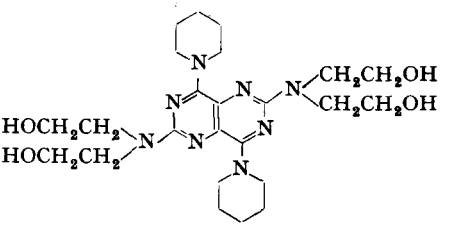
Short Communications

Effect of a pyrimido-pyrimidine derivative on non-specific enzymic stimulation *in vivo*

It has been shown that intraperitoneal injection of RNA or nucleotide mixtures, among other natural compounds, stimulates tyrosine–α-ketoglutarate transaminase activity of liver in a fashion similar to that observed following injection of tyrosine or hydrocortisone[1,2]. All of the RNA species tested were active and, therefore, it was obvious that this effect was non-specific. However, it was shown subsequently[3] that the effects of RNA were different for tryptophan pyrrolase and that RNA continued to exert a stimulation of tyrosine–α-ketoglutarate transaminase activity in adrenalectomized animals receiving hydrocortisone. Consequently, an attempt was made to find a compound which could inhibit the effects of RNA or the nucleotide mixture without affecting the stimulation caused by either tyrosine or hydrocortisone. Such a demonstration might lead to the conclusion that RNA or the nucleotides were acting, at least in part, by a mechanism other than simple stress.

This report shows the action of 2,6-bis(diethanolamino)-4,8-dipiperidino-pyrimido-(5,4-d)-pyrimidine,



This compound (obtained from the Geigy Corporation) may affect nucleoside metabolism as suggested by experiments of HOECKERTS AND BOEGELMAN[4] who found its action in myocardial tissue to be upon the level of ATP.

The RNA and the nucleotide mixture used in the present report have been described previously[1]. Enzymic activity was measured by the modified Briggs reaction[5] or by a spectrophotometric method[6]. Only the results of the Briggs reaction are reported; however, the spectrophotometric method produced similar results. One unit of enzymic activity is defined as the concentration of transaminase required to produce a reading of 1.0 at 850 mμ in a 3.0-ml reaction system at 37°. The reaction system consisted of the following components: 0.1 ml of a 35000 × *g* supernatant from a 10% homogenate, 30 μg pyridoxal phosphate, 12 μmoles diethyldithiocarbamate[6], 30 μmoles α-ketoglutarate, 12 μmoles of L-tyrosine and 0.2 *M* phosphate buffer to 3.0 ml. The reaction was initiated by tyrosine dissolved in the buffer after a 5-min preincubation. The reaction was stopped with addition of trichloroacetic acid and 1.0 ml of the resulting supernatant was used in the Briggs reaction.

Injections were made intraperitoneally and rats (150–175 g) were sacrificed 4 h after injection.

The results are shown in Table I. The trend of enzymic activity is similar when units of activity are based upon 100 g body weight or whole-organ weight. It is obvious that the pyrimidine compound does not affect the stimulation produced

TABLE I

EFFECT OF A PYRIMIDO-PYRIMIDINE DERIVATIVE UPON STIMULATION OF TYROSINE–α-KETOGLUTARATE TRANSAMINASE ACTIVITY *in vivo*

Expt.	*Number of observations*	*Tyrosine transaminase activity units*/g dry wt.*
Untreated	34	21 ± 5
Pyrimidine** derivative	34	84 ± 13
L-Tyrosine	7	104 ± 8
L-Tyrosine + pyrimidine derivative	7	133 ± 12
Hydrocortisone	6	131 ± 7
Hydrocortisone + pyrimidine derivative	6	131 ± 12
RNA	6	114 ± 8
RNA + pyrimidine derivative	6	74 ± 12
Nucleotide mixture	15	93 ± 7
Nucleotide mixture + pyrimidine derivative	15	66 ± 10

* That concentration of transaminase which gave a reading of 1.0 in a standard system using the modified Briggs method (see text). The value given is mean ± standard error of the mean.

** Injection concentrations are as follows: pyrimidine derivative, 600 mg/kg; L-tyrosine, 600 mg/kg; hydrocortisone acetate, 60 mg/kg; RNA, 300 mg/kg; and nucleotide mixture, 300 mg/kg.

either by L-tyrosine or by hydrocortisone, except to cause a slight elevation when added to tyrosine. Alone, the pyrimidine derivative exerts a non-specific stimulation presumably because of a stressing effect. However, when the compound is combined with RNA or the nucleotide mixture, a significant inhibition is observed. These results support the idea that RNA or the nucleotides stimulate enzymic activity by a mechanism which is, at least in part, different from stress resulting in the endogenous release of glucocorticoids.

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