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EFFECTS OF PRIMYCIN ON THE SYNTHESIS OF TRYPTOPHAN PYRROLASE

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Abstract—1. In the liver of adrenalectomized rats a subtoxic dose of primycin inhibits the synthesis of tryptophan pyrrolase induced by hydrocortisone.

2. Primycin does not substantially affect the enhancement of the activity of TP observed in the liver of adrenalectromized animals after administration of l-tryptophan.

3. The above effects of primycin are similar to those of actinomycin-D.

THE ANTIBIOTIC substance primicyn is produced from a Streptomyces culture.[1, 2] Its structure is not as yet known exactly but would appear to be a macromolecular guanidino-arabinoside.[3] In an earlier work[4] we pointed out that this substance is highly toxic in a variety of experimental animals after parenteral administration. Recently, Blum reported that primycin inhibits proliferation of *Euglena gracilis*, and of *Astasia longa*, and prevents the formation of an induced acid phosphatase in cells of protozoons.[5]

It is known that actinomycin-D inhibits the DNA-dependent synthesis of RNA[6] and that, in consequence, it arrests the adaptive synthesis of tryptophan pyrrolase (TP).[7] In view of the findings reported by Blum, we have investigated the effect of primycin on the formation of an adaptive enzyme present in a mammalian organism when an intact DNA molecule must be present *i.e.* the adaptive synthesis of TP. We have shown that primycin administered in subtoxic doses inhibits *in vivo* the hydrocortisone-induced synthesis of TP in the liver of adrenalectomized rats. In contrast, primicyn does not inhibit adaptation of the enzyme if this is induced by the substrate.

MATERIALS AND METHODS

Wistar female rats of 140–160 g body weight were bred at the Institute for Pathology, of the University of Medical Sciences, Debrecen. Before and during experiments the animals were allowed food and water *ad libitum*. Food was a compressed fodder with 22 per cent protein content, standardized by the Institute for Laboratory Animal Breeding.

After a 10-day period of examination, both suprarenal glands were removed retroperitoneally under ether anaesthesis.[8] After operation, drinking water was substituted for a 1 per cent salt solution. To reduce the toxicity of tryptophan, the animals selected for treatment with this compound for the induction of adaptation were given a pre-treated, beginning on the day of operation, with daily 5 mg/kg desoxycorticosterone acetate.[9]

The enzyme test was carried out on the fifth day after operation. The animal was killed by decapitation and its liver was rapidly removed and frozen in a mixture of dry ice and acetone. The TP content of the livers was determined according to Knox,[10] within 24 hr. For the induction of the adaptive process, 0·5 g/kg l-tryptophan was administered to the animals 4 hr prior to death, or in other cases 15 mg/kg of hydrocortisone acetate was injected i.p. 6 hr before death. Tryptophan (Reanal, p.a. grade) was used as a 5% suspension in 1% carboxyl-methylcellulose solution. The 0·3% aqueous suspension of hydrocortisone acetate was prepared by the dilution of the microcrystalline sample of Hydrocortisone manufactured by Köbányai Gyógyszerárugyár.

To inhibit adaptation, 1 hr before the administration of tryptophan or hydrocortisone, either primycin or actonomycin-D (0·5 mg/kg), was administered intraperitoneally. A 98 per cent pure preparation of primycin (Biochemicals Division of the Pharmaceutical Research Institute), was administered in a 0·02 per cent concentration in 1,2-propylene glycol, to facilitate solution.

Actinomycin-D was received as a gift from the National Joliot-Curie Institute for Radiation Biology, and was also used as a 0·02 per cent solution.

Desoxycorticosterone acetate (Decosteron, Köbányai Gyógyszerárugyár) was given by intramascular injection dissolved in oil.

RESULTS

Figure 1 shows that, referred to the base level column I, the TP level in the liver of the animals after administration of 15 mg/kg of hydrocortisone Column II is on average higher by a factor of 14·1.

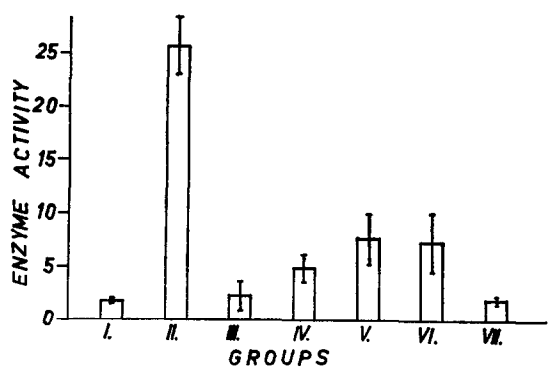

FIG. 1. Effects of primycin, and actinomycin-D, in female Wistar rats (140–160 g) on the rise of tryptophan pyrrolase enzyme level induced by 15 mg/kg of hydrocortisone acetate, given on the fifth day after adrenalectomy. Antibiotics were injected i.p. 1 hr before hydrocortisone (i.p.) and animals were killed 6 hr after hydrocortisone. The activity of the enzyme is expressed as μmoles of kynurenine formed by 1 g of liver/hr. Mean values, ± S.D. of data gathered on from four to ten animals are listed.

This vigorous response is suppressed in a high degree, i.e. 98 per cent (Column III) when actinomycin-D is administered i.p. in a 0·5 mg/kg dose. similarly, a strong suppressing effect, (87 per cent) is shown by primycin. This drug, when administered in a 0·5 mg/kg dose, substantially inhibits the increase of TP activity, induced by hydrocortisone, in the liver (column IV).

If the dose of the two antibiotics is halved (0·25 mg/kg), inhibition of enzyme adaptation induced by hydrocortisone weakens (columns V and VI). However, the combined effect of the reduced doses of the two antibiotics is an almost complete, i.e. 99 per cent inhibition of the adaptive rise of the enzyme (column VII), suggesting that the effects of actinomycin-D and primycin are additive.

A communication of Greengard *et al.*[7] reports that in the liver of adrenalactomized rats, actinomycin-D can inhibit only TP increase induced by cortisone, and does not affect enzyme adaptation by l-tryptophan. It is believed that there is an essential difference between the mechanism of TP rise according to whether this was brought about by hydrocortisone or by *l*-tryptophan.[7, 11] It is believed in order to gain better insight into the mechanism of action of primycin, its effect on the liver of animals in which the enhanced activity of TP had been induced by l-tryptophan was investigated. The enzyme level induced by l-tryptophan, and its changes due to administration of primycin, and actinomycin-D, are shown in Fig. 2.

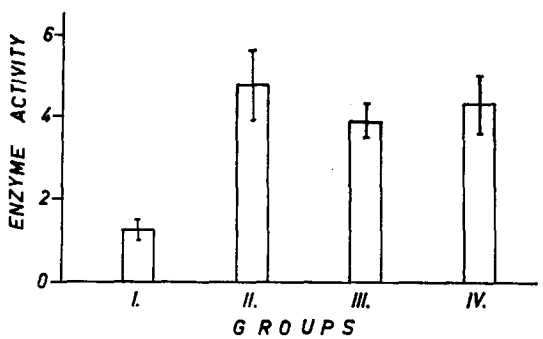

FIG. 2. Effects of primycin, and actinomycin-D, in female Wistar rats (140–160 g) on the rise of tryptophan pyrrolase enzyme level induced by 0·5 g/kg of l-tryptophan, on the fifth day after adrenalectomy. All animals were given intramuscular injections desoxycorticosteron acetate, (5 mg/kg/day), beginning on the day of the operation. The intraperitoneal injection of the antibiotics was given 1 hr before the administration of l-tryptophan, and the animals were killed 4 hr after i.p. injection of l-tryptophan.

In the livers of animals with suprarenal glands removed, the enzyme level rose substantially above the base value upon administration of 0·5 g/kg of l-tryptophan. This rise was not significantly inhibited by 0·5 mg/kg doses of either actinomycin-D or primycin, indicating that there is a certain similarity in the mechanism of the action of the two antibiotics. Further, this supports the conclusions arrived at by Greengard *et al.*[7]

DISCUSSION

Tryptophan pyrolase concentration in hepatic cells is determined by simultaneous processes, by the synthesis of the enzyme on the one hand, and by its degradation on the other.[14]

Several data seem to suggest that in adrenalectomized rats, the rise of the TP-level induced by hydrocortisone is directly due to a *de novo* synthesis of the enzyme protein.

The steroid triggers biochemical processes in the course of which the DNA-dependent synthesis of *m*-RNA is enhanced.[7] The fact that this causes increased TP-concentration due to stimulation by hydrocortisone, is supported by the observation that actinomycin-D effectively inhibits this process when the antibiotic given is within three hr after the introduction of the hormone.[14] The efficacity of the TP present in the liver of adrenalectomized rats can be enhanced by the oral or parenteral administration of l-tryptophan.[18] However, the mechanism of this change is essentially different from a hormone action insofar as it does not go hand in hand with an enhanced synthesis of *m*-RNA and the concomitant enhanced protein synthesis, since this change is the result either of a greater enzymatic activity[11] or that of a reduction of the degradation of the enzyme.[19] Accordingly, this process is not inhibited by actinomycin-D.[7, 13]

Our investigations seem to show that, among the two mechanisms listed, primycin inhibits the first one, i.e. the enzyme adaptation that can be induced by hydrocortisone, and does not affect the second one, because the effect of primycin is similar to that of actinomycin-D. This is supported by the synergism of the two antibiotic substances, (Fig. 1) to which Blum[5] has drawn attention.

In spite of this similarity in the actions of actinomycin and primycin, our experiments cannot furnish an unequivocal indication of the molecular point of attack of primycin. The synthesis of TP that can be induced by hydrocortisone may be inhibited by puromycin. However, it is known that puromycin specifically arrests the emergence of the peptide chains themselves and does not arrest the preceding step, the formation of *m*-RNA, in the reaction sequence leading to protein synthesis.[20–22] Primycin forms complexes with nucleic acids, first of all with DNA, and specifically with this single-stranded form.[5] Primycin prevents the hypochromism of DNA solutions which appear upon cooling after their thermal treatment.[5] Finally, the formation of a complex from primycin and DNA is suggested by our experimental results bearing upon viscosity, sedimentation characteristics, and thermo-denaturation points (Tm) of DNA solutions containing primycin.[23] With the latter, *in vitro*, effects of primycin in mind, it seems reasonable to suggest that the effect of this antibiotic on cell metabolism is more like that of actinomycin than that of puromycin.

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