Enhancement of rat brain tyrosine aminotransferase activity by cortisol

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Administration of cortisol to rats enhanced brain tyrosine:2-oxoglutarate aminotransferase activity by a mechanism excluding stabilization, but possibly involving enhanced synthesis, of the enzyme.

Although a number of studies concerning the properties of rat brain tyrosine:2-oxoglutarate aminotransferase (EC 2.6.1.5), including cofactor requirements, inhibition by catecholamines and other agents, intracellular compartmentation, reaction mechanism and structure, have been reported (Gibb & Webb, 1969; Miller & Litwack, 1969; Mandel & Aunis, 1974; Badawy *et al.*, 1980b), very little work has been done on its possible functions and regulation. In relation to this latter aspect, it has been reported that the enzyme activity is enhanced by acute administration of ethanol, tyrosine (Badawy *et al.*, 1980b) or cortisol (Laboritt & Thuret, 1977). On the other hand, Mandel & Aunis (1974) reported (without providing supporting data) that cortisol does not enhance the enzyme activity. In view of these latter two contradictory reports, we have performed in the present paper a detailed investigation of the effects of cortisol on rat brain tyrosine aminotransferase activity and found that it is enhanced.

Materials and methods

Locally bred male Wistar rats (150–170 g) were maintained on cube diet 41B (Oxoid, Basingstoke, Hants., U.K.) and water and were killed by decapitation between 14:00 and 14:30 h. The brains were rapidly removed (within 20 s of the death of the animals), immersed in liquid N₂ for 3 min and stored at –20°C for up to 24 h before analysis. Badawy *et al.* (1980b) found that freezing of brain for 24 h does not cause any significant changes in tyrosine aminotransferase activity.

Actinomycin D was a gift from Merck, Sharp and Dohme (Hoddesdon, Herts., U.K.), whereas all other chemicals (of the purest commercially avail-

able grades) were purchased from standard suppliers.

Chemicals were intraperitoneally administered. Cortisol acetate (2.5–50 mg/kg body wt.) was dissolved in dimethylformamide and appropriate control rats received an equal volume (1 ml/kg body wt.) of this solvent. Actinomycin D (0.7 mg/kg body wt.) or cycloheximide (10 mg/kg body wt.) was dissolved in 0.9% (w/v) NaCl and control rats received an equal volume (2 ml/kg body wt.) of 0.9% NaCl.

Brain tyrosine aminotransferase activity was determined in whole homogenates by the method (Badawy *et al.*, 1980a) used for the assay of the hepatic enzyme, but with some modifications. These were: (1) the incorporation into the 6.4 ml incubation mixture of 0.6 ml of a 16% (v/v) solution of Triton X-100 (in the 0.2 M-potassium phosphate buffer, pH 7.3); (2) pre-incubation of all components of the reaction mixture (except the 2-oxoglutarate solution) for 15 min at 37°C; (3) performing the incubation at 37°C for 45 min instead of the shorter time intervals used with the hepatic enzyme. Whole brain homogenates were prepared by homogenizing the frozen tissue in 4 vol. of 0.14 M-KCl/2.5 mM-KOH (at 0–2°C) for 1 min by using an Ultra-Turrax homogenizer. Portions (0.1 ml each) of this homogenate were then assayed.

The half-life of brain tyrosine aminotransferase was determined by the method previously described (Badawy *et al.*, 1980a) for that of the hepatic enzyme. Statistical analysis of results was performed by using Student's *t* test.

Results and discussion

The time course of the effects of administration of a 20 mg/kg body wt. dose of cortisol acetate on rat brain tyrosine aminotransferase activity is shown in Fig. 1. No significant changes in enzyme activity were observed at 1 or 2 h. By contrast, the first

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Fig. 1. Time course of the effects of cortisol administration on rat brain tyrosine aminotransferase activity. Cortisol acetate (20 mg/kg body wt.) was intraperitoneally administered at zero-time. The enzyme activity was determined as described in the Materials and methods section either in the absence (holoenzyme activity; ●) or in the presence (total enzyme activity; ○) of added (40 μ M) pyridoxal 5'-phosphate. Values are means $\pm$ S.E.M. for each group of four rats.

significant increases in both the holoenzyme and total enzyme activities (31 and 32% respectively; $P < 0.02$) occurred at 3 h. The above activities were then further increased, by 55% and 62% at 4 h, and by 71% and 59% at 5 h. After these latter maximum increases, the activities of the enzyme gradually returned to the basal values at 8 h. In experiments not shown here, it was found that tyrosine aminotransferase activities were not significantly altered at subsequent time intervals (up until 24 h) after cortisol administration. The failure of cortisol acetate to alter the enzyme activity at 2 h is in contrast with the enhancement reported by Laboritt & Thuret (1977). The latter authors, however, did not specify the form of cortisol administered; it is possible that they might have used a more rapidly absorbable water-soluble salt in view of their comparison of the cortisol effects with those of 0.9% NaCl.

The effects of administration of various doses of cortisol acetate were also examined at 5 h (Fig. 2).

Fig. 2. Effects of administration of various doses of cortisol on rat brain tyrosine aminotransferase activity. Rats received an intraperitoneal injection of either cortisol acetate (2.5–50 mg/kg body wt.) or an equal volume (1 ml/kg body wt.) of the solvent dimethylformamide and were killed 5 h later. The enzyme activity was determined as described in the Materials and methods section either in the absence (holoenzyme activity; ●) or in the presence (total enzyme activity; ○) of added (40 μ M) pyridoxal 5'-phosphate. Values are means $\pm$ S.E.M. for each group of four rats.

No significant changes in enzyme activities were observed with doses of up to 5 mg/kg body wt. The 7.5 mg/kg body wt. dose caused a 22% increase ($P < 0.001$) in the holoenzyme activity. Activities of both the holoenzyme and total enzyme were then increased in a dose-dependent fashion, with the 30 mg/kg body wt. dose causing the maximum increases (99% and 95% respectively; $P < 0.001$). These results therefore demonstrate the ability of administered cortisol to enhance rat brain tyrosine aminotransferase activity.

The cortisol induction of liver tyrosine aminotransferase has been much studied and it is now well established that the hormone does not stabilize this hepatic enzyme, but that it enhances its synthesis at the mRNA level (see e.g. Nickol *et al.*, 1978). To find out if cortisol acts similarly on the activity of the brain enzyme, further experiments were performed. The results in Table 1 show that the enhancement of both the holoenzyme and total enzyme activities observed at 5 h after cortisol administration (75% and 59% respectively; $P < 0.001$) was abolished by

Table 1. *Prevention by actinomycin D and cycloheximide of the enhancement of rat brain tyrosine aminotransferase activity by cortisol*

Rats were killed at 5 h after intraperitoneal administration of cortisol acetate (20 mg/kg body wt.) or an equal volume (1 ml/kg body wt.) of the solvent dimethylformamide. The animals were also pretreated, 30 min before the above two agents, with actinomycin D (0.7 mg/kg body wt.), cycloheximide (10 mg/kg body wt.) or an equal volume (2 ml/kg body wt.) of 0.9% (w/v) NaCl. The enzyme activity was determined as described in the Materials and methods section either in the absence (holoenzyme activity) or in the presence (total enzyme activity) of added (40 μ M) pyridoxal 5'-phosphate. The values given are means $\pm$ S.E.M. for each group of four rats. The holoenzyme and total enzyme activities in cortisol-treated rats are compared with those in control (dimethylformamide-treated) animals and the significance of differences is indicated as follows: * $P < 0.001$.

Treatment . . .	Tyrosine aminotransferase activity (μ mol of 4-hydroxyphenylpyruvate formed/h per g wet wt. of brain)			
	Dimethylformamide		Cortisol	
	Holoenzyme activity	Total enzyme activity	Holoenzyme activity	Total enzyme activity
Pretreatment				
0.9% NaCl	2.78 $\pm$ 0.18	3.72 $\pm$ 0.14	4.86 $\pm$ 0.09*	5.91 $\pm$ 0.20*
Actinomycin D	2.89 $\pm$ 0.24	3.66 $\pm$ 0.08	2.79 $\pm$ 0.17	3.45 $\pm$ 0.19
Cycloheximide	2.71 $\pm$ 0.15	3.75 $\pm$ 0.14	2.76 $\pm$ 0.05	3.84 $\pm$ 0.05

pretreatment of rats with either actinomycin D or cycloheximide. Neither agent exerted any significant effects ($P > 0.10$) on the basal enzyme activities in control (dimethylformamide-treated) rats. It may therefore be concluded that the cortisol-induced enhancement of rat brain tyrosine aminotransferase activity is sensitive to blockade by inhibitors of transcription and translation, and this is consistent with the hormone enhancing the mRNA-dependent synthesis of the enzyme.

To find out if cortisol alters the half-life of brain tyrosine aminotransferase, cycloheximide (10 mg/kg body wt.) was administered at 5 h after rats had already received an injection of cortisol acetate (20 mg/kg body wt.) or an equal volume of dimethylformamide and the enzyme activities were again determined at 1 and 2 h after the cycloheximide injection. The total enzyme activity (in μ mol of 4-hydroxyphenylpyruvate formed/h per g wet wt. of brain $\pm$ S.E.M. for each group of four rats) at 0, 1 and 2 h after cycloheximide administration to control (dimethylformamide-pretreated) rats was 3.42 $\pm$ 0.14, 2.76 $\pm$ 0.11 and 2.06 $\pm$ 0.05 respectively. The corresponding total enzyme activity of cortisol-pretreated rats was 5.88 $\pm$ 0.12, 4.57 $\pm$ 0.14 and 3.58 $\pm$ 0.10 respectively at 0, 1 and 2 h after cycloheximide administration. A plot of each of these two sets of data on semi-logarithmic paper revealed that the half-life of the total enzyme in control rats (2.63 h) was not much altered (2.53 h) by cortisol. Similar experiments and plots were also performed in relation to the holoenzyme and the half-life of this was found to be 2.61 and 2.64 h in control and cortisol-pretreated rats respectively. These results therefore suggest that cortisol does not

stabilize rat brain tyrosine aminotransferase. The half-lives of the basal enzyme (that in rats treated with 0.9% NaCl 3 h before cycloheximide) have previously been shown (Badawy *et al.*, 1980b) to be 2.38 and 2.49 h for the holoenzyme and total enzyme respectively.

In conclusion, the findings of the present paper have demonstrated the ability of administered cortisol to enhance rat brain tyrosine aminotransferase activity by a mechanism excluding stabilization of the enzyme, but possibly involving an increase in its synthesis. These findings and those (Badawy *et al.*, 1980b) demonstrating the ability of administered tyrosine to enhance this cerebral enzyme suggest that brain tyrosine aminotransferase may be subject to regulation by glucocorticoids and by its tyrosine substrate. Further work is, however, required to examine these possibilities.

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