TRYPTOPHAN FORCE-FEEDING: CHANGES IN THE ACTIVITIES OF ACETYLCHOLINESTERASE IN VARIOUS TISSUES OF WELL-FED NORMAL AND ADRENALECTOMIZED RATS

Atif M. Nakhla and Adhip P. Nandi Majumdar
Institute of Medical Biochemistry, University of Aarhus
DK-8000 Aarhus C, Denmark

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SUMMARY

The activity of acetylcholinesterase in the liver, heart, spleen, lungs and kidneys of well-fed normal and adrenalectomized rats was measured following a single tube-feeding of tryptophan. In well-fed normal rats, 30 min after tryptophan force-feeding, the enzyme activity in the heart and lungs was stimulated by 28 and 25% as compared to the water-fed control while in well-fed adrenalectomized rats acetylcholinesterase acticity in the heart, liver spleen and lungs was 40, 31, 22 and 15% increased, respectively over that of the corresponding control. In both groups of rats the enzyme activity in the kidney was unaffected by tryptophan. In the liver, spleen and heart of well-fed adrenalectomized rats the pattern of response for acetylcholinesterase to a tryptophan dose, over a period of 24-hr. was found to be biphasic. In well-fed adrenalectomized rats the tryptophan-mediated stimulation of acetylcholinesterase activity in the heart was found to be insensitive to actinomycin-D. The tryptophan-mediated stimulation of acetylcholinesterase activity in the heart of well-fed normal and adrenalectomized rats could not be related to the presence of an activator.

In recent years we have demonstrated that a single tube-feeding of tryptophan to well-fed adrenalectomized rats markedly stimulates amino acid incorporation into a number of hepatic proteins (1-3), as well as total proteins of liver, brain and kidneys (2). In our attempt to study the responsiveness of the brain to tryptophan under different physiological conditions, we have measured acetylcholinesterase (AChE) activity in the brain of intact and adrenalectomized rats after tryptophan force-feeding (4). We have observed that in both intact and adrenalectomized rats tryptophan enhances cerebral but not

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cerebellar AChE activity, and the magnitude of stimulation is higher in adrenalectomized rats as compared to the intact animals (4). The observation that tryptophan stimulates only cerebral AChE activity suggests further that the enzyme of different regions of the brain does not respond similarly to tryptophan stimulus. This prompted us to investigate the responsiveness of the enzyme to tryptophan in various other tissues (liver, heart, lungs, spleen and kidneys) of well-fed (non-fasted) normal and adrenalectomized rats.

MATERIALS AND METHODS

Adult male Wistar rats (220-250 g) were maintained on ad. lib. commercial laboratory diet throughout the experimental period. Bilateral adrenalectomy was performed 6 days before use. The animals were treated the same way as reported earlier (1,2). On the day of the experiment, rats were given by stomach tube either L-tryptophan (30 mg/100 g) in distilled water or an equivalent volume of water alone and killed at different intervals. The animals were killed by decapitation, and the liver, heart, lungs, spleen and kidneys were rapidly removed and frozen immediately on solid CO_2.

Acetylcholinesterase activity in various tissues was determined by the method of Ellman et al. (5) with acetylthiocholine as substrate as reported earlier (4).

RESULTS AND DISCUSSION

In the first series of experiments AChE activity in various organs of well-fed normal and adrenalectomized rats was measured after 0.5 hr of tryptophan force-feeding. In well-fed normal rats a further force-feeding of tryptophan enhanced the enzyme activity in the heart and lungs by 28 and 25%, respectively, but had no effect on the liver, spleen and kidneys (Table - 1). However, in the well-fed adrenalectomized rats AChE in the heart, lungs, spleen and liver was 40, 15, 22 and 31 increased, respectively, above the control following tryptophan treatment (Table - 1). In both normal

TABLE 1

Effect of tryptophan force-feeding on the activity of acetylcholinesterase in various tissues of well-fed normal and adrenalectomized rats.

Tissues	Acetylcholinesterase activity (μmoles per min per g of wet tissue)			
	Normal		Adrenalectomized	
	Control	Tryptophan treated	Control	Tryptophan treated
Heart	2.44 ± 0.11 (100)	3.14 ± 0.11 (128, P < 0.005)	2.07 ± 0.05* (100)	2.91 ± 0.04 (140, P < 0.001)
Lungs	1.12 ± 0.07 (100)	1.40 ± 0.09 (125, P < 0.05)	0.89 ± 0.06** (100)	1.02 ± 0.05 (115, P < 0.1)
Spleen	2.65 ± 0.11 (100)	2.34 ± 0.09 (88, NS)	2.38 ± 0.07 (100)	2.89 ± 0.13 (122, P < 0.01)
Liver	1.15 ± 0.10 (100)	1.25 ± 0.03 (109, NS)	1.06 ± 0.04 (100)	1.40 ± 0.03 (131, P < 0.001)
Kidneys	0.25 ± 0.02 (100)	0.24 ± 0.01 (98, NS)	0.20 ± 0.03 (100)	0.21 ± 0.02 (106, NS)

Intact normal and adrenalectomized rats were killed 0.5 h after water or tryptophan force-feeding. Each value represents the mean ± S.E.M. of 6-8 determinations from 3 rats. The values in the parentheses represent percentage of the respective water-fed control as well as P value. NS = non significant.

* $P < 0.025$, compared with water-fed normal control.

** $P < 0.05$, compared with water-fed normal control.

and adrenalectomized rats administration of tryptophan caused no enhancement in the enzyme activity in kidneys.

The above observation of a higher stimulatory effect of tryptophan on AChE activity in the heart, spleen and liver of

adrenalectomized rats as compared to the intact normal suggests that adrenalectomy enhances the responsiveness of the enzyme of these organs to tryptophan. This observation is comparable to that observed earlier with the brain, in which tryptophan caused 56% stimulation in cerebral AChE activity in adrenalectomized rats as opposed to 28% increment of the same in intact animals (4). The non-responsiveness of hepatic AChE of the well-fed normal rats to tryptophan is analogous to our earlier observation for protein synthesis in that tryptophan is found unable to stimulate amino acid incorporation into a number of hepatic proteins in well-fed normal rats (2). It appears to us that adrenal hormone(s) affects the tryptophan-mediated stimulation of AChE activity in the liver and probably in several other tissues as well. We have also observed that whereas adrenalectomy by itself lowers AChE activity in the heart and lungs it has no influence on the activity of the enzyme in the liver, spleen and kidneys (Table - 1). The recent observation that tryptophan and hydrocortisone compete for the same receptor protein in the rat liver cytosol (6) suggests further the possibility that at least in the liver the maximum biological activity of one compound might be impaired by the presence of the other. Recently, we have observed that pretreatment of well-fed adrenalectomized rats with cortisol lowers the tryptophan-mediated stimulation of amino acid incorporation into albumin and ferritin in vivo (7). However, exception to this phenomenon has also been observed. A number of hepatic enzymes in normal rats is shown to be stimulated by tryptophan (8-10). These observations suggest that whereas for certain protein the stimulatory effect of tryptophan could be seen in

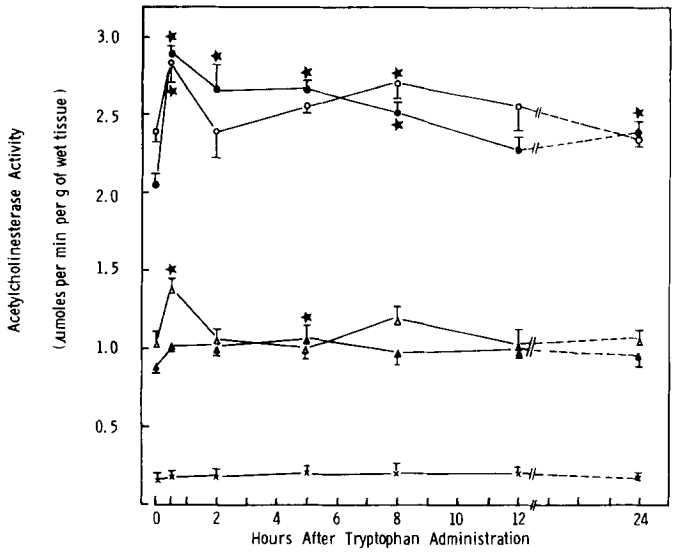

Fig. 1. Effect of a single tube-feeding of tryptophan to well-fed adrenalectomized rats on the activity of acetylcholinesterase in the liver (Δ——Δ); heart (●——●); spleen (o——o); lungs (▲——▲); and kidneys (x——x). Comparison with water-fed control. The rats were killed at 0.5, 2, 5, 8, 12 and 24 h after tryptophan administration. The water-fed controls (zero-time) were killed 0.5 hr after administration of water. Each value on the curve represents the mean ± S.E. of 6-8 determinations from 3 rats.

* Significantly different compared to the water-fed control at level of $P < 0.05$ or lower as judged by student's "t" test.

the absence of adrenal hormone(s), for others the presence of it is required for activation.

In the next series of experiments the time course of changes in AChE activity in various tissues of well-fed adrenalectomized rats were examined following a dose of tryptophan. The results are shown in Fig. 1. In the liver and spleen the pattern of response for AChE over a period of 24 hr was found to be biphasic, revealing peaks at 0.5 and 8 hr after tryptophan administration. At 24 hr the values were no longer different than the water-fed control. The biphasic response of the enzyme of liver and spleen is similar to that observed earlier for the

cerebral AChE (4), and for hepatic protein and RNA synthesis following a single tube-feeding of tryptophan (3). A slightly different picture was obtained with heart and lungs. AChE activity in the heart was increased by 40% ($P < 0.001$) after 0.5 hr of tryptophan force-feeding. The activity declined gradually over the next 11.5 hr,and then began to increase again (Fig. 1). At 24 hr the value was 16% ($P < 0.005$) higher than that of the initial water-fed control. In the lungs the enzyme activity increased more slowly, and 5 hr after tryptophan force-feeding the activity was 22% ($P < 0.05$) higher than in the control. No change in the activity in the kidney was observed over the 24 hr experimental period.

In our earlier studies of the effect of tryptophan on the level of hepatic microsomal cytochrome P-450 and AChE activity in the brain, we have observed that pretreatment of animals with actinomycin-D greatly lowers the tryptophan-mediated stimulation (10,4), indicating that ongoing RNA synthesis is required to attain the maximal stimulation by tryptophan. In order to determine whether a similar phenomenon would also prevail for the tryptophan-mediated stimulation of AChE activity in the heart of the well-fed adrenalectomized rats, groups of rats were injected with actinomycin-D or saline 30 min prior to water or tryptophan administration, and the enzyme activity was measured 8.5 hr afterward. The heart was chosen for the study because it showed the highest stimulation with tryptophan. Administration of tryptophan to non-antibiotic-treated rats caused 22% enhancement in the enzyme activity as compared to the water-fed control. When tryptophan was fed to actinomycin-D treated rats a similar 27% increment was also observed (Table 2). The results indicate

TABLE 2

Effect of tryptophan force-feeding on the activity of acetylcholinesterase in the heart of actinomycin-treated adrenalectomized rats.

Treatment	Acetylcholinesterase activity (μmoles per min per g of wet tissue)
Water-fed (control)	2.07 ± 0.54 (100)
Tryptophan	2.53 ± 0.68 (122; $P < 0.001$)
Actinomycin-D + water	2.19 ± 0.77 (105; N.S.)
Actinomycin-D + tryptophan	2.63 ± 1.13 (127; $P < 0.005$)

Well-fed adrenalectomized rats were injected with either saline or actinomycin-D (100 μg/100 g) 30 min prior to administration of water or tryptophan and killed 8.5 hr later. Each value represents the mean ± S.E.M. of 6 determinations from 3 rats. The figures in the parentheses represent percentage of the water-fed control as well as P value. Statistical comparisons were made with water-fed control.

that in the heart at least, the tryptophan-mediated stimulation could be observed in the absence of RNA synthesis.

To determine whether tissues of tryptophan-fed rats would contain an activator for AChE, the enzyme preparations from the heart of water- and tryptophan-fed rats were mixed in equal proportions. The enzyme activity of the mixture was compared with preparations from corresponding water- and tryptophan-fed rats. The results (Table 3) revealed that whereas the enzyme preparations from the heart of the tryptophan-treated normal and adrenalectomized rats had 34 and 42% higher activities, respectively, compared to the corre-

TABLE 3

Acetylcholinesterase activity in the heart following water or tryptophan treatment, and after mixing the two enzyme preparations.

Enzyme preparation	Acetylcholinesterase activity (μmoles per min per g of wet tissue)	
	Normal	Adrenalectomized
Water-fed (control)	2.88 ± 0.14 (100)	2.10 ± 0.11 (100)
Tryptophan-fed	3.87 ± 0.25 (134; P < 0.025)	2.99 ± 0.08 (142; P < 0.001)
1:1 Mixture (v/v, Control + Tryptophan-treated)	3.47 ± 0.20 (120; N.S.)	2.64 ± 0.01 (126; P < 0.005)

sponding water-fed control, the values for the mixture (control + tryptophan-fed, 1:1 v/v) were 20 and 26% over that of the respective water-treated controls. The degree of increments observed with the mixture-preparations was about half to those obtained with preparations from tryptophan-treated rats. Therefore, the tryptophan-mediated stimulation of AChE activity in the heart of both well-fed normal and adrenalectomized rats can not be related to the presence of an activator in the tissue.

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