Effect of ecdysone on glutamic decarboxylase activity in rat brain

Dose of ecdysone (μg/100 g body weight)	h of sacrifice	Enzyme activity dpm/mg protein $M^a \pm \sigma M$	P^b
Control	4	123 ± 5	
	12	124 ± 6	
	24	122 ± 8	
0.025	4	168 ± 10	0.01
	12	160 ± 20	0.05
	24	158 ± 13	0.01
0.1	4	156 ± 12	0.01
	12	162 ± 8	0.01
	24	148 ± 19	0.05
1.0	4	169 ± 6	0.01
	12	174 ± 15	0.01
	24	160 ± 21	0.05
5.0	4	171 ± 11	0.01
	12	169 ± 9	0.01
	24	165 ± 5	0.01

[a] $M = \sqrt{(m-M)^2/(N(N-1))}$. N, total number of determinations; m, each determination; M, mean; σ M, standard deviation. [b] Statistical analysis shows a significant difference between the treated and control animals for each corresponding time interval.

on protein synthesis or the increased enzyme activity is the result of an allosteric effect of ecdysone as a steroid, rather than as a hormone, is not known. In fact allosteric phenomena have been reported to be quite widespread in nature and several steroids have been implicated as capable of altering catalytic activities of enzymes through this mechanism[5]. In insects, ecdysone by inducing DOPA decarboxylase, has been shown to be implicated in processes leading to hardening and tanning of the cuticle. It is, therefore, of interest to compare the effect of ecdysone on glutamic decarboxylase activity in rat brain with that of its normal mode of action in insects[6].

Résumé. Nous avons étudié l'effet que produisait l'ecdysone, une hormone de la mue chez les insectes, sur l'activité de l'acide glutamique decarboxylase du cerveau de rat. Nous avons observé que l'activité de cet enzyme augmentait de façon significative chez les rats traités par cette hormone.

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A Non-Dialyzable Inhibitor of Proteolytic Activity in Soluble Extracts of *Escherichia coli*

In recent years, a number of polypeptide inhibitors of proteolytic activity have been isolated from mammalian pancreas[1,2], blood[3-5], and colostrum[6]. Inhibitors have also been observed in brain, ovary, breast, and bowel[7-10].

In view of the considerable quantities of trypsinogen and chymotrypsinogen released into the intestine from the pancreas[11], and in view of the survival of *Escherichia coli* in the intestinal tract in the presence of large quantities of such secreted proteinases, *E. coli* was selected and examined for its possible capacity to resist digestion by trypsin (T) and chymotrypsin (CT).

This communication reports the presence of a non-dialyzable, heat-stable inhibitor of tryptic and chymotryptic activity in the 90,000 *g* supernatant fraction of sonicated *E. coli* cells.

Five grams of *E. coli* strain ECB-6504, lyophilized (Worthington Biochemical Corporation, Freehold, New Jersey) were suspended in 120 ml of 0.1 *M* phosphate buffer, pH 7.0, and sonicated for 12 min. The suspension was then centrifuged at 90,000 *g* for 1 h to obtain a soluble 90,000 *g* supernatant fraction, which was stored frozen in aliquots until used. Assays for proteolytic and antiproteolytic activity were performed essentially according to the procedure of KUNITZ[12], using 0.5% casein as a substrate and twice crystallized T (Nutritional Biochemical Corporation, Cleveland, Ohio) and thrice crystallized CT (Worthington Biochemical Corporation, Freehold, New Jersey) as proteases. Protein nitrogens were performed according to the procedure of LOWRY et al.[13].

The inhibition of the tryptic and chymotryptic hydrolysis of casein by the 90,000 *g* supernatant fraction derived from sonicated *E. coli* cells is given in the Figure. Analogous curves have been obtained with extracts of mammalian normal and pathological breast, bowel, and with extracts of human glioma, adenocarcinoma of the ovary[10], and with astrocytoma extracts[8]. The soluble inhibitor is non-dialyzable (Table I) and is heat-stable (Table II). It can be sedimented with $(NH_4)_2SO_4$, indicating that the inhibitor is considerably larger than the pancreatic trypsin inhibitors isolated by NORTHROP and KUNITZ[1], and by KAZAL et al.[2] The bacterial inhibitor bears a

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Table I. The effect of dialysis on the inhibition of proteolytic activity by the 90,000 *g* supernatant fraction of *E. coli*

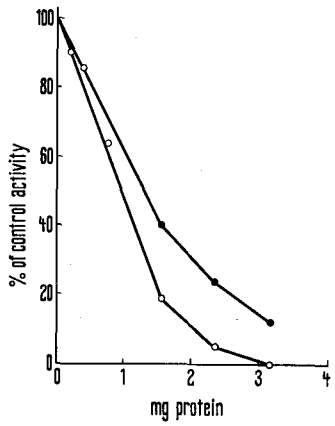
Incubation mixture	meq tyrosine released/ h/μg T or CT, $\times 10^3$	% of control activity
T, alone	0.975	100
T + 90,000 *g* supernatant fraction, undialyzed[a]	0.0	0
T + 90,000 *g* supernatant fraction, dialyzed[a]	0.108	9.6
CT, alone	0.825	100
CT + 90,000 *g* supernatant fraction, undialyzed[a]	0.292	35.5
CT + 90,000 *g* supernatant fraction, dialyzed[a]	0.296	35.1

[a] 2.33 mg protein.

Table II. The effect of heating on the inhibition of proteolytic activity by the 90,000 *g* supernatant fraction of *E. coli*

Incubation mixture	meq tyrosine released/ h/μg T or CT, $\times 10^3$	% of control activity
T, alone	1.07	100
T + 90,000 *g* supernatant fraction, unheated[a]	0.44	41.2
T + 90,000 *g* supernatant fraction, heated[a]	0.53	49.5
CT, alone	1.21	100
CT + 90,000 *g* supernatant fraction, unheated[a]	0.64	52.9
CT + 90,000 *g* supernatant fraction, heated[a]	0.71	58.7

[a] 1.56 mg protein. The supernatant fractions were heated for 10 min at 100 °C.



The inhibition of the tryptic and chymotryptic hydrolysis of casein by the 90,000 *g* supernatant fraction of sonicated *E. coli* cells. ○, T; ●, CT.

resemblance to the heat-stable inter-α-globulin[4] and α_2-globulin[5] observed in blood, which are also non-dialyzable.

The function of an inhibitor of proteolytic activity in an intestinal bacterium is subject to conjecture. It may function to protect the bacterium from degradation by the pancreatic proteases; it may regulate proteolytic activity in the *E. coli* cell; it may also serve as an evolutionary precursor to one or more of the mammalian or plant inhibitors of tryptic activity[14].

Résumé. Nous avons observé l'inhibition de l'activité tryptique et chymotryptique par extraits d'*Escherichia coli*. L'inhibition n'est pas détruit par chauffage et par dialyse.

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Inhibition of Deiodination of Diiodotyrosine in vivo; Relation to Catecholamine Biosynthesis

Various tyrosine analogues are specific inhibitors in vitro of the enzyme tyrosine hydroxylase[1], the enzymatic rate limiting step in catecholamine biosynthesis[2]. When given to animals[3] or man[4], most of these compounds inhibit noradrenaline (NA) production. The ring iodinated analogues of tyrosine, 3-iodo-L-tyrosine (MIT) and 3,5-diiodo-L-tyrosine (DIT) are among the most potent inhibitors of tyrosine hydroxylase in vitro[1]. However, when given to animals[5] or man[6], their inhibitory effect on catecholamine synthesis is weak. This lack of activity in vivo appears to be secondary to a rapid inactivation of these compounds by a specific deiodinating enzyme[5].

The present communication demonstrates that partial inhibition of the deiodination of the injected DIT by menadione (2-methyl-1,4-naphtoquinone) results in a marked fall in NA tissue levels.

Materials and methods. All compounds were administered by the i.p. route. DIT[7] was dissolved in 9‰ NaCl by the addition of 1 *N* HCl and the pH was adjusted to 2 with 0.5 *M* phosphate buffer pH 7. ^{131}I-labelled 3,5-diiodo-L-tyrosine was prepared according to Felber[8]. Fasted male Wistar rats, weighing 140–200 g, were used. Noradrenaline (NA) was essayed in tissues spectrofluorimetrically by the method of Crout et al.[9]

The rats were divided into 4 groups. Animals of group I received no drug. Rats of group II were given 2 injections of menadione (50 mg/kg) at intervals of 16 h. Rats of group III were given 2 injections of menadione (50 mg/kg)