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### NOTE ADDED IN PROOF

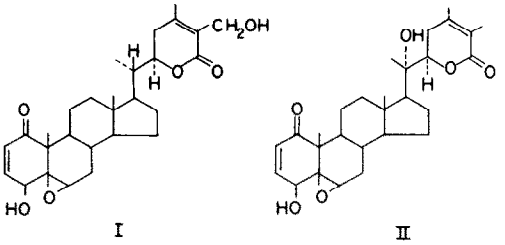
While studying the mechanism of $Zn^{2+}$ inhibition on NADPH oxidation the concentrations of all reactants were decreased as compared to the conditions of the experiment in Fig. 1. (Only the initial rate of the reaction was used so that Michaelis–Menton mechanisms could be applied.) Under these conditions $Zn^{2+}$ inhibited NADPH oxidation as is shown in Fig. 1, but the increase in absorption at 340 nm was not evident. Repeating the conditions of these previous experiments and scanning the reaction at 340 nm and 600 nm showed an increase in absorption at both these wavelengths when 60 μmoles of $Zn^{2+}$ were added to the reaction vessel. Increasing the temperature showed that this increase in absorption occurred at still lower levels of $Zn^{2+}$. From these new findings it appears that the increase in absorption at 340 nm in Fig. 1 may be due to micro-precipitation at these high levels of NADPH and $Zn^{2+}$. Further study is continuing in this laboratory into the possibility of NADPH and $Zn^{2+}$ interactions occurring even though the u.v. spectral analysis which is mentioned in this paper shows no perturbations.

Biochemical Pharmacology, Vol. 24, pp. 919–920. Pergamon Press. 1975. Printed in Great Britain.

## Mode of action of Withaferin A and Withanolide D

(*Received* 18 *August* 1974; *accepted* 3 *October* 1974)

Withaferin A(I) and Withanolide D(II) are steroidal lactones isolated from *Withania somnifera* Dun. Withaferin A was reported to retard growth of various experimental mouse tumours [1, 2]. It produces a mitotic arrest in the metaphase

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of dividing Ehrlich ascites carcinoma cells. Administration of withaferin A to Ehrlich ascites tumour bearing mice resulted in simultaneous disappearance of the tumour and acquisition of immunity towards a subsequent tumour implantation [3]. Chakraborti *et al.* [4] found that withanolide D, the major constituent in the plant occurring in West Bengal, India, has significant antitumour activity against cultured cells derived from human carcinoma of the nasopharynx (KB) and *in vivo* against Sarcoma-180 in mice. Shohat *et. al.* [5] reported from cytological studies that synthesis of DNA and RNA in Ehrlich ascites tumour cells was unaffected by withaferin A. The present study is a biochemical approach to establish the exact mechanism of tumour regression during withaferin A and withanolide D treatment.

DL-$^{14}$C-Phenylalanine and 2-$^{14}$C-uracil were obtained from Bhabha Atomic Research Centre, Trombay, India. Withaferin A and Withanolide D were kind gifts from Dr. S. K. Chakraborti, Department of Chemotherapy, of this research centre. Other chemicals used were of analytical grade.

Sarcoma-180 tumour cells were grown in ascites form in 4–6 week old Strain A male mice by intraperitonial transplantation. Seven to ten days following transplantation of the tumour, the animals were sacrificed and cells obtained as a pellet by centrifuging the ascites fluid. Cells were then washed twice with chilled normal saline.

To study the effect of the drugs on incorporation of $^{14}$C-phenylalanine into trichloroacetic acid (TCA)-insoluble proteins of Sarcoma-180 cells, the cells were incubated in Medium A (0·25 M sucrose, 0·025 M KCl, 0·005 M $MgCl_2$, 0·05 M Tris-HCL buffer, pH 7·4) with 0·3 μCi of DL-$^{14}$C-phenylalanine (45·6 mCi/m-mole) in a total volume of 0·1 ml at 37° with constant shaking. The drugs at a concentration of 40 μg/ml were added to one group while another was treated as a control. Four such pairs were incubated for 0 min, 30 min, 1 hr and 2 hr. The incubation mixtures were then applied to 25 mm discs of Whatman 3MM paper, dried and treated with 10% TCA. After 30 minutes, the discs were rinsed twice with 5% TCA, heated at 90°–95° for 15 min in 5% TCA and cooled to room temperature. The discs were then rinsed with 5% TCA followed by alcohol, alcohol–ether (1:1) mixture and finally with ether. The discs were finally dried and counted in a windowless gas flow counter.

To study the effect of the drugs on incorporation of 2-$^{14}$C-uracil into RNA of Sarcoma-180 cells, the cells were incubated in Medium A with 0·8 μCi of 2-$^{14}$C-uracil (46·7

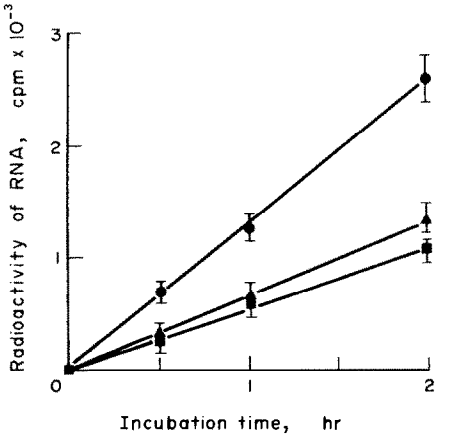


Fig. 1. Incorporation of $^{14}$C-uracil into RNA of Sarcoma-180 cells. ●—●- control; ▲—▲-treated with Withaferin A; ■—■-treated with Withanolide D. Mean values ± S.D. of five experiments are given. $P < 0.001$ with respect to the control.

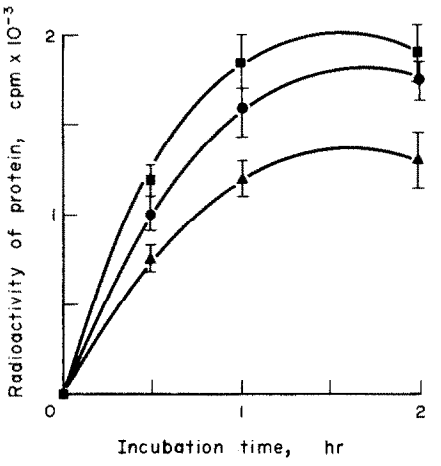


Fig. 2. Incorporation of DL-$^{14}$C-Phenylalanine into trichloroacetic acid insoluble proteins of Sarcoma-180 cells. ●—● control; ▲—▲-treated with Withaferin A; ■—■-treated with Withanolide D. Mean values ±S.D. of five experiments are given. Effect of Withanolide D is not significantly different from the control; Withaferin A $P < 0.01$ with respect to control.

mCi/m-mole) in a total volume of 0·1 ml at 37° with and without the drugs with constant shaking. After prescribed times of incubation, the mixtures were applied to 25 mm discs of Whatman 3MM paper, dried and then treated with ice cold 10% TCA. After 30 minutes the discs were rinsed several times with chilled 5% TCA and subsequently with chilled ethanol (containing 2% potassium acetate), ethanol–ether (1:1) mixture and finally with ether. The discs were dried and counted in a windowless gas flow counter.

Withaferin A and withanolid D were dissolved in dimethylformamide to a concentration of 4 mg/ml. During incubation control cells were treated with the same concentration of dimethylformamide (1%) as the treated cells.

From Fig. 1 it is evident that both withaferin A and withanolide D inhibit RNA synthesis of Sarcoma-180 ascites tumour cells; Within 30 min. of incubation RNA synthesis is inhibited more than 50 per cent by a dose of 40 μg/ml of both the drugs. Both the drugs are similar in structure and behave similarly towards inhibition of RNA synthesis. However, withaferin A inhibits protein synthesis of Sarcoma-180 cells, whereas protein synthesis is slightly stimulated in presence of this withanolide D. (Fig. 2).

Since mammalian cell mRNA is long lived, it is unlikely that inhibition of RNA synthesis would immediately affect protein synthesis of Sarcoma-180 cells. Thus from these results it is quite evident that withaferin A interferes not only with transcription but also with the translation process of these cells. Thus withaferin A and withanolide D, though similar in structure, differ in their mode of action. Present biochemical studies clearly reveal that inhibition of RNA synthesis by both of these drugs may be a vital cause of cell death in presence of these drugs, though cytological observations failed to show any change in biosynthesis and distribution of RNA in withaferin A treated cells when compared to controls [5].

The authors wish to express their sincere thanks to Prof. N. Chatterjee for his keen interest in the work. Thanks are also due to Dr. S. K. Chakraborti for helpful suggestions.

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Biochemical Pharmacology, Vol. 24, pp. 920–922. Pergamon Press, 1975. Printed in Great Britain.

# Effect of catecholamine analogs on the glycogen concentration in mouse parotid gland—Relationship to adenosine 3′,5′-monophosphate levels and increased deoxyribonucleic acid synthesis

(*Received* 15 *August* 1974; *accepted* 20 *September* 1974)

A single intraperitoneal injection of isoproterenol (IPR), a synthetic beta-adrenergic drug, produced after a lag period of 20 hr a marked increase in DNA synthesis in the mouse parotid gland [1]. Among the biochemical events preceding DNA synthesis there is an early decrease in the concentration of salivary glycogen [2]. There appeared to be a relationship between changes in hepatic glycogen level and the subsequent DNA synthesis produced in the mouse parotid by a number of catecholamine analogs [3].

Catecholamine-stimulated breakdown of glycogen by cardiac and smooth muscle is classified as a β-adrenergic response. There is a stimulation of adenylate cyclase and a rise in the tissue level of adenosine 3′,5′-monophosphate (cyclic AMP) [4] which results in the activation of phosphorylase [5].

Although catecholmines elevate tissue cyclic AMP levels by an apparent β-adrenergic mechanism, not all effects of catecholamines are associated with elevated levels of cyclic AMP. For example, in isolated rat liver cells [6] epinephrine and isoproterenol both elevated cyclic AMP levels, while only epinephrine increased gluconeogenesis. Also, catecholamines increased hepatic glycogenolysis by a cyclic AMP independent α-adrenergic mechanism [7]. Similarly, electrical stimulation of skeletal muscle results in an activation of phosphorylase without any detectable change in cyclic AMP level [8].

The present study uses a variety of catecholamine analogs to investigate the relationship between the initial decrease in glycogen concentration and changes in cyclic AMP level and DNA synthesis in mouse parotid.