Biochemical Pharmacology, Vol. 20, pp. 3481–3491. Pergamon Press, 1971. Printed in Great Britain

DRUG STIMULATION OF PUTRESCINE AND SPERMIDINE SYNTHESES

RELATIONSHIPS TO ENHANCEMENT OF RIBONUCLEIC ACID SYNTHESIS

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(*Received* 25 *March* 1971; *accepted* 30 *June* 1971)

Abstract—The enzymes that synthesize putrescine and spermidine and the rate of uridine-6-[^{3}H] incorporation were measured after a single treatment with various drugs, some of which are known to cause liver enlargement after chronic administration. The drugs examined were phenobarbital, 3-methylcholanthrene, 3,4-benzpyrene, clofibrate (atromid-S), and heparin. All these drugs produced elevations of both ornithine decarboxylase (L-ornithine carboxy-lyase; EC 4.1.1.17), the enzyme that catalyzes the formation of putrescine from ornithine, and S-adenosyl-L-methionine decarboxylase, the enzyme that catalyzes the formation of spermidine from putrescine and S-adenosyl-L-methionine. They also stimulated the incorporation of uridine-6-[^{3}H], an RNA precursor. Increased uridine-6-[^{3}H] incorporation followed enhanced ornithine decarboxylase activity and paralleled increased spermidine synthesis in most cases. Cycloheximide administered at the same time as phenobarbital completely abolished the induction of these enzymes.

NUMEROUS studies have shown that a single injection of a wide variety of drugs elicits enhancements of rat liver microsomal enzyme systems. These enzymes may then function in the metabolism of these drugs.[1] Prolonged treatment with such agents as phenobarbitol and 3-methylcholanthrene also produces hepatomegaly.[2] Chronic treatment with either 3-methylcholanthrene or 3,4-benzpyrene, also causes some liver enlargement,[1] and single injections produce alterations in the liver's protein synthetic apparatus, changes in the amount of nuclear RNA, and in the messenger activity of the nuclear RNA of the liver.[3] A recent report indicates that RNA levels and spermidine levels increase simultaneously in mouse liver after treatment with phenobarbital.[4] Putrescine and spermidine are polycationic compounds whose syntheses increase very early in rapid growth systems, i.e. regenerating rat and mouse liver, tumors, and developing embryos,[5–13] and in the liver after growth hormone administration.[14–16] Increased spermidine concentrations parallel increased RNA concentrations in those systems studied.[5,6,17]

If new putrescine and spermidine synthesis were necessary for growth and RNA synthesis, the enzymes involved in polyamine synthesis should be affected by drugs that produce liver enlargement. In both bacterial and mammalian systems, putrescine can be formed from the decarboxylation of ornithine and is catalyzed by ornithine decarboxylase (ODC).[18,19] S-adenosyl-L-methionine decarboxylase (SAMD) functions in spermidine synthesis by the decarboxylation of the methionine portion of the S-adenosyl-L-methionine (SAM) molecule. Then a propylamine moiety is transferred

from the decarboxylated SAM to putrescine to form spermidine.[20] In mammalian systems, the decarboxylation of SAM is putrescine-dependent and associated with the stoichiometric production of spermidine,[20] indicating a tight coupling of the decarboxylation and transfer reactions.

In this paper, ornithine decarboxylase (ODC) activity, S-adenosyl-L-methionine decarboxylase (SAMD) activity, and the incorporation of uridine-6-[^{3}H] were measured at 4, 24 and 48 hr after a single injection of drugs, some of which are known to produce hepatomegaly after chronic administration.

MATERIALS AND METHODS

DL-Ornithine-1-[^{14}C] monohydrochloride (3·10 mc/m-mole), DL-methionine-1-[^{14}C] (3·54 mc/m-mole), putrescine-1,4-^{14}C dihydrochloride (11·27 mc/m-mole) and uridine-6-^{3}H (10·4 mc/m-mole) were obtained from New England Nuclear Corp. Cycloheximide, the hydrochloride salts of putrescine, spermidine, and spermine, Tris, ATP, and heparin (grade 1) were obtained from Sigma. Atromid-S was purchased from Ayerst Laboratories. Phenobarbital sodium (USP) was purchased from Merck & Company. 3-Methylcholanthrene, 3,4-benzpyrene, and pyridoxal phosphate were obtained from Nutritional Biochemicals Corp.

4-Bromo-3-hydroxybenzyloxyamine dihydrogen phosphate (NSD-1055) was a gift from Smith & Nephew Research Ltd. *Escherichia coli* strain B cells were obtained from Grain Processing, Muscatine, Iowa.

Sprague–Dawley male rats (125–150 g) were ordered from Zivic–Miller. All rats were maintained in a room with a 0700–1900 hr photoperiod. To avoid the possibility of cyclical enzymatic changes, all animals were killed between 0900 and 1100 hr. Rats were killed by cervical fracture and the livers were removed, chilled immediately on ice, weighed, and homogenized in 5 vol. of 0·1 M sodium potassium phosphate buffer, pH 7·2, and 1 mM dithiothreitol. The homogenate was centrifuged at 20,000 *g* for 20 min, and the supernatant solution was used for enzymatic assays. The supernatant solution after 100,000 *g* centrifugation for 90 min gave identical enzyme activities to that found with a 20,000 *g* (20 min) supernatant solution. Therefore the supernatant solution after centrifugation at 20,000 *g* for 20 min was routinely used for enzymatic assays.

Determination of incorporation of uridine-6-[^{3}H]. Groups of 10 rats each were injected in the tail vein with 10 μc [^{3}H]uridine. They were killed 10 min later by cervical fracture, a 0·5-g sample of liver was removed, blotted, frozen in an ethanol–dry ice mixture, and stored at −20°. Isotope incorporation was determined by the procedures outlined by Fujioka *et al.*[21] A 10-min pulse has been shown to label only RNA (unpublished data). Samples were radioassayed in 0·5 ml of 1 M Hyamine in methanol, and 10 ml of Omnifluor toluene in a Packard model 3375 TriCarb liquid scintillation spectrometer.

Preparation of ^{14}COOH-S-adenosyl-L-methionine. Labeled ^{14}COOH-S-adenosyl-L-methionine was prepared from methionine-1-[^{14}C] and ATP: L-methionine-S-adenosyltransferase isolated from *E. coli* strain B cells (Grain Processing, Muscatine, Iowa). The general procedure for preparation of ATP: L-methionine-S-adenosyltransferase from *E. coli* as described by Tabor and Tabor[22] was followed. *E. coli* (150 g) was suspended in 350 ml of chilled 0·01 M potassium phosphate buffer (pH 7·0)

containing 5 mM 2-mercaptoethanol. Cells were sonicated for 15 min at 9 kc with a model 60 W MSE ultrasonic disintegrator, taking care to maintain the temperature below 10°. Saturated ammonium sulfate solution (pH 7·0, 5 mM 2-mercaptoethanol) was added to give a 33 per cent final saturation and allowed to stir overnight in the cold room. The precipitate was removed by centrifugation for 20 min at 12,000 *g* and discarded. The supernatant fluid was then brought to a 50 per cent final saturation of ammonium sulfate and stirred for 2 hr. After centrifugation, the precipitate (33–50% ammonium sulfate) was dissolved in 0·01 M potassium phosphate buffer (pH 7·0, 5 mM 2-mercaptoethanol) and stirred slowly overnight. This enzyme preparation was then used to synthesize ^{14}COOH-S-adenosyl-L-methionine from methionine-1-[^{14}C]. The reaction mixture and procedures for this preparation were the same as those previously described,[20] except that the *E. coli* enzyme preparation described above was used.

Determination of ornithine decarboxylase activity. Ornithine decarboxylase activity was determined by measuring the liberation of $^{14}CO_2$ from carboxyl-labeled substrate as previously described.[7,14] For each enzymatic determination, a 1-g liver sample was homogenized in 5 vol. of 0·05 M sodium–potassium phosphate buffer, pH 7·2, and 1 mM dithiothreitol.

The substrate concentration used (50 μM as L-ornithine) was nonsaturating. However, in some experiments, excess ornithine (2 mM) was used as substrate, and the same changes in enzyme activity were obtained after drug administration.

Ornithine decarboxylase activity in supernatant preparations from drug-treated and control rat livers was unaffected by dialysis for 4 hr against H_2O or by the addition of 1 mM Mg^{2+}, 1 mM Ca^{2+}, 0·05 M PO_4^{3-}, 1 mM SO_4^{3-}, and 1 mM HCO_3^-. Enzyme activity was the same whether assayed in 20,000 *g* or 100,000 *g* supernatant fractions.

All data are expressed as per cent of controls. Controls were litter mates that received saline or the solvent material for the particular drug that was administered. Drugs were administered in 0·5 ml of the appropriate solvent.

Assay for S-adenosyl-L-methionine decarboxylase activity. Enzyme activity was determined by measuring the liberation of $^{14}CO_2$ from ^{14}COOH-S-adenosyl-L-methionine. Incubations were carried out in 15-ml centrifuge tubes equipped with a rubber stopper supporting a polyethylene center well (Kontes Glass Company) that contained 0·2 ml of a 2:1 mixture of ethanolamine and 2-methoxyethanol. Incubation mixtures consisted of 150 μM ^{14}COOH-S-adenosyl-L-methionine, 2·5–5 mM sodium–potassium phosphate buffer, pH 7·2, 50 μM pyridoxal phosphate, 2·5 mM putrescine, and 0·05–0·15 ml (1·5–5 mg protein) of enzyme supernatant solution prepared as described above, to make a total volume of 0·2 ml. At least two different enzyme concentrations were run for each liver sample and the measured activity was always proportional to the amount of enzyme supernatant solution added. Duplicates for each concentration were also run.

The substrate concentration used (150 μM used as S-adenosyl-L-methionine) was nonsaturating. However, in some experiments, excess substrate (2 mM) was used and the same changes were observed between the experimental and control systems. Enzyme activity was unaffected by dialysis for 6 hr against 0·01 M phosphate buffer, pH 7·2, and 10^{-3} M pyridoxal phosphate. The enzyme was putrescine-dependent, however, as was the ventral prostrate enzyme described previously.[20]

The reaction mixture was shaken for 10 min at 37° in a Dubnoff metabolic shaker prior to addition of the substrate. After addition of substrate, the incubation was continued for 30 min under the same conditions. The reaction was stopped by careful injection of 0·25 ml of 1·0 M citric acid into the reaction mixture through the rubber stopper. The mixture was shaken for an additional 15 min to allow complete absorption of the evolved $^{14}CO_2$.

The center well was removed, placed in a vial containing 2 ml ethanol and 10 ml of Omnifluor toluene, and assayed for radioactivity in a Packard-TriCarb liquid scintillation counter. All values obtained were corrected by comparison against a reaction mixture (control-heated) enzyme assay. NSD-1055, an inhibitor of pyridoxal phosphate-dependent enzymes was also used to corroborate the blank values.

Determination of ^{14}C-spermidine formation from [^{14}C]putrescine. To determine if the evolution of $^{14}CO_2$ from ^{14}COOH-S-adenosyl-L-methionine was a reliable index of spermidine formation, [^{14}C]putrescine was added to the assay mixture in some experiments instead of ^{14}COOH-S-adenosyl-L-methionine.

Incubation conditions were the same as described above. The reaction was stopped with 1 ml of 10% TCA, ether extracted, and then treated with 0·25 ml of 0·1 N NaOH; the solution was saturated with a salt mixture (62·5 g anhydrous sodium sulfate and 9 g trisodium phosphate ground together and desiccated) and extracted into 1-butanol. The butanol phase was removed, acidified with a few drops of concentrated HCl, evaporated to dryness, and the residue dissolved in 0·2 ml of 0·1 N HCl. An aliquot of this solution was applied to 3 MM Whatman chromatography paper and run at 3000 V for 1·5 hr in a 0·1 M citric acid–NaOH buffer, pH 4·3.[6] Recovery rate of spermidine was determined by adding known amounts of [^{14}C]spermidine to reaction mixtures without labeled substrate and extracting as described. Ninety per cent of authentic spermidine was recovered into butanol in this system and the appropriate correction was made. The spermidine spot was cut out and placed into a vial with 5 ml ethanol. After 30 min, 10 ml Omnifluortoluene was added and radioactivity was measured. The site of the spermidine on the chromatography paper was determined by drawing a glass rod dipped into a ninhydrin–acetone–acetic acid–cadmium mixture along the chromatograph and developing the color in a drying oven for 15 min. Standards were also run and stained, and were detected at the same distance from the origin.

Indeed there was no difference in the two methods, and $^{14}CO_2$ evolution from ^{14}COOH-S-adenosyl-L-methionine was a reliable index of spermidine formation.

RESULTS

Effect of phenobarbital on putrescine, spermidine and RNA synthesis. A group of 10 rats received intraperitoneally phenobarbital (100 mg/kg) at 4, 24 and 48 hr prior to assaying their livers for enzymatic activity and for RNA synthesis. ODC and SAMD were measured in 20,000 *g* supernatant solutions. For the determination of the incorporation of uridine-6-^{3}H, rats were injected with 10 μc uridine-6-^{3}H (10·4 mc/m-mole), killed 10 min later, and the amount of radioactivity in acid-insoluble TCA fractions was determined.[21] These procedures were followed for each drug that was injected.

Within 4 hr after a single intraperitoneal injection of phenobarbital, ODC activity

in rat liver had doubled (Fig. 1). Ornithine decarboxylase activity remained elevated 24 hr after drug treatment, but returned to basal level by 48 hr.

S-adenosyl-L-methionine decarboxylase activity was not elevated at 4 or 24 hr after drug treatment, but exhibited over a 2-fold enhancement in activity by 48 hr. Increased incorporation of uridine-6-[^{3}H] (130 per cent of controls) was also detectable 48 hr after drug treatment.

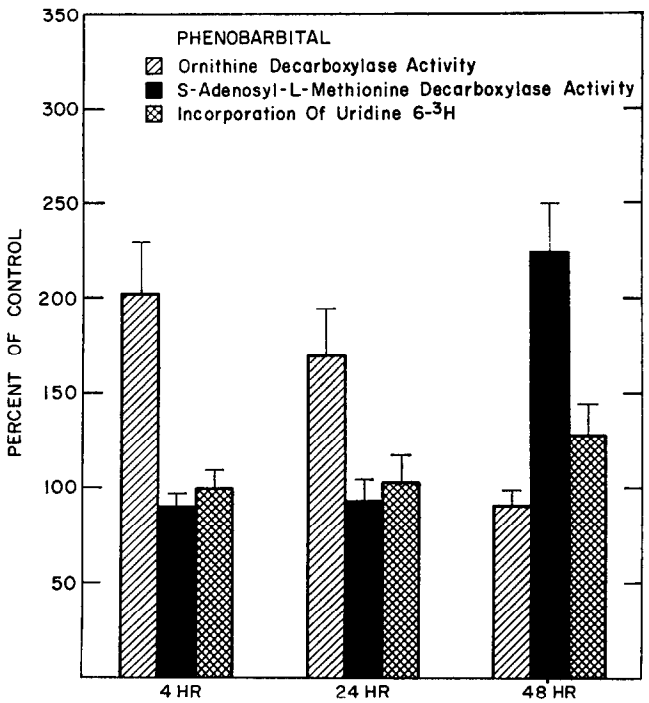

FIG. 1. Groups of 10 rats each received 100 mg/kg of phenobarbital intraperitoneally and were killed 4, 24, and 48 hr thereafter. Supernatant solutions of their livers were assayed for ornithine decarboxylase and S-adenosyl L-methionine decarboxylase activities. Control values for these enzymes expressed as millimicromoles $^{14}CO_2$/30 min/g wet wt. ± S.E.M. were 2·6 ± 0·5 and 43 ± 7·1 respectively. The incorporation of uridine into RNA was determined in the following manner: each rat was injected with 10 μc uridine-6[^{3}H] (10·4 mc/m-mole), killed 10 min later, a 0·5-g sample homogenized as outlined by Fujioka *et al.*,[21] and the radioactivity of the acid-insoluble TCA fraction determined. Incorporation of uridine-6-[^{3}H] into control livers was 85,000 ± 10,000 counts/min/g wet wt.

Effects of 3-methylcholanthrene and 3,4-benzpyrene on putrescine and spermidine synthesis and on RNA synthesis. 3-Methylcholanthrene showed an early effect on putrescine synthesis, whereas 3,4-benzpyrene caused a much later increase of ODC (Figs. 2, 3). Within 4 hr after 3-MC (Fig. 2), ODC activity was 175 per cent of controls, by 24 hr was 300–400 per cent of controls, and was still over 200 per cent of controls within 48 hr after drug administration (Fig. 2).

After 3-MC, spermidine synthesis as measured by the activity of SAMD was enhanced by 24 hr (125 per cent of controls), and markedly elevated by 48 hr (225 per cent of controls). Increased uridine-6-^{3}H incorporation was elevated within 48 hr after 3-MC, when it was 170 per cent of controls (Fig. 2).

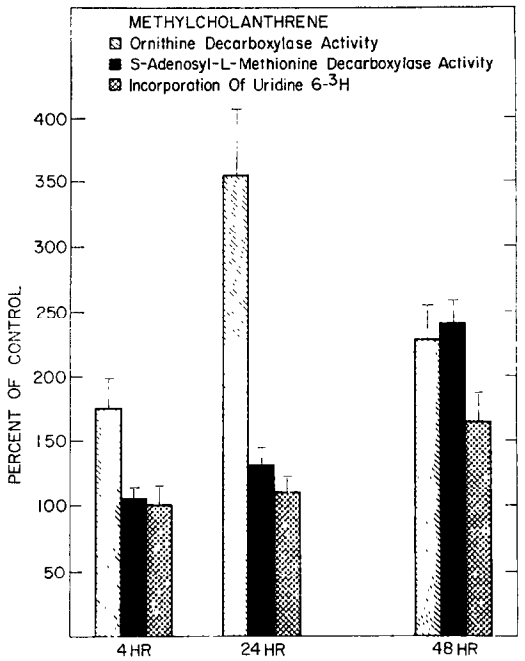

FIG. 2. Groups of 10 rats each received 50 mg/kg of intraperitoneal injections of 3-methylcholanthrene in sesame seed oil. Controls received only the sesame seed oil. They were killed 4, 24, and 48 hr thereafter. Supernatant solutions of their livers were assayed for ornithine decarboxylase and S-adenosyl-L-methionine decarboxylase activities. Control values for these enzymes expressed as millimicromoles $^{14}CO_2$/30 min/g wet wt. ± S.E.M. were 2·4 ± 0·5 and 42 ± 5·6, respectively. The incorporation of uridine into RNA was determined in the following manner: each rat was injected with 10 μc uridine-6-[^{3}H], killed 10 min later, a 0·5-g liver sample was processed according to Fujioka *et al.*,[21] and the radioactivity of the acid-insoluble TCA fraction determined. Uridine-6-[^{3}H] incorporation into control livers was 85,000 ± 10,000 counts/min/g wet wt.

After 3,4-benzpyrene injection (Fig. 3), there were no early changes in the activities of either enzyme in the polyamine biosynthetic pathway, nor in incorporation of uridine-6-^{3}H. Within 24 hr, however, ODC activity increased to 250 per cent of controls and within 48 hr increased to over 650 per cent of controls. Both SAMD activity and uridine-6-[^{3}H] incorporation showed marked elevations at 48 hr: i.e. SAMD activity, 220 per cent of controls; uridine-6-[^{3}H] incorporation, 170 per cent of controls.

Effect of heparin. Heparin administration stimulated an early increase in ODC activity (Fig. 4). ODC activity increased to 300 per cent of controls by 24 hr and returned to control level by 48 hr after drug administration.

SAMD activity showed an early activation and was 125 per cent of controls within 4 hr after heparin injection, 135 per cent within 24 hr, and still 120 per cent of controls within 48 hr (Fig. 4).

Within 24 hr there was some slight increase in uridine-6-[^{3}H] incorporation, and within 48 hr a 25 per cent increase in uridine-6-[^{3}H] incorporation was detectable (Fig. 4).

Effect of atromid-S. Within 4 hr after atromid-S injection, ODC activity was 300

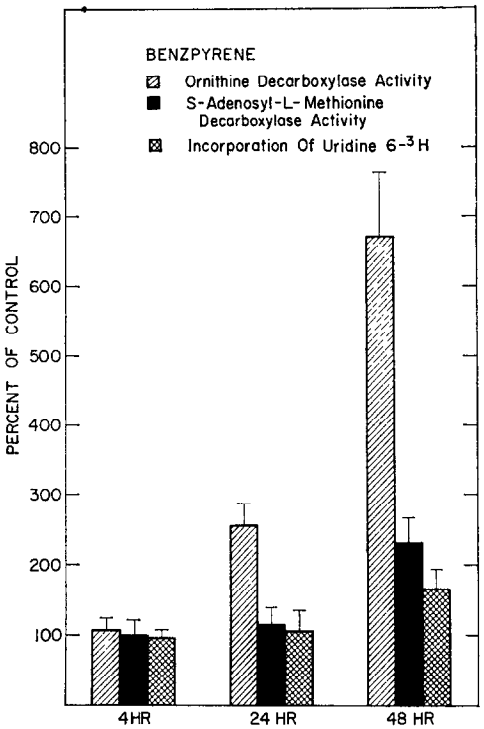

FIG. 3. Groups of 10 rats each received 50 mg/kg of intraperitoneal injections of 3,4-benzpyrene in sesame seed oil. Controls received only the sesame seed oil. They were killed 4, 24 and 48 hr thereafter. Supernatant solutions of their livers were assayed for ornithine decarboxylase and S-adenosyl-L-methionine decarboxylase activities. Control values for these enzymes expressed as millimicromoles $^{14}CO_2$/30 min/g wet wt. ± S.E.M. were 1·8 ± 0·4 and 43 ± 6·8 respectively. The incorporation of uridine into RNA was determined in the following manner: each rat was injected with 10 μc uridine-6-[^{3}H], killed 10 min later, a 0·5-g liver sample processed according to Fujioka *et al.*,[21] and the radioactivity of the acid-insoluble TCA fraction determined. Uridine-6-[^{3}H] incorporation into control livers was 85,000 ± 10,000 counts/min/g wet wt.

per cent of controls (Fig. 5). ODC activity was similar to that of controls at 24 and 48 hr after atromid-S administration.

SAMD activity was 125 per cent of controls at 24 hr after atromid-S, and was 130 per cent of controls within 48 hr.

Incorporation of uridine-6-[^{3}H] was elevated as early as 4 hr, when it was 120 per cent of controls. By 24 and 48 hr, uridine-6-[^{3}H] incorporation had risen to 130 and 140 per cent of controls respectively.

Cycloheximide inhibition. When cycloheximide (50 mg/kg) was given at the same time as phenobarbital, the enhancements of ODC and SAMD were suppressed (Table 1). ODC activity was so low after cycloheximide that it was undetectable at 4, 24 and 48 hr after drug administration. SAMD was about 25 per cent of controls at 4 hr, and was nondetectable at 24 and 48 hr after cycloheximide.

DISCUSSION

An early indication of tissue stimulation is the elevation within a tissue of ornithine decarboxylase activity,[7,10,12] the enzyme which catalyzes the formation of putrescine

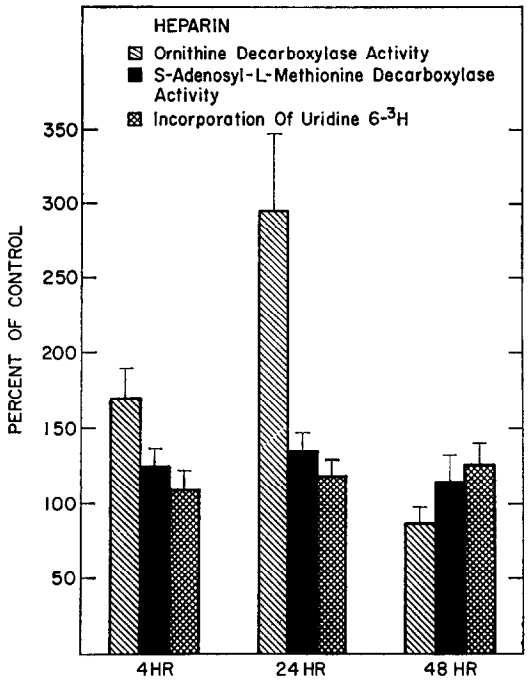

FIG. 4. Groups of 10 rats each received 50 mg/kg of intraperitoneal injections of heparin and were killed 4, 24 and 48 hr thereafter. Supernatant solutions of their livers were assayed for ornithine decarboxylase and S-adenosyl-L-methionine decarboxylase activities. Control values for these enzymes expressed as millimicromoles $^{14}CO_2$/30 min/g wet wt. ± S.E.M. were 2·7 ± 0·5 and 45 ± 8·1 respectively. The incorporation of uridine into RNA was determined in the following manner: each rat was injected with 10μc uridine-6-[^{3}H], killed 10 min later, a 0·5-g liver sample processed according to Fujioka *et al.*,[21] and the radioactivity of the acid-insoluble TCA fraction determined. Uridine-6-^{3}H incorporation into control livers was 85,000 ± 10,000 counts/min/g wet wt.

and which is the initial step in the biosynthetic pathway of polyamines.[18,20] All the drugs administered in these studies, with the exception of benzpyrene, produced an elevation in rat liver ornithine decarboxylase activity within 4 hr of administration. However, the magnitude of the response and the temporal aspect of the elevation varied considerably. For instance, phenobarbital and atromid-S produced a 2- to 3-fold increase of ornithine decarboxylase activity within 4 hr, but within 24 hr the phenobarbital-stimulated liver still exhibited elevated enzyme activity, whereas the atromid-S-stimulated liver did not. In both cases, the ornithine decarboxylase activity had returned to the control level within 48 hr. Heparin or methylcholanthrene administration produced an increment in ornithine decarboxylase activity within 4 hr and a further increment in activity within 24 hr. Thereafter, ornithine decarboxylase activity declined to the control level in the liver of the heparin-treated animals, but was still twice the control level in the methylcholanthrene-treated animals.

Benzpyrene had no effect on liver ornithine decarboxylase activity within 4 hr, but at 24 and 48 hr ornithine decarboxylase activity was 260 and 670 per cent of controls respectively. Therefore, although ornithine decarboxylase activity is stimulated in all cases, the temporal pattern of this stimulation is varied, indicating that these drugs stimulate ornithine decarboxylase activity by action at different levels or sites in the

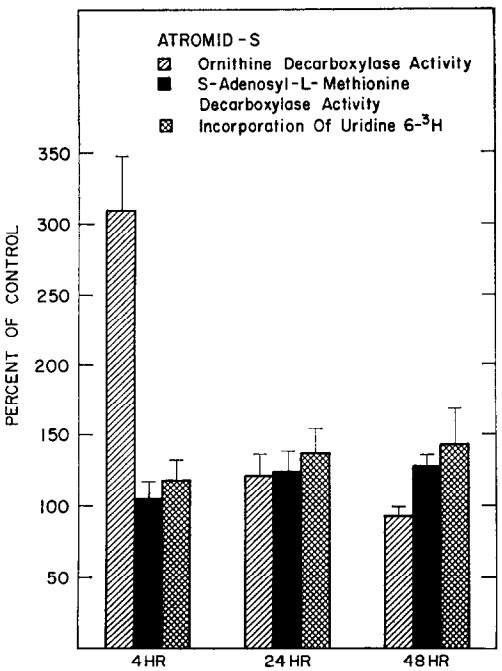

FIG. 5. Groups of 10 rats each received 50 mg/kg of intraperitoneal injections of atromid-S in sesame seed oil. Controls received only the sesame seed oil. They were killed 4, 24 and 48 hr thereafter. Supernatant solutions of their livers were assayed for ornithine decarboxylase and S-adenosyl-L-methionine decarboxylase activities. Control values for these enzymes expressed as millimicromoles $^{14}CO_2$/30 min/g wet wt. ± S.E.M. were 2·6 ± 0·5 and 44 ± 6·0 respectively. The incorporation of uridine into RNA was determined in the following manner: each rat was injected with 10 μc uridine-6-[^{3}H], killed 10 min later, a 0·5-g liver sample processed according to Fujioka *et al.*,[21] and the radioactivity of the acid-insoluble TCA fraction determined. Uridine-6-^{3}H incorporation into control livers was 85,000 ± 10,000 counts/min/g wet wt.

TABLE 1. EFFECTS OF CYCLOHEXIMIDE ON ORNITHINE DECARBOXYLASE AND S-ADENOSYL-L-METHIONINE DECARBOXYLASE ACTIVITIES IN RAT LIVER AFTER PHENOBARBITAL ADMINISTRATION*

Time after cycloheximide (hr)	$^{14}CO_2$ (mμmoles/30 min/g wet wt.)	
	Ornithine decarboxylase activity	S-adenosyl-L-methionine decarboxylase activity
0	2·6 ± 0·5	43·0 ± 7·1
4	< 2·0	9·6 ± 2·3
24	< 2·0	< 4·0
48	< 2·0	< 4·0

* Cycloheximide (50 mg/kg) was administered to groups of five rats each immediately after the injection of phenobarbital. At 4, 24 and 48 hr thereafter, the rats were killed and supernatant solution of their livers was assayed for ODC and SAMD activities as described in the text.

cell. This is in line with evidence that indicates varying patterns of microsomal enzyme induction with phenobarbital as opposed to methylcholanthrene and other polycyclic hydrocarbons.[2]

Variations in the extent and timing of S-adenosyl-L-methionine decarboxylase activation after various drug administrations were noted also. S-adenosyl-L-methionine decarboxylase, an enzyme involved in spermidine synthesis, shows a later increment than that of ornithine decarboxylase in most cases, but appears to increase in concert with uridine-6-[^{3}H] incorporation. This corroborates a recent report which showed a constant relationship between the levels of spermidine and RNA in mouse liver after phenobarbital treatment.[4] The most dramatic increases in the S-adenosyl-L-methionine decarboxylase activity occurred in the liver 48 hr after either phenobarbital, methylcholanthrene or benzpyrene administration. Atromid-S or heparin administration did not result in a highly significant increase in S-adenosyl-L-methionine activity at any time measured. However, because both these drugs stimulated ornithine decarboxylase activity, spermidine synthesis in all likelihood was enhanced due to an increased putrescine pool. A rise in ornithine decarboxylase activity appears to be an accurate indication of new putrescine accumulation.[7,11,23,24] Chronic atromid-S administration results in liver enlargement[25] but chronic heparin administration is not tolerated (unpublished data). Heparin was used in this study because of a report that it caused marked stimulation of mitotic activity in normal rat liver parenchymal cells,[26] and also a reported stimulation of DNA synthesis *in vitro* in thymocyte nuclei.[27]

There was some correlation between active polyamine biosynthesis and active uridine-6-[^{3}H] incorporation. The largest increment in uridine-6-[^{3}H] incorporation occurred in the liver within 48 hr of methylcholanthrene administration, and this occurred concomitantly with the highest S-adenosyl-L-methionine decarboxylase activity (240 per cent of controls) recorded in this study. Benzpyrene also produced marked increases of ornithine decarboxylase, S-adenosyl-L-methionine decarboxylase, and uridine-6-[^{3}H] incorporation, all at 48 hr after drug administration.

Failure to find increased enzyme activities after simultaneous phenobarbital and cycloheximide administration may mean that *de novo* synthesis of both enzymes is responsible for the elevated activities after phenobarbital administration alone. However, cycloheximide can have other effects besides the inhibition of protein synthesis,[28] so these data must be interpreted with caution.

It appears, then, that a series of drugs known to alter various biochemical parameters within the cell all stimulate the polyamine biosynthetic pathway to various degrees and with different time courses. Second, uridine-6-[^{3}H] incorporation, and thus presumably RNA synthesis, are stimulated to some extent under all the experimental regimens, but are the most stimulated in those systems with highly stimulated polyamine synthesis. Third, maximal stimulation of putrescine synthesis seems to precede the enhancement of the uptake of RNA precursor, whereas spermidine synthesis parallels the uptake of RNA precursor. More studies would be necessary in order to determine if there are minute differences in the onset of spermidine synthesis and the enhancement of the incorporation of RNA precursor.

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