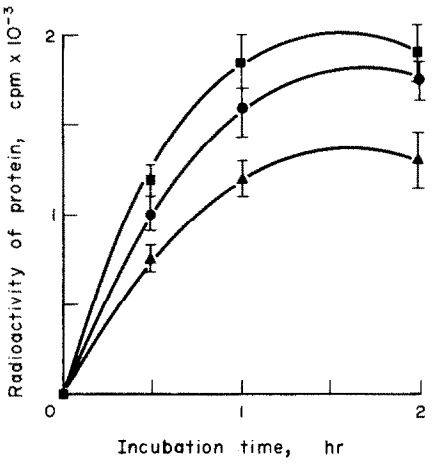

Fig. 2. Incorporation of DL-^{14}C-Phenylalanine into trichloroacetic acid insoluble proteins of Sarcoma-180 cells. ●—● control; ▲—▲-treated with Withaferin A; ■—■-treated with Withanolide D. Mean values ±S.D. of five experiments are given. Effect of Withanolide D is not significantly different from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

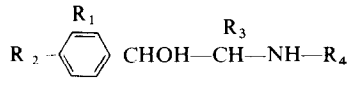
A single intraperitoneal injection of isoproterenol (IPR), a synthetic beta-adrenergic drug, produced after a lag period of 20 hr a marked increase in DNA synthesis in the mouse parotid gland [1]. Among the biochemical events preceding DNA synthesis there is an early decrease in the concentration of 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.

Table 1. Effect of catecholamine analogs on the concentration of cyclic AMP and glycogen and on DNA synthesis in mouse parotid gland*



Compound injected		R_1	R_2	R_3	R_4	Cyclic AMP (pmoles/mg DNA)	Glycogen (μg/g wet wt)	DNA synthesis†
I	(3)	OH	OH	H	$CH(CH_3)_2$	11,403 ± 2207	66 ± 5	+++
I‡	(3)	OH	OH	H	$CH(CH_3)_2$	407	136 ± 18	+
II	(3)	OH	OH	H	$CH(CH_3)_2$	1031 ± 407	121 ± 15	+++
III	(3)	OH	OH	H	$CH(CH_3)_2$	9281 ± 2239	43 ± 6	+++
IV	(4·5)	OH	OH	H	$(CH_2)_3CH_3$	5701	39 ± 10	+++
V	(1·5)	OH	OH	H	$CH(CH_3)CH_2CH_3$	6947	44 ± 8	+++
VI	(4·5)	OH	OH	CH_3	$CH(CH_3)_2$	5886	52 ± 1	++
VII	(3)	OH	OH	CH_2CH_3	$CH(CH_3)_2$	220	140 ± 27	−
VIII	(1·5)	OH	OH	H	CH_2CH_3	320 ± 29	36 ± 4	−
IX	(1·5)	OH	OH	CH_3	CH_3	298	59 ± 13	−
X	(4·5)	OH	H	H	CH_2CH_3	215	89 ± 17	−
XI	(1·5)	H	OH	CH_3	CH_3	402 ± 68	96 ± 16	+
XII	(4·5)	H	H	CH_3	CH_2CH_2OH	410	230 ± 37	−
XIII	(3)	CH_2OH	OH	H	$C(CH_3)_3$	707	137 ± 37	+
XIV	(2)	H	H	H	$CH(CH_3)_2$	299 ± 22	178 ± 28	+++
XV	(2)	Cl	Cl	H	$CH(CH_3)_2$	142	159 ± 29	+++
XVI§	(1·5)					383 ± 57	61 ± 4	+
H_2O						329 ± 18	130 ± 11	−

* Cyclic AMP and glycogen were determined at 15 min and 2 hr after analog injection on the pooled glands from three and six mice respectively. Other conditions were as described in text. Standard errors of the means are given where three or more separate determinations were made. Numbers in parentheses are the amounts of each compound injected (mg). DNA synthesis was stimulated 30-fold by IPR (I).

† The actual values for the level of thymidine incorporation into DNA will be published elsewhere.

‡ Propranolol, 1 mg, was administered 10 min prior to compound I.

§ Pilocarpine.

Male Fels A or Porton mice where used when 3–4 months old and weighing 26–30 g. Animals were kept on a 12-hr light and dark schedule and fed *ad lib.* until 2 hr before the experiment when food was withdrawn. Compounds were administered intraperitoneally. Mice were killed by cervical dislocation and the parotid glands dissected out, cleaned and frozen in liquid nitrogen in 30–45 sec. Cyclic AMP was purified and measured by the method of Butcher [9]. Glycogen and DNA specific activity were determined as described by Malamud and Baserga [2] and Kirby *et al.* [3] respectively. All determinations were carried out in duplicate.

The following compounds were kindly supplied free of charge: racemicisoproterenol (I), L(+)isoproterenol (II), D(−)isoproterenol (III), STO-4, 1424 (V), Lz-F-104 (VI), R-007-XL (VIII) and Sor-N-49 (IX) by Sterling-Winthrop, Rensselaer, N.Y.; SN4-151 (IV) by Frinton Laboratories, South Vineland, N.J.; isoetharine (VII) by Riker Laboratories, Loughborough, England; salbutamol (XIII) by Allen and Hanburys Ltd, Ware, England and propranolol by ICI Ltd., Alderley Park, England. *p*-Hydroxyephedrine (XI) and dichloroisoproterenol (XV) were supplied by Aldrich Chemical Co., and S40032 (X) and S37260 (XII) were obtained from Alfred Bader Chemicals. 1-Phenyl-2-isopropylaminoethanol (XIV) was synthesized as previously described by Kirby *et al.* [3].

Kirby *et al.* [3] have shown that the ability of a number of catecholamine analogs to stimulate DNA synthesis in combined mouse salivary glands was correlated with the ability to produce glycogenolysis in the liver. Since the DNA response in mouse salivary glands occurs primarily in the parotid [1] and changes in glycogen level were not determined in the salivary glands, the effects of certain of these analogs plus additional new ones on cyclic AMP levels, glycogen stores and DNA synthesis in the parotid were determined. The results are shown in Table 1.

Cyclic AMP levels 15 min after injection of the various compounds are shown, as this is the time of peak response to IPR (not shown). Compounds which did not raise cyclic AMP levels at 15 min had no effect on these levels at any of several other points studied (not shown).

Analogs were classified as α- or β-agonists or antagonists according to Ariens [10]. A number of β-adrenergic agonists produce large increases in cyclic AMP levels (I, III, IV, V and VI) and these compounds also caused glycogen depletion. Analogs II, XIII, XIV and XV, which have β-adrenergic agonist and/or antagonist actions in many systems, produced at most a very small rise in cyclic AMP and did not stimulate glycogenolysis. Propranolol, a β-adrenergic blocking agent, prevented the increase in cyclic AMP produced by IPR (I) and also inhibited its effect on glycogen breakdown. Compounds lacking both hydroxyl groups on the aromatic ring appeared to increase the level of parotid glycogen. Compounds VIII and X, which are mixed α- and β-agonists, and IX and XI, which are primarily α-agonists, all caused glycogenolysis, although removal of a hydroxyl group from the aromatic ring partially inactivates. Pilocarpine (XVI), which acts indirectly on the parotid through the superior cervical ganglion, also caused glycogenolysis. However, none of these compounds caused an increase in either DNA synthesis or cyclic AMP level.

These results suggest that increased cyclic AMP levels might be essential for the effect of β-adrenergic agonists on glycogenolysis, while α-adrenergic agonists act through a

cyclic AMP independent mechanism to stimulate glycogen breakdown. Since different analogs can cause glycogenolysis and not stimulate DNA synthesis and *vice versa*, it is clear that there is no correlation between these two biochemical events.

Acknowledgements—During the course of part of this work, F. R. B. was a United States Public Health Service Postdoctoral Fellow (F02-CA-43,880) in the laboratory of Dr. Van R. Potter and J. P. D. a Postdoctoral Fellow of the Damon Runyon Fund for Cancer Research in the laboratory of Dr. R. Baserga. Financial support was provided for F. R. B. in part by an American Cancer Society Grant (IN-45N) and the National Cystic Fibrosis Research Foundation. J. P. D. was supported by the Scottish Hospital Endowments Research Trust (HERT 422) during part of this work and is presently supported by a grant from the Medical Research Council (G. 973/954/B).

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Biochemical Pharmacology, Vol. 24, pp. 922–923. Pergamon Press, 1975. Printed in Great Britain.

The effect of heating rat liver cytosol on oestrogen-induced tryptophan oxygenase activity

(*Received* 14 *June* 1974; *accepted* 3 *October* 1974)

Recently it has been shown that thermal activation of rat liver cytosol in the presence of tryptophan as stabilizer results in a considerable increase in the total enzyme units of tryptophan oxygenase [1]. During our studies on the effect of treatment with steroid hormones upon rat liver tryptophan oxygenase we initially compared the activities of non-activated and thermally activated tryptophan oxygenase. Besides large differences in the absolute values for the activities of the enzyme in the activated and non-activated state, we observed considerable differences in holoenzyme activation after induction of apoenzyme synthesis by oestrogens dependent on whether the liver cytosol had been heated or not (Table 1).

Female or male Wistar rats, weighing 180–200 g, were injected subcutaneously with 0·1 ml of an oestradiol benzoate solution in arachis oil (for doses see Table 1) for 15 days. The last injection was given one hour before decapitation. The livers were removed and homogenized in three volumes of 0·14 M potassium chloride containing 0·01 M L-tryptophan. Preparation of the cytosol and thermal activation (heating of the cytosol at 55° for 5 min) were carried out according to Schutz and Feigelson [1]. Estimation of tryptophan oxygenase activity was based on the combined rates of formylkynurenine plus kynurenine formation [1], which were measured by recording optical densities at 321 and 360 nm with a Zeiss PM QII spectrophotometer at 37°. The experimental design (complete randomization of rats and treatments) met the requirements for a statistical analysis of results by means of analysis of variance.

Treatment of female rats with oestradiol benzoate caused a highly significant increase in tryptophan oxygenase activity when the heated cytosol was used as the enzyme source,

Table 1. Activities of non-activated and thermally activated rat hepatic tryptophan oxygenase after treatment with oestradiol benzoate for 15 days

Treatment dose (μg/kg)	Tryptophan oxygenase activity (μmoles (formylkynurenine + kynurenine)/gm liver/hr ± SEM) Female rats		Male rats	
	Activated	Non-activated	Activated	Non-activated
Placebo (8)*	8·4 ± 0·6	2·6 ± 0·1	7·3 ± 0·3	2·1 ± 0·1
O.B.† (8) 50	13·5 ± 0·4‖	2·7 ± 0·1	11·9 ± 0·5‖	2·9 ± 0·2‡
O.B. (8) 100	13·3 ± 0·5‖	2·8 ± 0·3	12·7 ± 0·3‖	3·2 ± 0·2§

* Number of rats for each experiment.
† Oestradiol benzoate.
Statistical significance as compared to placebo values calculated by means of analysis of variance: ‡ $P < 0.05$, § $P < 0.01$, ‖ $P < 0.001$.