THE EFFECT OF TWO CARCINOSTATIC AGENTS ON THE CHEMICALLY INDUCED STIMULATION OF AMINO ACID INCORPORATION IN A MAMMALIAN SYSTEM

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Pretreatment of rats with 3-methylcholanthrene (3MC) or phenobarbital (PB) stimulates the incorporation of free amino acid into protein in a liver microsomal system (Gelboin and Sokoloff, 1961) dependent on endogenous or exogenous messenger RNA (Gelboin, 1964; Kato, et al., 1965).

The recent success in inhibiting the growth of tumors in animals and in tissue culture, with a whole range of terephthalanilide derivatives (Kensler, 1963; Burchenal, et al., 1963) and with derivatives of indolyl-3-acetic acid (Norman and Schultz, 1965; Schultz and Norman, 1965) prompted this investigation into the effect of representative samples of these carcinostatic drugs on the stimulation of amino acid incorporation in a subcellular system which is normally brought about by pretreatment with PB or 3MC. The present paper shows that n-pentyl indolyl-3-acetate (pIAA) when co-administered with PB prevented the PB-induced stimulation of amino acid incorporation in vitro. Simultaneous pretreatment with N, N'-bis (p-(N'-methylamidino) phenyl) terephthalamidine (NSC-57155) and with PB or 3MC only prevented the 3MC-induced stimulation of amino acid incorporation into protein in the rat liver sub-cellular system.

Materials and Methods - 3MC was purchased from Eastman Kodak, Rochester, N. Y., PB from the Merck Company, pIAA from the Cyclo Chemical Corporation, Los Angeles, Calif. We are grateful to Dr. John Venditti of the National Cancer Institute for a generous supply of NSC-57155

Animals used were female Sprague-Dawley rats weighing 160 g. for work with PB, and male Sprague-Dawley rats weighing 45 g. for experiments with 3MC. The rats treated with PB were given intraperitoneal injections (80 mg/Kg.) for three successive days and were fasted on the third day. Those treated with 3MC were given one intraperitoneal injection (50 mg/Kg. in corn oil) and were then fasted for one day. Simultaneous or alternative pretreatment of the animals with NSC-57155 (30 mg/Kg) was given daily for three successive days in the PB experiments, or once in the 3MC experiments. pIAA (500 mg/Kg) was administered intraperitoneally in a vehicle of distilled water-'Tween 20'-1,2-propanediol (Norman and Schultz, 1965) at the time of the first PB treatment and at the same time as the 3MC treatment. Control animals were injected with the same volumes of the appropriate vehicle. The rats were killed by decapitation, the livers were removed, homogenized and centrifuged as described by Gelboin (1964). The resuspended microsomal fractions were adjusted to the same protein concentrations as measured by the Lowry *et al.* (1951) method.

Results - Fig. 1 shows that the amino acid incorporation in the preincubated, polyU-dependent microsomal system is greatly enhanced in incubations containing microsomes from PB treated rats. Since Mg^{++} plays a role in maintaining the integrity of the incorporation system (Campbell *et al.*, 1964), the possible effect of various pretreatments on the optimal Mg^{++} concentration was determined by carrying out the preincubation procedures at four different levels of added $MgCl_2$. There was no detectable shift in Mg^{++} requirement for the *in vitro* system which could have been linked to the pretreatment of the animals *in vivo*. The effect of pretreatment with pIAA in conjunction with PB was to prevent the enhancement of amino acid incorporation brought about by PB treatment alone. NSC-51755 pretreatment together with PB had little effect on the stimulation of amino acid incorporation. The effect of pIAA or NSC-51755 treatment alone was a lowering of the amino acid incorporation below the level of the control over the whole range of Mg^{++} concentration

tested. In the non-preincubated system (at the optimal level of 5 μmoles $MgCl_2$ per incubation) dependent on endogenous RNA, the microsomal system from PB-treated rats was more active in incorporating amino acid than the

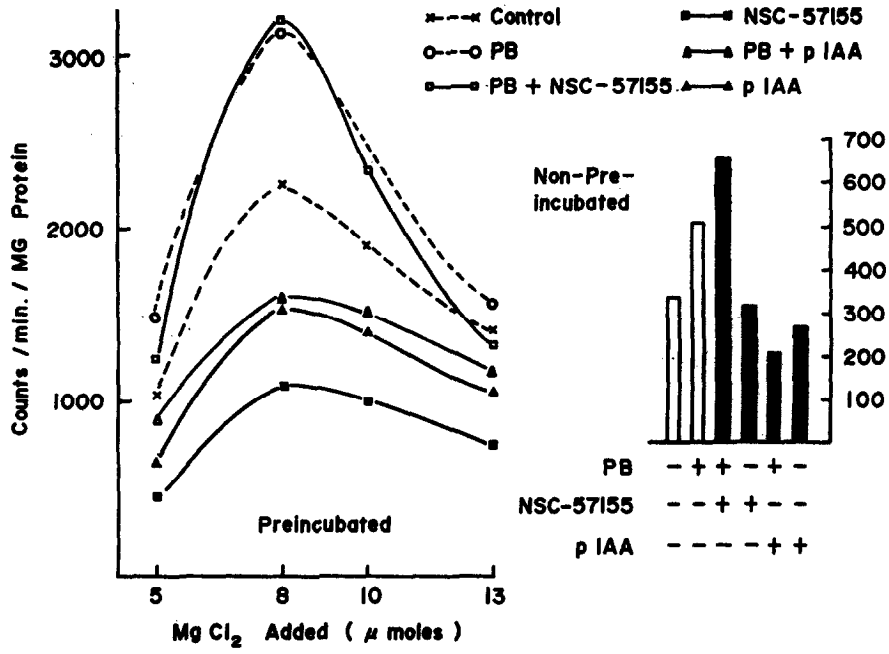

Fig. 1. Effect of treatment with carcinostatic agents on phenylalanine incorporation in vitro in liver microsomal preparations from control and PB-treated rats.

Incubations (duplicate or triplicate) in total volume of 0.85 ml contained the following components (in μmoles unless otherwise indicated): potassium phosphate (pH 7.4), 10; magnesium chloride, 5-13; sucrose, 75; ATP, 1.25; GTP, 0.25; phosphocreatine, 20; creatine phosphokinase, 0.125 mg.; reduced glutathione (neutralized with potassium hydroxide), 50; uniformly labeled L-(^{14}C)-phenylalanine of specific activity 10 μc/μmole, 0.025; microsomal protein, 3.6 mg.; supernatant protein, 1.2 mg. The mixtures were incubated at 37°C for 15 minutes. Preincubations for 12 minutes had all the components in the mixture except for labeled phenylalanine. After preincubation, polyuridylic acid, 0.15 mg. additional phosphocreatine 20, and creatine phosphokinase, 0.125 mg., and labeled phenylalanine were added to the system which was then incubated as above. The reaction was stopped with cold 10% w/v trichloroacetic acid containing 1 mg/ml unlabeled L-phenylalanine. The precipitated proteins were purified essentially as by Siekevitz (1952) and were then redissolved in hyamine, transferred to vials and counted in a toluene-PPO-POPOP scintillation solution in a liquid scintillation counter (Nuclear-Chicago, Model 724/5).

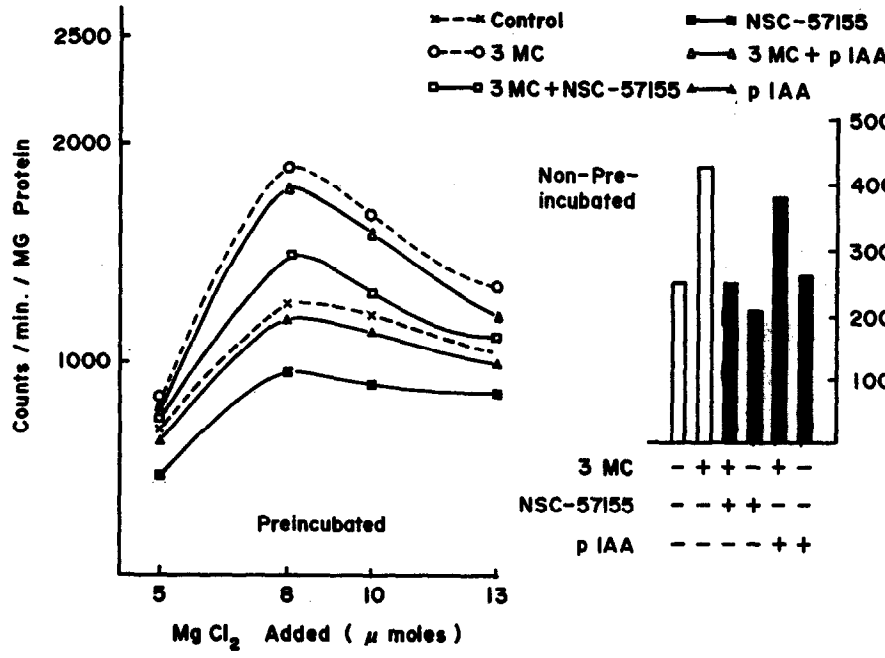

Fig. 2. Effect of treatment with carcinostatic agents on phenylalanine incorporation in vitro in liver microsomal preparations from control and 3MC-treated rats. Experimental details as given under Fig. 1.

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control. Simultaneous pretreatment of animals with PB and pIAA not only prevented the stimulation of amino acid incorporation by PB, but lowered the incorporation activity below the level of the controls as in the preincubated system. The co-administration of PB and NSC-51755, on the other hand, slightly enhanced the stimulation of endogenous RNA-directed amino acid incorporation beyond the level observed with PB treatment alone. pIAA or NSC-51755 treatment alone, as in the preincubated system brought about a lowering of the amino acid incorporation below the level of the control.

The results of the corresponding experiment with 3MC are shown in Fig. 2. Pretreatment with 3MC stimulated the preincubated polyU-sensitive microsomal amino acid incorporation system. This stimulation by 3MC treatment in vivo was not modified by simultaneous pretreatment with pIAA. However, the combined pretreatment with 3MC and NSC-51755 reduced the stimulating

effect on amino acid incorporation observed with 3MC alone by about 65%. Pretreatment of animals with pIAA alone did not appreciably deviate from the control levels of polyU-directed phenylalanine incorporation, whereas NSC-51755 did lessen the incorporation in the system below the level of the control.

These results ran parallel with the effect of combined 3MC and pIAA or NSC-51755 pretreatment on the non-preincubated, endogenous RNA-dependent incorporation system. With pIAA co-administration, the stimulating effect of 3MC on the phenylalanine incorporation was not impaired, whereas co-administration of 3MC and NSC-51755 prevented the 3MC induced stimulation of phenylalanine incorporation.

Discussion - The administration of PB or 3MC increased the rat liver microsomal amino acid incorporation in vitro in both the non-preincubated, and the preincubated polyU-dependent system. The effects of co-administering pIAA were confined to the PB-stimulated system, which was almost unaffected by NSC-51755. The latter drug, however, did depress incorporation in the 3MC-stimulated system. These results suggest that there may be different mechanisms involved in the PB and 3MC stimulation of amino acid incorporation. Furthermore, the results seem to indicate that, since the co-administration of either pIAA and PB or NSC-51755 and 3MC affected the enhanced endogenous RNA-directed, as well as the increased polyU-dependent amino acid incorporation in the same manner, the explanation of the effect probably involves an interference in the sensitivity of the microsomal system to RNA, rather than changes in the synthesis of mRNA. This is consistent with the findings of Pine, et al. (1963) and Ochoa, et al. (1964), regarding the action of a terephthalanilide (NSC-38280) on growth inhibition of E. Coli, and impairment of protein synthesis in cell-free extracts of L1210 mouse ascites cells, respectively.

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