP) in rat liver. Early works from our laboratory revealed that administration of a physiological dose of glucocorticoids is essential to the substrate- or L-tryptophan-mediated stimulation of TAT in the liver of adrenalectomized rats [6–8]. In contrast to TAT the DBcAMP-mediated induction of L-serine:pyruvate aminotransferase (EC 2.6.1.51) is inhibited by cortisone acetate at a dose which stimulates TAT in rat liver [9,10]. The present study demonstrates that a physiological dose of cortisone acetate restores the reduced response of the enzyme to the DBcAMP-mediated induction in adrenalectomized rats nearly to the level of intact animals.

2. Materials and methods

2.1. *Animals and their treatments*

Female Wistar rats weighing 120–150 g were maintained at 25°C under the controlled lighting (12 hr) and darkness (12 hr) on a laboratory chow (Altromin GmbH, Lage) and water ad libitum. Adrenalectomy was performed bilaterally under ether anesthesia and the animals were fed the chow and physiological saline as a drinking water usually for 6 to 8 days until the animals were killed. They were sacrificed between 08^{00} and 13^{00} h in order to avoid the possible diurnal alteration in the enzyme activity [11]. Glucgon (Serva Feinbiochemica), DBcAMP, actinomycin D (Boehringer Mannheim) were injected intraperitoneally.

2.2. *Preparation of enzyme extracts and the enzyme assay*

Rats were killed by decapitation and the livers were quickly removed, chilled over ice, weighed and homogenized in 5 vols. of ice cold 50 mM sodium pyrophosphate buffer (pH 7.6) containing 0.14 M KCl, 1 mM EDTA and 10 μM pyridoxal 5′-phosphate in a Ultra-Turrax homogenizer at 2000 rev/min for 20 sec. The homogenate was centrifuged at 770 *g* for 10 min and the resultant supernatant was further diluted with 3 vols of the homogenization buffer for the assay of tyrosine aminotransferase by the method of Rosen et al. [12]. The units of enzyme activity were defined as μmoles of *p*-hydroxyphenylpyruvate formed per hr under the assay conditions.

2.3. *Plasma glucocorticoid determination*

Glucocorticoids in rat plasma were determined by the competitive protein-binding radioassay [13] using Cortipac radioassay kit (Amersham Buchler GmbH & Co. KG).

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3. Results

Fig.1 illustrates the change in the inducibility of TAT by DBcAMP and plasma level of glucocorticoid following adrenalectomy of rats. Degree of induction by the cyclic AMP rapidly decreases following operation with a half-time of about 40 min and reaches a constant level by 5 hr, at which time, however, DBcAMP is still able to induce the enzyme to an extent of 40% of the unoperated control level. The reduced postoperational response even persists for 8 days without any decline. Plasma glucocorticoid level decreases more rapidly and pronounced; it reaches a minimal level within 60 min after operation. As shown in fig.2 the response of the enzyme to DBcAMP is considerably lower in adrenalectomized rats than in the intact animals at all dose levels of DBcAMP tested. Given cortisone acetate at a dose of 0.2 mg/100 g body weight simultaneously with nucleotide inducer, which alone does not influence the basal enzyme level (fig.2), the reduced inducibility appreciably restores at all dose levels of the inducer. In all these three groups of rats, however, the efficiency of DBcAMP, as estimated from the half-maximal dose of DBcAMP, was almost identical (2 mg/100 g body weight). These results clearly demonstrate a 'potentia-

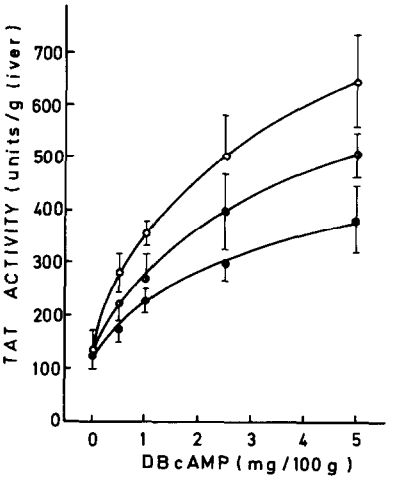

Fig.2. DBcAMP-dose depence of TAT induction in the liver of intact, adrenalectomized or cortisone-treated adrenalectomized rats. Intact rats, adrenalectomized rats at 8 days after operation and such animals received cortisone acetate (0.2 mg/100 g) were injected with varying dose levels of DBcAMP and the enzyme was assayed 3 hr later. Data are mean ± S.E. for 5 or 6 rats. (○) intact; (●) adrenalectomized; (◑) cortisone-treated adrenalectomized rats.

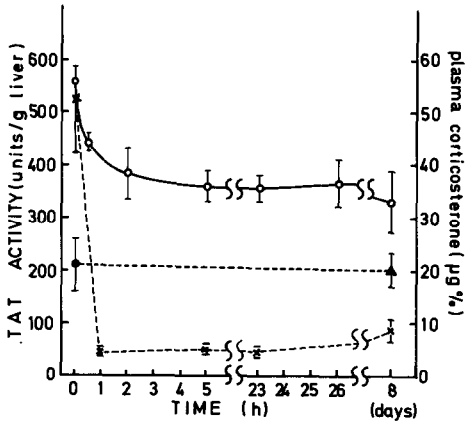

Fig.1. Change in the inducibility of TAT by DBcAMP following adrenalectomy. Rats at various times after adrenalectomy were injected with DBcAMP (3 mg/100 g) and the enzyme in the liver was assayed 3 hr later. Data are mean ± S.E. for 5 or 6 rats. (○—○) induced by DBcAMP; (●) intact rats without DBcAMP; (▲) rats at 8 days after operation, without DBcAMP; (X---X) plasma corticosterone level.

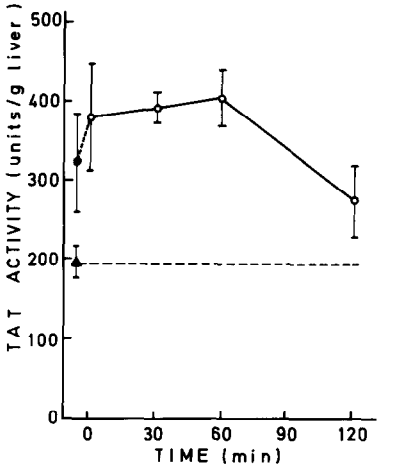

Fig.3. Change in the inducibility of TAT by DBcAMP following injection of cortisone acetate. Adrenalectomized rats at various times after cortisone acetate injection (0.2 mg/100 g) received DBcAMP (3 mg/100 g) and the enzyme was assayed 2 hr later. Data are mean ± S.E. for 5 or 6 rats. (○—○) induced by DBcAMP after cortisone acetate; (●) induced by DBcAMP before cortisone acetate; (▲) basal level without DBcAMP.

ting' effect of cortisone acetate on the response of TAT to the DBcAMP-mediated induction. The response of the enzyme to glucagon can be equally potentiated by such a dose of cortisone acetate (data are not shown). Lower doses of cortisone acetate failed to show such an effect. The inducibility of the enzyme by DBcAMP was then followed at various times after cortisone injection into adrenalectomized rats (fig.3). The potentiating effect of cortisone acetate continues for at least 60 min and is no longer detectable 120 min after the glucocorticoid injection.

Fig.4 represents the effect of such a dose of cortisone acetate on the time course of DBcAMP-mediated TAT induction. Cortisone acetate, which alone does not affect the TAT level (fig.3), promotes the initial rate of enzyme induction by DBcAMP and the activity reaches a maximum 3 hr after injection of drugs as is the case of control animals without cortisone. Given cortisone at the time of activity peak (3 hr), the rate of the following spontaneous activity decline is significantly prevented. However, cortisone has no effect on the rate of activity decay caused by

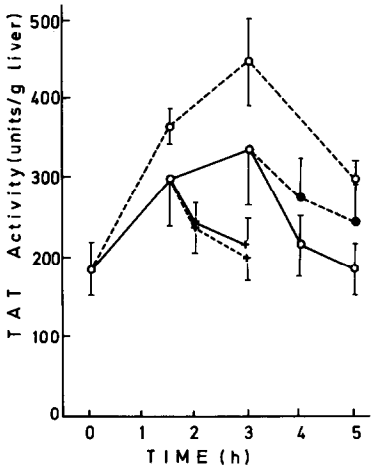

Fig.4. Time course of the effect of cortisone acetate on the DBcAMP-mediated induction of TAT. Adrenalectomized rats were injected with DBcAMP (3 mg/100 g), cortisone acetate (0.2 mg) or cycloheximide (0.3 mg) alone and in combination as follows: DBcAMP at zero time alone (○—○), DBcAMP and cortisone acetate at zero time (○---○), DBcAMP at zero time and cortisone acetate 3 hr later (●---●), DBcAMP at zero time and cycloheximide at 1.5 hr without (X—X) and with cortisone acetate (X---X). Data are mean ± S.E. for 5 or 6 rats.

Table 1
Effect of actinomycin D on the induction of tyrosine aminotransferase by DBcAMP in the presence and absence of cortisone acetate

Administration	Actinomycin D	Tyrosine aminotransferase (units/g liver)
None	–	228.5 ± 43.5
DBcAMP	–	325.0 ± 40.7
	+	212.0 ± 20.6
DBcAMP	–	432.0 ± 58.0
+ Cortisone acetate	+	179.5 ± 22.0

Adrenalectomized rats received actinomycin D (100 μg/100 g) 30 min prior to DBcAMP (3 mg/100 g) or DBcAMP plus cortisone acetate (0.2 mg/100 g) and the enzyme was assayed 3 hr after the inducers. Data are mean ± S.E. for 5 rats.

cycloheximide injection at a rapidly inducing phase (1.5 hr).

Actinomycin D at a dose of 100 μg/100 g completely depresses the effect of DBcAMP both in the presence and absence of cortisone acetate (table 1). The result indicates that RNA synthesis is involved both in the cortisone-dependent and -independent induction of TAT by DBcAMP.

4. Discussion

The impaired response of TAT to DBcAMP (or glucagon) in adrenalectomized rats can be restored by injecting a low dose of cortisone acetate, implicating a physiological role of glucocorticoids in the cyclic AMP-mediated induction of the enzyme. Since the reduced inducibility in adrenalectomized rats, about 40% of that in intact rats, persists for at least 8 days after operation, the possibility can be excluded that this 40% fraction of the enzyme induction is due to the remaining m-RNA or other factors required for TAT translation, that have been produced in the liver in response to endogenous glucocorticoids prior to adrenalectomy. Thus two actinomycin D-sensitive sites, glucocorticoid-dependent and - independent sites, are likely to be involved in the TAT induction by cyclic AMP. As the effective doses of DBcAMP do not differ from each other among the animals with and without glucocorticoids (fig.2), it seems probable

that cortisone acetate increases the amount of an unknown key factor which degrades rapidly but is required in common for the enzyme induction by cyclic AMP both in the presence and absence of glucocorticoids. In the later case this factor might be regulated by substances other than glucocorticoids. Whereas independent sites in target cells are suggested for the action of glucocorticoids and cyclic AMP [14], there have been evidences for a synergistic effect between these hormones on TAT induction [2–4]. It has been evidenced from in vivo and in vitro experiments that glucocorticoids do not facilitate the uptake of cyclic AMP by liver cells [4,5,15]. Recently Krone et al. [5] described a rapid decline in the induced level of TAT by a repeated injection of DBcAMP to basal one in adrenalectomized rats, which is prevented by hydrocortisone, whereas no such a decline was observed by ourselves (data not shown) and by Wicks et al. [2] in rat liver under the similar conditions and by Butcher et al. [4] in H-35 hepatoma cell culture. In all these previous works glucocorticoids were applied in such an amount, that it alone can induce the enzyme markedly. In the present work we found a 'potentiating' effect of cortisone on the cyclic AMP-mediated induction of the enzyme by administrating a low dose of the glucocorticoid, which alone does not induce the enzyme. It seems likely that the previously observed synergistic effect is, in part at least, a reflex of this potentiating effect. Present result using cycloheximide as an inhibitor of TAT biosynthesis implicates that the effect of a small dose of cortisone acetate is not due to the stabilization of enzyme but to the stimulating action on the de novo TAT synthesis. The question, however, is to be solved directly in more elaborated experimental systems.

5. Acknowledgements

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